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CLINICAL AND BIOLOGICAL ASPECTS OF MUCINOUS COLORECTAL CANCER

NIEK HUGEN
CLINICAL AND BIOLOGICAL ASPECTS
OF MUCINOUS COLORECTAL CANCER

Niek Hugen
About the cover
Cancer therapy is increasingly being tailored to the needs of the individual patient, depending upon both patient and tumor characteristics. Tumor histology is one of the most easily accessible features, although not yet widely used in clinical practice. Mucinous adenocarcinoma is found in one in every eight colorectal cancer patients. A raised awareness of the various tumor subtypes might improve prediction of prognosis and might lead to more-tailored approaches to patient management.

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CLINICAL AND BIOLOGICAL ASPECTS
OF MUCINOUS COLORECTAL CANCER

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CLINICAL AND BIOLOGICAL ASPECTS OF MUCINOUS COLORECTAL CANCER

Doctoral thesis

to obtain the degree of doctor
from Radboud University Nijmegen
on the authority of the Rector Magnificus,
according to the decision of the Council of Deans
to be defended in public on Thursday, January 7, 2016
at 14.30 hours

by

Niek Hugen

born on March 25, 1989
in Doetinchem
Paranimfen
Marieke de Kuyper-de Ridder
Jasper van Lieshout
To my parents
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INTRODUCTION
General Introduction
General Introduction

Colorectal cancer

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer related mortality worldwide. The lifetime risk of developing CRC is 5% in the Netherlands, thereby comprising a major burden for health care. CRCs are classified into histological classification groups by the pathologist according to their differentiation. The majority of CRCs are the common adenocarcinomas, often referred to as adenocarcinomas not otherwise specified (AC). In 10-15% of CRC cases patients suffer from mucinous adenocarcinoma (MC), which is characterized by the presence of extracellular mucus, that comprises at least 50% of the tumor volume (Figure 1). In only 1% of CRCs a signet-ring cell carcinoma (SRCC) is reported. The hallmark of SRCC is a tumor containing abundant mucus in more than 50% of its cells. The relatively rare occurrence of MC and SRCC makes these subtypes less studied and conclusions and implications have mainly been drawn from small studies. Also, the histological subtype often has not been addressed in clinical trials and clinical practice is generally extrapolated to all histological subtypes.

Figure 1. Haematoxylin and eosin stained tumor, from left to right adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma.

Diagnostics and treatment of CRC have been subjected to continuous development over the past few decades. New surgical techniques have been introduced and neo-adjuvant and adjuvant therapies have improved disease free survival and overall survival. Also, standardized pathological assessment has improved reporting back to the clinic. Technological advances in imaging and introduction of new targeted cancer treatments initiated the era of personalized medicine. Molecular and genetic analyses of tumors for specific mutations that may aid in the prediction of prognosis have become one of the main focuses of research. This, however, resulted in overlooking the relevance of information that was readily available, the histological subtype.

The debate concerning the prognostic impact of the MC and SRCC subtypes has been ongoing ever since the introduction of the classification of CRCs according to their histological differentiation. Lack of numbers of patients and inaccurate registries have posed problems in determining clinical relevance. Nonetheless, MC and SRCC patients were considered prognostic unfavorable tumors and resistance to therapies was suspected.
**Tumor development and diagnosis**

CRC develops when normal cell division and cell growth is distorted and becomes uncontrolled. Mutations in regulatory genes such as proto-oncogenes, tumor suppressor genes or genes involved in DNA proofreading and repair result in carcinogenesis. Tumors progress from local to invasive, eventually having the potential to spread throughout the body leading to metastatic disease. CRC is diagnosed after physical examination, colonoscopy and imaging such as computed tomography (CT) or magnetic resonance imaging (MRI). Preoperative staging and determination of tumor characteristics from imaging and biopsy is important, since it enables the multidisciplinary team of clinicians to make appropriate decisions concerning treatment.

**Staging**

The TNM classification system is the currently used method to classify the invasiveness of disease. Depth of tumor penetration through the bowel wall (T-stage), lymph node involvement (N-stage) and distant metastasis (M-stage) result into a further classification of stages of disease. Stage I and II tumors are local tumors that are limited to the bowel wall, while stage III tumors have already spread to local lymph nodes. Presence of metastases leads to classification as stage IV disease, the most advanced stage. Preoperative and postoperative pathological staging highly influences the decision-making process concerning the optimal treatment.

![Figure 2. TNM classification and staging system.](image-url)
Treatment

For colorectal cancer patients, decisions regarding treatment are made in a multidisciplinary board, usually consisting of a surgical oncologist, a pathologist, a radiation oncologist, a medical oncologist and a radiologist. For each individual patient the therapeutic plan and follow-up strategy are discussed according to national and international guidelines.

Surgery

The main treatment for CRC is surgical removal of the tumor. For colon cancer an open or laparoscopic resection is performed according to principles of complete mesocolic excision. This implies that the tumor is removed plus a section of normal tissue on both sides of the tumor, as well as nearby lymph nodes that can be found in the mesentery. Surgical treatment of rectal cancer has changed over the past decades. While the initial surgery focused on removal of the tumor only, introduction of total mesorectal excision (TME), involving resection of the tumor together with the fatty tissue surrounding the rectum, has led to an impressive decline in local tumor recurrences and is considered standard of care nowadays (Figure 3).\(^6\) The main goal of TME surgery is to obtain a radical resection, including not only the tumor, but surrounding deposits as well, and should result in a circumferential resection margin (CRM) that is free of tumor. The CRM is defined as the shortest distance from an affected region to the mesorectal fascia (MRF).

![Figure 3. TME surgery, sagittal plane.](image)

Neo-adjuvant treatment

Although chemotherapy as a neo-adjuvant treatment option is currently studied for locally advanced colon cancer, only rectal cancer patients are potentially eligible for additional preoperative treatment nowadays.\(^7\) Adequate staging of the rectal tumor is of importance for assigning patients to the right therapeutic regimen. The TME trial demonstrated that short-term preoperative radiotherapy with 5x5 Gy followed by TME surgery led to a reduction of local recurrences, but did not affect overall survival.\(^8\)-\(^10\) Nowadays, short-term radiotherapy is recommended for all T1-T3 tumors with clinical evidence of node positive disease. Patients who have a threatened MRF (T3-T4) and are at risk of a positive CRM are eligible for a different regimen of neo-adjuvant treatment. A total of 45-50 Gy is given to these patients in fractions of 1.8-2 Gy, usually in combination with chemotherapy for the full duration of radiotherapy. This treatment causes tumor regression and enables the surgeon to perform a radical resection. Surgery usually takes place after an eight to ten weeks interval. Patients demonstrating a good response can even be treated with local excision, and a wait-and-see approach has been proposed for patients with a pathological complete response. \(^11\), \(^12\)
Adjuvant treatment
In the Netherlands adjuvant systemic treatment is recommended for stage III colon cancer patients. Patients who receive adjuvant systemic treatment are usually treated with a combination of 5-fluorouracil (5-FU), leucovorin and oxaliplatin.\textsuperscript{13, 14} In selected high-risk stage II patients (T4 stage, perforation or obstruction at presentation, less that 10 lymph nodes examined or angio-invasion) adjuvant chemotherapy is also recommended.\textsuperscript{15} No benefit of adjuvant chemotherapy has been demonstrated for rectal cancer patients.\textsuperscript{16}

Metastatic disease
Approximately 20\% of all CRC patients have distant metastases upon first presentation and 30-40\% of patients treated for potentially curable CRC relapses.\textsuperscript{3, 17-19} Tumor metastases influence survival and add to disease morbidity. The predominant metastatic sites of CRCs are the liver, lung and peritoneum but various other metastatic sites such as bone, spleen, brain and distant lymph nodes have been described.\textsuperscript{20-26} Localization of metastatic disease strongly influences survival.\textsuperscript{27} Patients with isolated liver metastases can be treated curatively with 5-year survival rates of 40\%.\textsuperscript{28, 29} Unfortunately, no more than 20\% of patients with liver metastases are amenable to potentially curative resection since tumor size, location, multifocality and inadequate hepatic reserve are limiting factors. A small proportion of patients with lung metastases undergoes resection of their metastatic lesion. When metastatic lesions are no longer resectable, patients progress to a palliative setting, in which cure is no longer aimed for, but rather elongation of survival becomes the focus of therapy. Patients then receive palliative chemotherapy, of which studies demonstrated less benefit in MC compared with AC patients (Figure 4).\textsuperscript{27, 30, 31}

<table>
<thead>
<tr>
<th>Study</th>
<th>Chemotherapy regimen</th>
<th>Weight</th>
<th>Hazard ratio IV, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negri et al. (2005)</td>
<td>Capecitabine, irinotecan and oxaliplatin</td>
<td>26.8%</td>
<td>1.49 (1.02-2.19)</td>
</tr>
<tr>
<td>Catalano et al. (2009)</td>
<td>Capecitabine, irinotecan and oxaliplatin</td>
<td>22.8%</td>
<td>1.59 (1.05-2.40)</td>
</tr>
<tr>
<td>Mekenkamp et al. (2012)</td>
<td>Capecitabine, irinotecan, oxaliplatin, bevacizumab and cetuximab</td>
<td>50.4%</td>
<td>1.78 (1.35-2.35)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>100%</td>
<td>1.66 (1.36-2.02)</td>
</tr>
</tbody>
</table>

\(\text{Chi}^2 = 0.57, df = 2 (P = 0.75); I^2 = 0\% \text{ Test for overall effect: } Z = 5.04 \text{ (P < 0.0001)}\)

Figure 4. Meta-analysis of studies on benefit from palliative chemotherapy in CRC patients.
General Introduction

Outline of the thesis

This thesis discusses the clinical relevance of the mucinous and signet-ring cell subtypes within the spectrum of CRCs on a molecule to man to mankind-based evaluation. The distinct clinical and pathological appearance of MC suggests a different oncogenic development, but etiology is not well understood. In Chapter 1 we aim at elucidating different etiological aspects of MC, by describing worldwide variations in prevalence of MC and by analyzing the occurrence of MC in several patient subgroups. In Chapter 2 we present an extensive review of studies on the molecular background of MC and compare findings from colorectal MC with MCs from other organs. Findings from two population-based epidemiological studies form the basis of the clinical part of this thesis. Assessment of clinicopathological characteristics, prognostic impact and benefit from adjuvant chemotherapy for stage III colon cancer is described in Chapter 3 for MC and in Chapter 4 for SRCC. Findings from these studies are further explored in the following chapters. Treatment of rectal cancer patients has changed rapidly over the past two decades. Improved survival and lower recurrence rates have been reported, but this has not been assessed in the mucinous subtype previously. In Chapter 5 the benefits of modern rectal cancer treatment are analyzed for MC patients. MC is a poor prognostic factor in advanced-stage disease. Variations in metastatic spread are considered a possible explanation for this difference. Chapter 6 presents a detailed analysis of the metastatic patterns of AC, MC and SRCC through an autopsy study that was validated with results from the TME trial. Chapter 7 discusses the differences in genetic background between MC and AC, using genome-wide copy number data from various cohorts. Finally, Chapter 8 forms the discussion of this thesis. It reviews the advances that have been made regarding MC and puts results from this thesis in a broader clinical context.
References

General Introduction


Chapter 1

INSIGHT INTO MUCINOUS COLORECTAL CARCINOMA: CLUES FROM ETIOLOGY

REVIEW

N. Hugen, J.J.P. van Beek, J.H.W. de Wilt and I.D. Nagtegaal

Annals of Surgical Oncology, 2014;21:2963-2970
Abstract

The prognostic impact of mucinous carcinoma (MC) in colorectal cancer (CRC) has been the subject of debate ever since the introduction of the classification of tumors according to their histological differentiation. MC is a distinct clinical and pathological entity within the spectrum of CRC and accounts for approximately 10-15% of cases. Factors involved in MC development have not been completely understood, but clinical observations may lead to a better insight into the etiology of MC.

In this article we provide an in-depth review of the literature regarding etiological aspects of MC. We show that there are worldwide differences in the prevalence of MC, with low rates in Asian countries and higher rates in the Western world. Moreover, MC is more commonly diagnosed in patients suffering from inflammatory bowel diseases or Lynch syndrome and an increased rate of MC is observed in patients with radiotherapy-induced CRCs. These findings are suggestive of a different oncogenic development.

Identification of conditions that are associated with MC generates insight into the etiological pathways leading to the development of this special subtype.
Etiology of mucinous colorectal carcinoma

Introduction

Worldwide, approximately 125,000 patients present with a colorectal mucinous adenocarcinoma (MC) annually, which is around 10% of all colorectal cancers (CRC). In this patient group, a higher rate of female patients is present. Moreover, tumors are frequently found in the proximal colon and are diagnosed at a higher stage compared with the more common adenocarcinoma (AC). Also, the pattern of metastatic spread of MC is different. Conflicting results have been reported in the literature regarding the prognostic impact of MC, but overall survival only seems to be different in rectal cancer patients. The response to systemic therapies varies between MC and the common adenocarcinoma (AC), and a poorer outcome has been reported for MC in metastatic disease.

To fulfill the definition of MC as emphasized by the World Health Organization, more than 50% of the tumor should consist of extracellular mucus. The etiology behind the development of MC is not well understood. Molecular and genetic analyses revealed differences between MC and AC, suggesting a different oncogenic development. This idea is enforced by the increased incidence of MC in specific risk groups, i.e. patients with hereditary cancer and inflammatory bowel disease.

Identification of conditions that are associated with higher rates of MC generates insight into the etiological concepts leading to the development of this special subtype and may improve understanding of resistance to therapies. In this review we aim at providing more insight into the differences in worldwide prevalence of MC and analyze the occurrence of MC in several clinical patient subgroups.

Geographical variations in prevalence of MC

It is generally accepted that MC is found in 10-15% of CRC cases. However, CRC incidence rates differ worldwide and it appears that distribution of histological subtypes also shows international variations (Figure 1). Studies from various parts of the world reported MC to occur in 1.6-25.4% of all CRCs. The prevalence of MC in large population-based studies ranged from 3.9% in Asia to 10-13.6% in the Western World. Five-year prevalence proportions of CRC per region as estimated by GLOBOCAN 2012 were not linked to differences in the distribution of MC. Moreover, a large national cancer database study from the USA demonstrated that the distribution of histological subtypes was similar in white, African American and other races. This indicates that the reported differences in prevalence of MC appear to be influenced by other factors, such as lifestyle and dietary conditions, rather than by genetic variations between races.
Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation of the intestines. Patients with long-standing IBD, such as Crohn’s disease (CD) or ulcerative colitis (UC) are at increased risk of developing CRC. The cumulative risk of CRC in UC was estimated to be 18% at 30 years after the onset of disease, but recent updates reported a declining risk as a result of aged cohorts. MCs represent a relatively high proportion of IBD-associated CRCs. Most studies from the nineties reported frequencies between 17% and 37% (Table 1), but a large study that included a recent cohort of patients (1990-2005) reported that 17% of IBD-associated CRCs was MC, which was not considered significantly different from the standard population. The latter may be the result of improved screening and treatment of IBD patients. Combination of findings from the literature (n = 614) showed that MC was found in approximately one quarter of IBD-associated CRC patients (23.0% in CD and 21.3% in UC).

Chronic inflammation is likely to play an important role in carcinogenesis in IBD and CRC occurs primarily in areas affected by inflammation, but mechanisms of carcinogenesis are not yet completely understood.
Etiology of mucinous colorectal carcinoma

understood. Generation of reactive oxygen and nitrogen species by inflammatory cells during hypoxia causes DNA damage and increases mutation rates. This leads to activation of oncogenes and inactivation of tumor suppressor genes, causing chromosomal instability. The generally accepted mechanism accounting for CRC development, in which a sequence of molecular alterations leads to progression from dysplasia to adenocarcinoma, applies to CRC in IBD as well. However, there are profound differences in timing and frequency of these events. Loss of APC, for example, is a common early event in the sporadic CRC pathway, but is less frequent and usually occurs later in the IBD-associated CRC development. The mutation and loss of heterozygosity of p53, occur at an earlier stage in IBD-associated CRC and is already present in non-cancerous mucosal tissue, whereas p53 mutations occur relatively late in sporadic CRC. The high frequency of p53 mutated alleles in non-dysplastic mucosa in chronic colitis has been linked to oxidative stress from reactive oxygen and nitrogen species generated during inflammation. MC is associated with low p53 overexpression, but has a low APC mutation rate, similar as IBD-associated CRC. Microsatellite instability (MSI), which is found in a higher frequency in MC, is also an early event in IBD mucosa, occurring even before dysplasia is detected. In vitro and animal studies demonstrated that epigenetic silencing of the mismatch repair (MMR) system occurs during chronic inflammation and oxidative stress in IBD. Paradoxically, the MMR system is less active during times of high mutational risk, increasing susceptibility for CRC development. This phenomenon links IBD to MSI-CRC development. The disturbance in cytokine levels as seen in chronic inflammatory conditions, causes upregulation of MUC2, that is commonly found in MC. Further identification of molecular changes could improve understanding of a possible final common pathway in IBD patients leading to MC.

Table 1. Reports on mucinous histology among patients suffering from inflammatory bowel disease-associated colorectal cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Study period</th>
<th>Patients in study (n)</th>
<th>MC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>CD</td>
</tr>
<tr>
<td>Gyde</td>
<td>1980</td>
<td>1944-1976</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Savoca</td>
<td>1990</td>
<td>1967-1987</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Choi</td>
<td>1994</td>
<td>1957-1991</td>
<td>80</td>
<td>28</td>
</tr>
<tr>
<td>Rubio</td>
<td>1997</td>
<td>1951-1996</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>Mayer</td>
<td>1999</td>
<td>1983-1995</td>
<td>39</td>
<td>8</td>
</tr>
<tr>
<td>Svrcek</td>
<td>2007</td>
<td>1990-2005</td>
<td>57</td>
<td>16</td>
</tr>
<tr>
<td>Brackmann</td>
<td>2009</td>
<td>1973-2005</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td>Higashi</td>
<td>2011</td>
<td>1985-2009</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Watanabe</td>
<td>2011</td>
<td>1978-1998</td>
<td>121</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>614</td>
<td>95</td>
</tr>
</tbody>
</table>

† Overall weighted percentages according to number of patients in each study
MC, mucinous adenocarcinoma; CD, Crohn's disease; UC, ulcerative colitis

Includes both mucinous and signet-ring cell carcinoma
§ Includes both mucinous and signet-ring cell carcinoma
† Includes both mucinous and signet-ring cell carcinoma

27
Chapter 1

One of the possible explanations for the worldwide differences in prevalence of MC, is the geographic variation of IBD. IBD is much more common in Europe, Australia and North-America, compared with Asia. Australia has incidence rates of IBD that are among the highest in the world and the prevalence of MC in Australia is very high as well. Japan, China and South-Korea on the contrary, have lower rates of IBD and are also associated with a lower prevalence of MC. In Asian countries that become more westernized, IBD appears to be emerging; therefore an increase in incidence of MC in the next decades would further support the theory that development of MC might be related to inflammation.

**MC following radiotherapy**

Radiotherapy is an important modality in cancer treatment, however, induction of secondary malignancies is unfortunately one of the unwanted late effects of radiotherapy. Radiation colitis is a frequent side-effect of radiotherapy and can ultimately result in CRC. Many cases of CRC following radiotherapy of the pelvis have been reported. Most reports described the occurrence of rectal cancer in the presence of radiation proctocolitis with latency periods ranging from 5 to 30 years. There seems to be an association between radiation-induced carcinoma and the presence of MC. Several studies noted a significant increase in MC in CRC cases following radiotherapy. In the largest cohort, 26% of 72 radiation-induced CRCs were MC, and the increase seems to be even more profound for rectal cancer. Our literature search revealed 180 cases of radiation-associated CRCs, of which 69 (38%) were MC (Table 2). The frequency of MC was higher in rectal cancers patients with 52% of radiation-associated tumors being MC. In support of these results, an experimental animal study by Denman et al. found that colonic carcinomas induced by radiotherapy were all MC.

The mechanisms behind the higher frequency of MC in radiation-associated CRC are unknown, but it is considered possible that either radiation-induced inflammation or acquisition of DNA damage may facilitate mucinous differentiation. CRC usually starts off as a benign polyp, that develops into a carcinoma through a multistep process of genetic and molecular alterations as the result of DNA damage. In contrast, radiation-induced colorectal carcinomas may arise de novo from flat mucosa. Ionizing radiation can cause DNA damage directly, or through the formation of reactive oxygen and nitrogen species. Genetic susceptibility, such as a deficiency in DNA repair, leading to hypersensitivity to the carcinogenic risk of radiation has been described. Eventually, accumulation of multiple changes converts a stable genome of a normal cell into an unstable genome characteristic of a tumor. A high rate of mutated p53 in radiation-induced CRCs is indicative for this instability. This has been found in several radiation-induced MCs as well. Interestingly, Japanese atomic bomb survivors, who have been exposed to radiation as well, did not show a higher frequency of MC despite an increased risk for CRC. Therapeutic radiation dosages are higher than dosages to which atomic bomb survivors were exposed, but it is unknown whether a dose-relation may cause differences in histological subtype. There was a small number of high-dose cases in the atomic bomb survivors but radiation dosages generally did not exceed 1 Gy, thereby not completely excluding radiation-associated DNA damage as a causative factor in MC development. The incidence of MC in CRC patients exposed to radiation after the Chernobyl or Fukushima Daiichi nuclear disasters have not been reported to date.
Radiotherapy enhanced inflammation is another theory for the development of MC. Inflammation of irradiated tissue leads to the production of free radicals by phagocytes. Since free radicals are associated with tumor promotion, radiation proctocolitis provides a tumor promoting environment, similar to that found in the prolonged inflammation in IBD. Cells try to minimize DNA damage from reactive oxygen species, but in vivo evidence suggests that this defense mechanism is not sufficiently initiated during the acute inflammatory response in radiation. Cells that are directly irradiated are not the only ones to exhibit a response. Non-irradiated cells adjacent to exposed cells
often respond, which is called the ‘bystander effect’. It can result in cell death, mutagenesis and oncogenic transformation, leading to CRC eventually. Similarly to IBD, an imbalance of the antioxidant response in intestinal mucosa occurs during inflammation. Resemblance in increased activation of mucosal cytokines such as interleukin (IL)-2, -6 and -8 in diseased and normal segments of colon is seen in radiation proctocolitis and IBD patients. Therefore, inflammation may be an important factor in the higher frequency of MC in radiation-associated CRC as well.

**Table 3.** Reports on mucinous histology among colorectal cancer patients diagnosed with Lynch syndrome (verified through germ line mutation analysis and/or confirmation of MSI tumor after fulfilling clinical criteria).

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Lynch patients in study (n)</th>
<th>MC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jass¹⁰⁸</td>
<td>1995</td>
<td>62</td>
<td>31.3</td>
</tr>
<tr>
<td>Young¹⁰⁹</td>
<td>2001</td>
<td>72</td>
<td>22.2</td>
</tr>
<tr>
<td>Shia¹¹⁰</td>
<td>2003</td>
<td>24</td>
<td>33.3</td>
</tr>
<tr>
<td>Valle¹¹¹</td>
<td>2007</td>
<td>30</td>
<td>40.0</td>
</tr>
<tr>
<td>Jin¹¹²</td>
<td>2008</td>
<td>8</td>
<td>25.0</td>
</tr>
<tr>
<td>Chiang¹¹³</td>
<td>2010</td>
<td>50</td>
<td>24.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>246</td>
<td>28.5†</td>
</tr>
</tbody>
</table>

† Overall weighted percentage according to number of patients in each study

MC in Lynch syndrome patients

Lynch syndrome (LS), previously known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominantly inherited cancer predisposition syndrome associated with an elevated risk of cancer. LS is caused by inactivating germ line mutations in genes that encode the mismatch repair (MMR) system. Five LS genes have been identified: MLH1, PMS2, MSH2, MSH6 and EPCAM. The lifetime risk of developing CRC is estimated to be 35-80% and individuals with LS have a high susceptibility to develop other cancers as well. Loss of MMR mechanisms leads to microsatellite instability (MSI), which is the hallmark of LS. MSI is detected in about 15% of all CRCs, 3% is caused by LS, the other 12% are sporadic cases. LS-associated CRCs have a high rate of MC compared with sporadic CRC, approximately 22-40% of LS-associated CRCs is MC (Table 3). It has been described that LS-associated MCs have a better survival compared with their non-mucinous counterparts, suggesting a different prognostic impact of MC in LS. LS-associated CRCs are associated with lower rates of APC and p53 mutations than sporadic CRCs and this low mutation rate of APC and p53 is also seen in MC patients. This finding substantiates the concept of an alternative oncogenic mechanism leading to MC development, different from the classic adenoma-carcinoma sequence.
Conclusion

Since the introduction of the classification of CRCs according to their histological differentiation, the debate concerning the impact of MC has been ongoing. It is widely acknowledged that MC constitutes a distinct clinicopathological entity within the spectrum of CRC, but factors involved in MC development have not been completely understood. This review described conditions in which high frequencies of MC have been found and analyzed the worldwide distribution of MC.

The finding of a higher prevalence of MC in various hereditary and acquired conditions associated with CRC is suggestive of a different oncogenic development. It seems plausible that the development of MC occurs through divergent mechanisms. Lifestyle and dietary conditions may explain worldwide variations in prevalence of MC. Also, inflammatory conditions seem to influence MC differentiation in CRC arising in IBD and radiotherapy-treated patients.

The exact mechanisms causing MC differentiation in these subgroups are unknown, but genetic and epigenetic changes may be responsible for the development of MC. Further studies are needed to evaluate these factors in MC. Also, it is unknown to what extent tumors arising under inflammatory conditions or in LS patients show overlapping molecular alterations with sporadic CRC. CRCs in LS patients are associated with a better prognosis than other types of CRC and microsatellite instable MCs have a better prognosis than microsatellite stable MCs. However, further determination of the prognostic impact of other conditions enhancing mucinous differentiation and relevance for response to various therapies may help to identify patient subgroups that have a poor outcome or are less responsive to additional treatments such as chemotherapy or radiotherapy.

Continuing efforts to elucidate the etiology of MC will eventually increase understanding of the mucinous phenotype and may improve prognostication and therapeutic options in these patients.
Chapter 1

References


Chapter 1


Etiology of mucinous colorectal carcinoma

Chapter 1


Chapter 2

THE MOLECULAR BACKGROUND OF MUCINOUS CARCINOMA BEYOND MUC2

REVIEW


The Journal of Pathology: Clinical Research, 2014;1:3-17
Chapter 2

Abstract

The increasing interest of the oncology community in tumor classification and prediction of outcome to targeted therapies has put emphasis on an improved identification of tumor types. Colorectal mucinous adenocarcinoma (MC) is a subtype, that is characterized by the presence of abundant extracellular mucus that comprises at least 50% of the tumor volume and is found in 10-15% of colorectal cancer patients. MC development is poorly understood, however, the distinct clinical and pathological presentation of MC suggests a deviant development and molecular background. In this review we identify common molecular and genetic alterations in colorectal MC. MC is characterized by a high rate of MUC2 expression. Mutation rates in the therapeutically important RAS/RAF/MAPK and PI3K/AKT pathways are significantly higher in MC compared with non-mucinous adenocarcinoma (NMC). Furthermore, MC shows higher rates of microsatellite instability and is more frequently of the CpG island methylator phenotype (CIMP).

Although the majority of MCs arise from the large intestine, this subtype also develops in other organs, such as the stomach, pancreas, biliary tract, ovary, breast and lung. We compared findings from colorectal MC with tumor characteristics of MCs from other organs. In these organs, MCs show different mutation rates in the RAS/RAF/MAPK and PI3K/AKT pathways as well, but a common mucinous pathway cannot be identified. Identification of conditions and molecular aberrations that are associated with MC generates insight into the aetiology of this subtype and improves understanding of resistance to therapies.
Introduction

Rapid development of individualized therapy for cancer patients has led to an increased attention for tumor subtypes. The search for therapeutically relevant pathways has been ongoing and molecular classification of cancer has become an important component in clinical decision-making. Identification of the molecular background of tumors is one of the key challenges in cancer research, as it improves understanding of tumor development and may predict responsiveness to therapies.

Annually, approximately 1.2 million patients develop CRC worldwide and the non-mucinous adenocarcinoma (NMC) forms the vast majority of these patients. However, in 10-15% of cases mucinous adenocarcinoma (MC) is diagnosed. MC is a subtype that is characterized by the presence of abundant extracellular mucus comprising at least 50% of the tumor volume. Compared with NMC, MCs are more frequently found in the proximal colon and have a higher stage at presentation. Moreover, MCs have a distinct metastatic pattern and are less responsive to palliative chemotherapy. The relatively rare occurrence of colorectal MC renders it a less well-studied entity and MC development is not well understood. Nevertheless, the distinct clinical and pathological presentation suggest a deviant development and molecular background.

Although the majority of MCs arises from the gastrointestinal tract, they are also found in various other organs. Overexpression of MUC2 is a common finding in MCs, but it does not explain the distinct biology of these tumors. Identification of conditions and molecular aberrations that are associated with MC may generate insight into the pathways leading to the development of this subtype and improves understanding of resistance to therapies. In this review we identify common molecular and epigenetic alterations in colorectal MC and compare findings with MCs from other organs.

Methods

Review of literature

The literature was searched with a Boolean search term combination until December 2013, using PubMed and EMBASE. Titles and abstracts were evaluated to identify relevant studies, which were assessed in full text. Reference lists of retrieved studies were explored for further relevant publications. Only studies that contained data on molecular or genetic characteristics and that compared MC and NMC (at least five patients per subtype) were selected. Studies that did not adhere to the definition of MC as reported in the guidelines of the World Health Organization were excluded from the analyses. Overlap between study populations was assessed and in case of overlap only the most recent data was used for analysis. Differences between categorical outcomes were calculated using the risk ratio (RR) and corresponding 95% confidence interval (CI). Heterogeneity was assessed by means of the $I^2$ statistic. The existence of publication bias in the meta-analyses was assessed using funnel plots.
The Cancer Genome Atlas Project

The Cancer Genome Atlas (TCGA) project was established to profile genomic changes in different cancer types. Data on 32 somatic recurrently mutated genes in CRC was published in 2012 by the TCGA group, and data from this study was available online. Data on somatic mutations that were involved in the RAS/RAF/MAPK and PI3K/AKT pathways was downloaded on December 22, 2013. We only selected samples that were designated as either MC or NMC. A total of 28 MCs and 160 NMCs were identified from the TCGA data set of this publication. Fisher’s exact test was used for comparing mutation rates between MC and NMC. Statistical analyses were two-sided and \( P \) values < 0.05 were considered significant.

Molecular determinants in MC

In CRC development acquisition of mutations leads to abnormal cell division and uncontrolled cell growth. There are several well-recognized molecular pathways in CRC development. Chromosomal instability (CIN), microsatellite instability (MSI) and hypermethylation of CpG islands are genetic instability pathways involved in carcinogenesis. Mutations in targets of the RAS/RAF/MAPK and PI3K/AKT pathways are common findings in CRC. These important drivers of cancer development are of prognostic and predictive importance and are being explored for targeted therapies.

**MUC2**

Secreted gel-forming mucins are epithelial glycoproteins that play a role in physiological processes of the gastrointestinal tract. They are encoded by the \( MUC2 \), \( MUC5AC \), \( MUC5B \) and \( MUC6 \) genes on chromosome 11p15.5. \(^{12}\) \( MUC2 \) is of particular interest in regard to its role in CRC as the expression of \( MUC2 \) is generally decreased in CRC. \(^{13}\) Interestingly, an increase of \( MUC2 \) has been observed in MCs, which also explains the mucinous appearance of these tumors. \(^{14-16}\) A meta-analysis by Li et al. demonstrated a higher rate of \( MUC2 \) positivity in MC compared with NMC (RR 2.10 95% CI 1.30-3.40). \(^{17}\) Overexpression of \( MUC2 \) was one of the first molecular aberrations that distinguished MC from NMC and is related to the low methylation status of the promoter of the \( MUC2 \) gene in MC. \(^{18}\)

**Microsatellite instability**

Loss of mismatch repair (MMR) mechanisms causes MSI, which is the hallmark of Lynch syndrome-associated tumors. Lynch syndrome (previously known as hereditary non-polypsis colorectal cancer, HNPCC) is an autosomal dominantly inherited cancer predisposition syndrome, caused by germ line mutations in MMR genes. MC accounts for 22-40% of Lynch syndrome-associated CRCs. \(^{19}\) MSI is also found in approximately 12% of CRC patients who do not suffer from a hereditary predisposition. \(^{20}\) The prevalence of MC has been reported to be 11-77% in sporadic MSI CRC patients (weighted average of 34%, Table 1). \(^{21-34}\) Studies that directly compared sporadic MSI and Lynch syndrome-associated CRCs found a higher rate of MC in sporadic MSI CRCs than in Lynch syndrome-associated CRCs. \(^{24,35}\) A better survival in MC patients has been reported for tumors exhibiting MSI compared with microsatellite stable tumors. \(^{36-38}\) However, comparison of MSI rates between studies is difficult, as a wide variety of markers for determining MSI status is used.
MSI can also occur through hypermethylation of the hMLH1 promoter region, which is seen in CRCs that display the CpG island methylator phenotype (CIMP). CIMP is characterized by hypermethylation of CpG islands in the promoter region of genes involved in carcinogenesis, leading to epigenetic silencing. Studies found 36-41% of MCs to be CIMP positive, compared with only 12-18% in NMC (Supplementary Figure S1).

Tanaka et al. demonstrated that MCs more frequently have MSI or CIMP or BRAF mutations than NMCs (54% versus 28%) and since the various characteristics are correlated, this is indicative for MC arising from an alternative oncogenic pathway. The sequence of these mechanisms is not yet completely understood.

**KRAS**

Mutations in *KRAS* lead to an epidermal growth factor receptor (EGFR) independent disturbance of the RAS/RAF/MAPK pathway, that regulates cell proliferation and survival and is a prognostic factor in CRC. Conflicting results have been reported in the literature regarding the incidence of *KRAS* mutations in MC. Rates of mutant *KRAS* are varying between 7-65% in MC versus 5-50% in NMC. Often, results were not statistically significant, possibly due to lack of power. Eighteen studies were included in an analysis on *KRAS* status in MC and NMC and *KRAS* mutations were found in MC more frequently (RR 1.27, 95% CI 1.14-1.41; Figure 1).

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Patients with MSI in study</th>
<th>MC (%)</th>
</tr>
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<tbody>
<tr>
<td>Kim</td>
<td>1994</td>
<td>18</td>
<td>33.3</td>
</tr>
<tr>
<td>Bocker</td>
<td>1996</td>
<td>11</td>
<td>36.4</td>
</tr>
<tr>
<td>Gafà</td>
<td>2000</td>
<td>44</td>
<td>36.4</td>
</tr>
<tr>
<td>Young</td>
<td>2001</td>
<td>42</td>
<td>42.9</td>
</tr>
<tr>
<td>Hawkins</td>
<td>2002</td>
<td>43'</td>
<td>41.9</td>
</tr>
<tr>
<td>Shia</td>
<td>2003</td>
<td>35'</td>
<td>11.4</td>
</tr>
<tr>
<td>Sarli</td>
<td>2004</td>
<td>22</td>
<td>77.3</td>
</tr>
<tr>
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<td>14</td>
<td>28.6</td>
</tr>
<tr>
<td>Chang</td>
<td>2006</td>
<td>19'</td>
<td>31.6</td>
</tr>
<tr>
<td>Meng</td>
<td>2007</td>
<td>12'</td>
<td>50.0</td>
</tr>
<tr>
<td>Ashktorab</td>
<td>2008</td>
<td>6</td>
<td>33.3</td>
</tr>
<tr>
<td>Kim</td>
<td>2010</td>
<td>135'</td>
<td>15.6</td>
</tr>
<tr>
<td>Kakar</td>
<td>2012</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td>Day</td>
<td>2013</td>
<td>134'</td>
<td>43.3</td>
</tr>
<tr>
<td>Total</td>
<td>549</td>
<td></td>
<td>34.1±</td>
</tr>
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</table>

Table 1. Reports on mucinous adenocarcinoma (MC) among patients with sporadic colorectal cancer with microsatellite instability (MSI).

† Bethesda panel was used for determination of MSI status

§ Overall weighted average according to number of patients in each study
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**BRAF**

Mutated **BRAF** is another molecular aberration that is more frequently found in MC patients. **BRAF** is the downstream effector of **KRAS** and is also involved in the RAS/RAF/MAPK pathway. In various studies mutational **BRAF** was found in 0-46% of MC patients, whereas 6-25% of NMC tumors displayed mutated **BRAF** (RR 2.04, 95% CI 1.67-2.51; Figure 2). B**raf** mutations lead to constitutive activation of the RAS/RAF/MAPK signalling pathway. A hotspot for **BRAF** mutations involves replacement of a single amino acid, V600, located within the kinase domain and accounts for 80% of **BRAF** mutations in CRC. **BRAF** mutations are highly correlated with CIMP, with approximately 60-80% of CIMP tumors having **BRAF** mutations. **BRAF** mutations are also frequently found in sporadic MSI CRC, but not in Lynch syndrome-associated CRC.

**PIK3CA**

Activating mutations in **PIK3CA** occur in approximately 13% of CRCs (Figure 3). **PIK3CA** encodes a catalytic subunit of PI3K and is a positive regulator of the PI3K/AKT pathway, which is involved in cell growth, survival, proliferation and motility. The PI3K pathway is normally inhibited by tumor suppressor gene **PTEN**. **PIK3CA** is more commonly mutated in MC (9-50%) than in NMC (7-12%) and a RR of 1.79 (95% CI 1.46-2.19) was found for MC in an analysis on mutational **PIK3CA** status. Also, **PIK3CA** mutations occur more frequently in tumors that are localized in the proximal colon, as are MCs. **PIK3CA** mutations are common in combination with **KRAS** mutations and are associated with high levels of CIMP, which are both linked to MC. An association between **PIK3CA** mutation and MSI has not been demonstrated. In the literature conflicting results have been published regarding **PTEN**. A study by Day et al. that analyzed mutational status of **PTEN** found a higher frequency of **PTEN** mutations in MC (10% in MC versus 5% in NMC); however, studies that analyzed cytoplasmic expression of **PTEN** did not always find a difference between MC and NMC.

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**Figure 1.** Relative risk for **KRAS** mutation in studies comparing colorectal mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC). CI, confidence interval.
Molecular background of mucinous carcinoma

Figure 2. Relative risk for \(BRAF\) mutation in studies comparing colorectal mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC). CI, confidence interval.

Figure 3. Relative risk for \(PIK3CA\) mutation in studies comparing colorectal mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC). CI, confidence interval.

TCGA

Besides findings from the literature, also unpublished data collected by TCGA offers possibilities to compare mutation rates in CRC. In 188 CRCs (28 MC and 160 NMC) the mutational status of genes involved in the RAS/RAF/MAPK and PI3K/AKT pathway was assessed (Figure 4). Also data on MSI was available. In concordance with the literature, MCs more often displayed MSI and a higher rate of \(BRAF\) and \(PIK3CA\) mutations was found in MC. Mutation rates for other genes were not significantly different. Inclusion of TCGA data into the analyses on mutational status did not significantly alter RR for \(BRAF\) (RR 2.24 95% CI 1.84-2.72), \(KRAS\) (RR 1.26 95% CI 1.13-1.40) and \(PIK3CA\) (RR 1.82 95% CI 1.50-2.20). Mutations in \(ERBB2\) (which encodes HER-2), are considered uncommon in CRC and were found in only 7.1% and 6.3% of MC and NMC samples respectively. In conclusion, data from TCGA confirmed differences in mutation rates between MC and NMC of several genes that were also reported in the literature.

Mucinous colorectal pathway

Findings from the literature and TCGA suggest that MC and NMC differ on a molecular basis (Figure 5). An increased rate of mutations is seen in MC in the RAS/RAF/MAPK and PI3K/AKT pathways. \(KRAS\), \(BRAF\)
and PIK3CA are more frequently mutated in MC compared with NMC, leading to constitutive activation of these pathways. No differences in expression of the cell surface receptors EGFR or HER-2, that are upstream of these pathways, have been reported between MC and NMC in the literature. Although MSI, CIMP and activation of the RAS/RAF/MAPK and PI3K/AKT pathways are distinctive features of MC, the relationship between these characteristics and mucus production has not yet been elucidated. There is no data on a molecular link between MSI or CIMP and overexpression of MUC2. However, various in vitro studies demonstrated that both the RAS/RAF/MAPK and PI3K/AKT pathway are involved in MUC2 upregulation in colon cancer cell lines and indicated that MUC2 production can be inhibited by a MEK inhibitor. In another cell line, however, upregulation of MUC2 was considered independent of MAP kinase. Recently, Walsh et al. reported data on 722 CRC patients which supported the association between overexpression of MUC2 and activation of the RAS/RAF/MAPK pathway via BRAF and KRAS mutations. They also found that MUC2 overexpression was associated with a deficient MMR system and CIMP. Especially the latter is surprising, as it indicates an increase in protein expression in an environment in which excessive silencing of gene promoters is present. These findings strongly suggest that overexpression of MUC2 in MCs is related to other molecular aberrations, but further evaluation is needed.

**Compare and contrast**

Besides the colorectal variant, MC is also found in tumors originating from other organs. MC has been described in patients suffering from carcinoma of the esophagus, stomach, small intestine, pancreas, biliary tract, gall bladder, ovary, endometrium, urinary bladder, breast and lung. It is unknown whether MCs from different organs share common molecular characteristics. Hanski et al. previously demonstrated that overexpression of the MUC2 gene was found in MCs from different organs. The rare occurrence of MC in most organs is reflected by the limited number of studies regarding this
Molecular background of mucinous carcinoma

In this section MCs from variant organs are described, dependent on availability in the literature.

**Mucinous gastric carcinoma**

MC is one of the five main subtypes in the WHO classification system of gastric adenocarcinomas and comprises approximately 2-5% of all gastric cancers. Since most studies use the Laurén classification system, which divides gastric carcinoma in an intestinal and diffuse subtype, there is little data on gastric MC. Identical to colorectal MC, gastric adenocarcinoma is designated mucinous if more than 50% of the tumor consists of extracellular mucus. Gastric MCs are more often diagnosed at a more advanced stage of disease than NMC, resulting in a poorer outcome. Similar to colorectal MC, gastric MC is also associated with MUC2 overexpression. Also, a higher rate of MSI is found in MC when compared with NMC (average of 14% versus 11%, RR 1.51 95% CI 1.03-2.21, Supplementary Figure S2). HER-2 overexpression and ERBB2 gene amplification are less common in MC than in NMC (1% versus 6%). A higher rate of loss of heterozygosity of 18q, which is associated with adverse outcome, has been reported for gastric MC compared with NMC (52% versus 21%). Expression of PTEN seems to be less altered in gastric MC, compared with NMC; Kang et al. found that 27% of NMCs displayed loss of PTEN whereas none of the MCs did. Gastric MC is associated with lower rates of EGFR overexpression compared with NMC (5-11% versus 26-31%). Conversely, one small study by Liu et al. found an EGFR mutation in 2 out of 7 MCs. Additionally, this study found no KRAS mutations in MC, while 12% of NMCs had a KRAS mutation.

**Mucinous non-cystic pancreas carcinoma**

Mucinous non-cystic carcinoma of the pancreas is a variant of ductal adenocarcinoma and is usually referred to as colloid carcinoma. In pancreatic colloid carcinoma mucus accounts for more than 50% of the tumor. It is considered an uncommon subtype and arises almost exclusively from the intraductal papillary mucinous neoplasm (IPMN). The rare occurrence is a limiting factor on knowledge of the molecular background of colloid carcinoma, but Adsay et al. demonstrated a low mutational rate of KRAS (25%) in a small colloid carcinoma cohort, whereas KRAS is mutated in > 90% of ductal adenocarcinomas. As in colorectal MC, colloid carcinoma of the pancreas is associated with a high expression frequency of MUC2 compared with ductal adenocarcinomas. However, in contrast with colorectal MC, MSI is not a common finding in colloid carcinoma of the pancreas. Lüttges et al. found only one case of MSI among 12 colloid carcinomas.

**Mucinous carcinoma of the gall bladder and extrahepatic bile ducts**

MCs of the gall bladder and biliary ducts contain more than 50% extracellular mucus by definition of the WHO classification system. In a population-based study on biliary tract cancers MC was found in 5% of cases. This study by Rashid et al. also found a higher rate of MSI in MCs (33%) from the biliary tract, compared with NMCs (2%). A recent study by Dursun et al. on 606 gall bladder carcinomas reported MC in 2.5% of cases. MUC2 expression, which is typically negative in NMC of the gall bladder, was positive in 86% of MCs. However, none of the MCs displayed MSI in this study.
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Mucinous ovarian carcinoma

NMC of the ovary forms the majority of ovarian carcinomas and mainly consists of serous, clear cell and endometrioid carcinomas. MC is diagnosed in approximately 11-14% of ovarian carcinomas. MC is more frequently found in an early stage of disease and is associated with a better survival than NMC. Compared with CRC, the ovarian variant of MC is an ill-defined entity and is usually classified as MC when the tumor has an ‘intestinal’ or ‘cervical gland-like’ phenotype. Unlike in the colon, ovarian carcinoma is labeled mucinous when either intracellular or extracellular mucus is present, without requiring any strict quantification of the mucus component. Practically, this means that the group of ovarian MC comprises those phenotypes that are defined as both NMC and MC in the colon. In the literature presence of either intracellular or extracellular mucus is generally not mentioned nor quantified.

In ovarian cancer KRAS is more frequently mutated in MC (10-71%), than in NMC (2-25%, Supplementary Figure S3). BRAF mutations are rare in ovarian carcinoma, with only 0-9% of MC and 0-4% of NMC showing this mutation. There seems to be no significant role for MSI in the mucinous differentiation, with MSI in 0-55% of MC and in 2-62% of NMC (Supplementary Figure S4). For PIK3CA and PTEN, literature is limited. Campbell et al. reported that 8% of NMCs exhibited a PIK3CA mutation, whereas none of the MCs did. PTEN mutations were found in up to 10% of ovarian carcinomas, but this was not different between histological subtypes. ERBB2 amplification does occur in ovarian carcinoma, but no obvious differences between MC (28%) and NMC (19%) have been found. CIMP has been examined to a limited extent in ovarian carcinoma. The interpretation of data concerning ovarian MC is further complicated by the fact that a considerable part of MC consists of metastases from primary tumors originating elsewhere in the body, mainly from the gastrointestinal tract. Because differentiation between a primary MC and metastasis is difficult, it is possible that a proportion of carcinomas that are considered ovarian MC are in fact metastatic CRC. This might impede interpretation of the reported data, but it could also explain the high frequency of KRAS mutations in MC.

Mucinous lung carcinoma

Invasive mucinous adenocarcinoma (IMA) of the lung (formerly mucinous bronchioalveolar carcinoma) was separated from the non-mucinous subtype in the new international multidisciplinary classification system based on major clinical, pathological, and genetic differences between both subtypes. IMA, however, is not the pulmonary equivalent of MC from the gastrointestinal tract, as mucus is found intracytoplasmic in this tumor. The colloid carcinoma which is characterized by abundant extracellular mucus, shows more resemblance with colorectal MC. Pulmonary colloid carcinoma is a rare subtype (found in less than 0.5% of lung carcinomas) and is often found as a mixture with other NMC subtypes. KRAS and EGFR mutations are the two most frequently mutated proto-oncogenes in adenocarcinoma of the lung, whereas BRAF mutations and MSI are rare in lung carcinoma. The pathogenic mechanisms behind colloid carcinoma are largely unknown, but MUC2 is found to be strongly expressed. Moreover, a study by Liu et al. found a higher rate of KRAS mutations and a lower rate of EGFR mutations in colloid tumors, compared with other subtypes. Since EGFR tyrosine kinase inhibitors are of particular interest for lung cancer treatment, more insight into the molecular background of subtypes could improve targeting therapy.
Molecular background of mucinous carcinoma

Mucinous breast carcinoma
According to the WHO classification system of breast carcinomas, MC of the breast is found in 7% of breast cancers and consists of clusters of tumor cells floating in pools of extracellular mucus. In the literature a pure and mixed variant of MC have been distinguished. Pure MC of the breast consists exclusively of MC and represents approximately 2% of all breast cancers. The mixed variant of MC shows an admixture with another component (usually infiltrating ductal carcinoma, IDC). Compared with IDC, pure MC is a less aggressive subtype that is rarely associated with lymph node metastases. Comparison at the molecular level shows that MC is transcriptionally distinct from IDC. MC is more homogenous at the genetic level and shows less genetic instability than most other types of breast cancer. MC of the breast is associated with higher rates of MUC2 expression than IDC. MC also has a higher rate of estrogen receptor (ER) expression (73-94% versus 26-82%, Supplementary Figure S5) and is associated with more progesterone receptor (PR) expression (63-90% versus 47-74%, Supplementary Figure S6). For MC, less HER-2 overexpression has been reported compared with NMC (0-14% versus 20-41%, Supplementary Figure S7). Studies that included small numbers of MC demonstrated that mutated PIK3CA, which is found in 16-33% of IDCs, is not a common finding in MC (0-13%, Supplementary Figure S8). Mutations of BRAF and KRAS are not common in breast cancer (0-3% and 2-5%) and associations with MC have not been studied. Unlike in colorectal MC, MSI is a rare phenomenon in MC of the breast, occurring only sporadically (0-3%). Studies evaluating EGFR mutations in breast cancer have not focused on MC.

Comparison with CRC
A common mucinous pathway cannot be identified for MC from different organs (Figure 5). However, in general limited data is available for non-colorectal MC. There are differences between MC and NMC in mutation rates of targets of the RAS/RAF/MAPK and PI3K/AKT pathways. Also differences in expression of EGFR, HER-2, ER and PR have been found in non-colorectal MCs. The association between these molecular characteristics and the mucinous phenotype is not well studied in non-colorectal MC. However, in vitro studies with lung cancer cell lines showed that cell treatment with epidermal growth factor resulted in an increased expression of MUC2. Conversely, blockage of the PI3K/AKT pathway in gastric cancer cell lines resulted in an increase in MUC2 expression, indicating the need for further clarification of the regulatory mechanisms behind MUC2 expression in MCs. MSI is another distinctive tumor characteristic of colorectal MC, but has only been reported at a higher rate in MCs from the stomach and biliary tract. Since various molecular characteristics have been associated with either worse or improved prognosis, differences in these pathways may explain deviant tumor behavior of MC in different organs.

Conclusions and implications
The era of personalized medicine has led to an emerging interest in tumor subtypes and the molecular background of malignancies. The distinct clinicopathological presentation and the impaired response to systemic therapies are suggestive of a different molecular background of colorectal MC, but development of this subtype is not well understood. This review recapitulated alterations in several
therapeutically important pathways of CRC and compared findings with the literature regarding MCs from other organs.

Overexpression of MUC2, leading to abundant mucus production, is a molecular key feature of MC, but it does not explain the distinct clinical behavior of MC. Review of the literature demonstrated that MC showed a higher rates of mutations in \textit{BRAF}, \textit{KRAS} and \textit{PIK3CA} than NMC and higher rates of CIMP and MSI were found in MC. Funnel plots did not demonstrate publication bias (figures not shown). These findings suggest that mutations in the RAS/RAF/MAPK and PI3K/AKT pathways are involved in MC development.

\textbf{Figure 5.} EGFR, HER-2 and ER with downstream the RAS/RAF/MAPK and PI3K/AKT pathway.

(A) Mutation rates of \textit{KRAS}, \textit{BRAF} and \textit{PIK3CA} are different between mucinous carcinoma (MC) and non-mucinous carcinoma (AC) in colorectal cancer. (B). An increase or decrease in mutation or expression rates of components of the RAS/RAF/MAPK and PI3K/AKT pathway has been observed in MC when compared with AC in different tumor types.
Previously, it has been reported that MC is more commonly found in tumors arising under inflammatory conditions and in patients with a hereditary predisposition for CRC. A higher rate of MC was observed in patients suffering from inflammatory bowel diseases or Lynch syndrome and in patients who developed CRC following radiotherapy. It is unknown to what extent these factors contribute to MC development, but they indicate that epigenetic changes may well influence MC development.

From a therapeutic perspective, colorectal MC has a worse outcome than NMC when treated with palliative chemotherapy for advanced-stage disease. Interestingly, there is no difference in benefit from adjuvant chemotherapy in MC patients. MSI tumors have been associated with less responsiveness to 5-FU chemotherapeutic treatment, but this does not explain the discrepancy between the adjuvant and palliative setting. In rectal cancer, resistance of MC to radiotherapy or chemoradiotherapy is suspected, given the poorer rate of tumor downstaging. Also, the metastatic pattern is different between MC and NMC patients. This indicates that not only phenotype, but also tumor behavior is different between histological subtypes.

As the definition of MC in CRC requires that at least 50% of the tumor consists of mucus, it is not inconceivable that tumor heterogeneity may have influenced findings from the literature. It is possible that molecular aberrations have remained unnoticed due to dilution by non-mucinous tumor elements. However, no study has attempted to address this problem by focusing solely on pure MC samples in CRC. Moreover, since CRC can develop via CIN and MSI it would be interesting to analyze molecular aberrations stratified by these different pathways. Unfortunately, this was not feasible as insufficient data was available in the literature.

This review also compared colorectal MC with MCs from other organs. The definition of MC is not unambiguous between different organs, as it sometimes refers to tumors containing abundant intracellular mucus or a combination of intra- and extracellular mucus. MC is less prevalent in other organs than in the colorectum, which was reflected by the limited amount of literature on molecular differences between subtypes in these tumors. A common mucinous pathway could not be identified, but between MC and NMC differences in mutation rates of components of the RAS/RAF/MAPK and PI3K/AKT pathways were found in most organs. Alterations in these pathways may be associated with MUC2 overexpression. Interestingly, the genetic instability pathway of MSI, which is a predominant characteristic of mucinous CRC, could not be linked to MCs in every other organ.

Further identification of molecular aberrations may lead to the development and implementation of targeted therapies, but could also explain resistance of tumors to such therapies. Moreover, identification of the molecular background of MC may improve prognostication and could lead to a better prediction of response to local and systemic therapies.
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References


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Molecular background of mucinous carcinoma

Molecular background of mucinous carcinoma


162. Halford, S.E. et al. MSI-low, a real phenomenon which varies in frequency among cancer types. *J Pathol* 201, 22-31 (2010).


Molecular background of mucinous carcinoma

Supplemental material

Supplementary Figure S1. Relative risk for CpG island methylator phenotype in studies comparing colorectal mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC). CI, confidence interval.

Supplementary Figure S2. Relative risk for microsatellite instability in studies comparing mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC) of the stomach. CI, confidence interval.

Supplementary Figure S3. Relative risk for KRAS mutation in studies comparing mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC) of the ovary. CI, confidence interval.
### Supplementary Figure S4.
Relative risk for microsatellite instability in studies comparing mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (NMC) of the ovary. CI, confidence interval.

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<th>NMC Events</th>
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<tr>
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<td>7</td>
<td>5</td>
<td>245</td>
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Total (95% CI): 12 54 78 470 100% 0.91 (0.56 - 1.47)

Heterogeneity: \(\chi^2 = 0.96, \text{df} = 4 \ (P = 0.92); I^2 = 0\%

Test of overall effect: \(Z = 0.40 \ (P = 0.69)\)

### Supplementary Figure S5.
Relative risk for estrogen receptor expression in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast. CI, confidence interval.

<table>
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<th>Study</th>
<th>MC Events</th>
<th>IDC Events</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diab 1999</td>
<td>1123</td>
<td>1211 35741 43587</td>
<td>10.0%</td>
<td>1.12 (1.10 - 1.14)</td>
<td>1999</td>
</tr>
<tr>
<td>Zhang 2013</td>
<td>91</td>
<td>117 3657 5575</td>
<td>0.8%</td>
<td>1.19 (1.07 - 1.31)</td>
<td>2013</td>
</tr>
<tr>
<td>Park 2010</td>
<td>68</td>
<td>88 2110 3328</td>
<td>0.6%</td>
<td>1.22 (1.09 - 1.37)</td>
<td>1991</td>
</tr>
<tr>
<td>Saverio 2008</td>
<td>10748</td>
<td>11422 253520 338479</td>
<td>85.0%</td>
<td>1.26 (1.25 - 1.26)</td>
<td>2006</td>
</tr>
<tr>
<td>Tseng 2013</td>
<td>84</td>
<td>93 1715 2674</td>
<td>0.6%</td>
<td>1.42 (1.31 - 1.51)</td>
<td>2009</td>
</tr>
<tr>
<td>Bae 2011</td>
<td>240</td>
<td>265 1552 2455</td>
<td>1.6%</td>
<td>1.43 (1.36 - 1.50)</td>
<td>1998</td>
</tr>
<tr>
<td>Cho 2008</td>
<td>18</td>
<td>23 55 105</td>
<td>0.1%</td>
<td>1.49 (1.13 - 1.98)</td>
<td>2001</td>
</tr>
<tr>
<td>Cao 2012</td>
<td>227</td>
<td>309 1028 2202</td>
<td>1.3%</td>
<td>1.57 (1.45 - 1.71)</td>
<td>2004</td>
</tr>
<tr>
<td>Zhou 2013</td>
<td>27</td>
<td>31 11 27</td>
<td>0.1%</td>
<td>2.14 (1.33 - 3.44)</td>
<td>2013</td>
</tr>
<tr>
<td>Hsu 2005</td>
<td>10</td>
<td>11 20 76</td>
<td>0.0%</td>
<td>3.45 (2.27 - 5.26)</td>
<td>2003</td>
</tr>
</tbody>
</table>

Total (95% CI): 12636 13580 299409 309508 100% 1.25 (1.25 - 1.26)

Heterogeneity: \(\chi^2 = 259.48 \text{df} = 9 \ (P < 0.0001); I^2 = 97\%

Test of overall effect: \(Z = 89.30 \ (P < 0.0001)\)

### Supplementary Figure S6.
Relative risk for progesterone receptor expression in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast. CI, confidence interval.

<table>
<thead>
<tr>
<th>Study</th>
<th>MC Events</th>
<th>IDC Events</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park 2010</td>
<td>55</td>
<td>88 1881 3328</td>
<td>0.6%</td>
<td>1.11 (0.94 - 1.30)</td>
<td>2010</td>
</tr>
<tr>
<td>Diab 1999</td>
<td>830</td>
<td>1221 26588 45587</td>
<td>8.6%</td>
<td>1.11 (1.07 - 1.16)</td>
<td>1999</td>
</tr>
<tr>
<td>Hsu 2005</td>
<td>6</td>
<td>11 36 76</td>
<td>0.1%</td>
<td>1.15 (0.64 - 2.08)</td>
<td>2005</td>
</tr>
<tr>
<td>Zhang 2013</td>
<td>81</td>
<td>117 3340 5575</td>
<td>0.8%</td>
<td>1.16 (1.02 - 1.31)</td>
<td>2013</td>
</tr>
<tr>
<td>Cho 2008</td>
<td>17</td>
<td>23 64 105</td>
<td>0.1%</td>
<td>1.21 (0.91 - 1.62)</td>
<td>2008</td>
</tr>
<tr>
<td>Zhou 2013</td>
<td>28</td>
<td>31 20 27</td>
<td>0.1%</td>
<td>1.22 (0.95 - 1.57)</td>
<td>2013</td>
</tr>
<tr>
<td>Saverio 2008</td>
<td>9309</td>
<td>11422 220668 338497</td>
<td>85.9%</td>
<td>1.25 (1.24 - 1.26)</td>
<td>2008</td>
</tr>
<tr>
<td>Cao 2012</td>
<td>202</td>
<td>309 1053 2202</td>
<td>1.5%</td>
<td>1.37 (1.25 - 1.50)</td>
<td>2012</td>
</tr>
<tr>
<td>Tseng 2013</td>
<td>74</td>
<td>93 1555 2674</td>
<td>0.6%</td>
<td>1.37 (1.23 - 1.52)</td>
<td>2013</td>
</tr>
<tr>
<td>Bae 2011</td>
<td>215</td>
<td>265 1405 2455</td>
<td>1.6%</td>
<td>1.42 (1.33 - 1.52)</td>
<td>2011</td>
</tr>
</tbody>
</table>

Total (95% CI): 10817 133580 256610 309508 100% 1.24 (1.23 - 1.25)

Heterogeneity: \(\chi^2 = 56.77 \text{df} = 9 \ (P < 0.0001); I^2 = 84\%

Test of overall effect: \(Z = 48.20 \ (P < 0.0001)\)
Supplementary Figure S7. Relative risk for HER-2 expression in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast. CI, confidence interval.

Supplementary Figure S8. Relative risk for PIK3CA mutation in studies comparing infiltrating ductal carcinoma (ICD) and mucinous adenocarcinoma (MC) of the breast. CI, confidence interval.
Part II

TREATMENT AND BIOLOGY
Chapter 3

PROGNOSIS AND VALUE OF ADJUVANT CHEMOTHERAPY IN STAGE III MUCINOUS COLORECTAL CARCINOMA


Annals of Oncology, 2013;24:2819-24
Abstract

Colorectal mucinous adenocarcinoma (MC) has been associated with impaired prognosis compared with non-mucinous adenocarcinoma (NMC). Response to palliative chemotherapy is poor in metastatic disease, but the benefit of adjuvant chemotherapeutic treatment has never been assessed in large patient groups. This study analyzes overall survival and efficacy of adjuvant chemotherapy in terms of survival in patients following radical resection for MC.

This population-based study involved 27,251 unselected patients diagnosed with colorectal carcinoma between 1990 and 2010 and recorded in a prospective pathology-based registry. Kaplan-Meier analysis and log-rank testing were used to estimate survival. Cox proportional hazard model was used to calculate multivariate hazard ratios for death.

MC was found in 12.3% ($n = 3052$) of colorectal tumors with a different distribution compared with NMC, with 24.4% located in the rectum and 54.3% in the proximal colon (versus 38.0% and 30.6%, $p < 0.0001$). NMC was more often classified as stage I disease than MC (20.5% versus 10.9%, $p < 0.0001$). After adjustments for covariates, MC was associated with a higher risk of death only when located in the rectum (hazard ratio 1.22, 95% confidence interval 1.11-1.34). Multivariate regression analysis showed a similar survival after adjuvant chemotherapy for stage III MC and NMC patients.

The poor prognosis for MC is only present in rectal cancer. In the adjuvant setting there is no difference in the efficacy of chemotherapy between MC and NMC; therefore, current adjuvant treatment recommendations should not take histology into account.
Prognosis and adjuvant chemotherapy in mucinous colorectal carcinoma

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer-related mortality worldwide.\(^1\)\(^,\)\(^2\) The majority of cases presents with adenocarcinoma not otherwise specified,\(^3\) but in approximately 10-15\% mucinous carcinoma (MC) is reported.\(^4\)\(^,\)\(^5\) At histological examination MC is composed of more than 50\% extracellular mucus.\(^3\) MC more often affects younger patients,\(^6\)\(^,\)\(^7\) is more frequently seen in the proximal colon\(^6\)\(^,\)\(^8\) and in females\(^6\) and usually has a more advanced stage at presentation.\(^7\)\(^-\)\(^10\) The prognostic significance of MC, however, is considered controversial. Most studies demonstrated that patients with MC have a poorer prognosis compared with those with a non-mucinous histology (NMC), which is contradicted by others.\(^4\)\(^,\)\(^6\)\(^,\)\(^10\)\(^-\)\(^19\) Especially rectal MCs have been associated with impaired overall survival (OS).\(^6\)\(^,\)\(^7\) A recent systematic review found a small, but significantly shorter survival in MC versus NMC (hazard ratio 1.05, 95\% confidence interval (CI) 1.02-1.08).\(^16\)

Fluorouracil-based adjuvant chemotherapy has been demonstrated to be beneficial in stage III colon cancer.\(^15\)\(^,\)\(^20\)\(^,\)\(^21\) Due to the increased stage of presentation of MC, many patients with MC have an indication for adjuvant therapy.\(^11\)\(^,\)\(^22\)\(^,\)\(^23\) However, several studies demonstrated reduced responsiveness of metastatic MC to chemotherapy in the palliative setting, subsequently leading to a worse OS.\(^14\)\(^,\)\(^23\)\(^-\)\(^25\) Due to relative resistance to systemic treatment, therapeutic benefit may be reduced in patients with MC. In the adjuvant setting, the only study taking tumor type into account suggests an equal benefit for MC.\(^10\)

The aim of this study is to establish the role of MC in OS for CRC patients and to identify whether tumor type should be taken into account in decision-making concerning adjuvant chemotherapy following radical resection.

Patients and methods

A database containing 27,251 CRC patients diagnosed between 1990 and 2010, was extracted from the population-based Eindhoven Cancer Registry (ECR). Clinicopathological data were recorded by the registration clerks from the hospital records as routine registration for the ECR. Data on follow-up of vital status were retrieved by linkage to the nationwide population registries network. Patients without a histological confirmation of the primary tumor (\(n = 566\)) were excluded from this study (Consort diagram in Supplementary Figure S1). Patients were also excluded if they had a previous malignancy within five years preceding the diagnosis of CRC (except for basal cell skin cancer or in situ carcinomas) (\(n = 1275\)). Tumors demonstrating a mixed histological picture or undifferentiated carcinoma (\(n = 112\)), and tumors that were classified as signet-ring cell carcinoma or other than adenocarcinoma (\(n = 805\)) were excluded. Appendiceal carcinomas were also excluded from the study (\(n = 173\)). Information about hereditary syndromes was not available in the database.

The following variables were available for this study: age, sex, year of diagnosis, primary tumor location and histology (based upon WHO ICD-O classification), stage at presentation according to
the TNM classification (version dependent on year of diagnosis), surgery performed, (neo)adjuvant therapy, socio-economic status (SES), co-morbidity and survival time or time from diagnosis until 31 December 2011. To compare adjuvant chemotherapy in stage III, we excluded patients who died within 30 days after surgery for these analyses \( n = 145 \). As grade assignment is largely subjective with few or no defined criteria, and is considered inappropriate for mucinous tumors, this variable was not taken into account. Co-morbidity at diagnosis was registered according to a modified version of the Charlson co-morbidity index, which included among other cardiovascular disease, pulmonary disease or diabetes mellitus. Tumors were classified as proximal if they were found in the cecum, ascending colon or transverse colon up to the splenic flexure and were classified as distal if they were found in the descending or sigmoid colon. SES was defined by the ECR upon postal code, combining mean household income and mean value of the residence. Postal codes were divided into three SES subcategories: low, intermediate and high. Institutions, such as nursing homes, were assigned to a separate category. Patients received adjuvant chemotherapy in accordance with national therapeutic guidelines applicable at that time, which was 5-fluorouracil or capecitabine, with the addition of oxaliplatin (FOLFOX or CAPOX regimen) from 2005 on. In the Netherlands, neo-adjuvant radiotherapy was standard of care for rectal cancer patients since 2000 for T2-T4 tumors and concomitant chemoradiotherapy was given to patients with locally advanced tumors.

**Statistical analysis**

The \( \chi^2 \) test was used to compare clinicopathological characteristics. Primary outcome was OS, which was defined as the interval between the date of diagnosis until the date of death of any cause or until the date of last follow-up. Patients who were still alive at the end of follow-up were censored. OS curves were generated according to the Kaplan-Meier method and equality of distributions was compared with the log-rank test. Multivariate analysis of OS was performed using the Cox proportional hazard model. Covariates were identified after study of the literature. All tests of significance were two tailed: differences at \( P \) values of < 0.05 were considered significant. Statistical analyses were performed with the statistical software package SPSS 19.0 (SPSS, Inc., Chicago, IL).

**Results**

**Patient characteristics**

Patient and tumor characteristics are presented in Table 1. MC was found in 12.3% patients and females accounted for 52.4% MC versus 45.9% NMC \( p < 0.0001 \). MC was less often located in the rectum (24.4% MC versus 38.0% NMC, \( p < 0.0001 \)) and more frequently in the proximal colon (54.3% versus 30.6%, \( p < 0.0001 \)). At time of diagnosis, NMCs were more often classified as stage I tumors (20.5% versus 10.9%, \( p < 0.0001 \)).

**MC as a prognostic factor**

In the rectum, there was a significant difference in OS between MC and NMC (5-year OS 41.0% versus 51.2%, \( p < 0.001 \); Figure 1), most prominent in stage I (77.4% versus 60.9%, \( p = 0.001 \)) and III (48.5% versus 35.6%, \( p < 0.0001 \); Supplementary Figures S2-S5). The multivariate analysis showed that rectal
### Table 1. Demographic and clinicopathological features of patients with colorectal carcinomas diagnosed between 1990 and 2010.

<table>
<thead>
<tr>
<th>Features</th>
<th>Mucinous n = 3052 (12.3%)</th>
<th>Non-mucinous n = 21 845 (87.7%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>1598 (52.4)</td>
<td>10 017 (45.9)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1454 (47.6)</td>
<td>11 828 (54.1)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Median</td>
<td>70 years</td>
<td>69 years</td>
<td></td>
</tr>
<tr>
<td>&lt; 45</td>
<td>102 (3.3)</td>
<td>611 (2.8)</td>
<td></td>
</tr>
<tr>
<td>45-59</td>
<td>515 (16.9)</td>
<td>4171 (19.1)</td>
<td></td>
</tr>
<tr>
<td>60-74</td>
<td>1331 (43.6)</td>
<td>10080 (46.1)</td>
<td></td>
</tr>
<tr>
<td>≥ 75</td>
<td>1104 (36.2)</td>
<td>6983 (32.0)</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>1658 (54.3)</td>
<td>6685 (30.6)</td>
<td></td>
</tr>
<tr>
<td>Distal colon</td>
<td>621 (20.3)</td>
<td>6563 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>744 (24.4)</td>
<td>8301 (38.0)</td>
<td></td>
</tr>
<tr>
<td>Colon, unknown</td>
<td>29 (1.0)</td>
<td>296 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Number of co-morbidities</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>0</td>
<td>1354 (44.4)</td>
<td>10 438 (47.8)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>743 (24.3)</td>
<td>5333 (24.4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>227 (7.4)</td>
<td>1540 (7.0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28 (0.9)</td>
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<td>4383 (20.1)</td>
<td></td>
</tr>
<tr>
<td>Co-morbidity</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>720 (24.3)</td>
<td>4878 (22.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>221 (7.5)</td>
<td>1754 (8.3)</td>
<td>0.001</td>
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<tr>
<td>Diabetes Mellitus</td>
<td>340 (11.5)</td>
<td>2234 (10.5)</td>
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</tr>
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<td>Socio-economic status</td>
<td></td>
<td></td>
<td>0.012</td>
</tr>
<tr>
<td>Low</td>
<td>849 (28.7)</td>
<td>5666 (26.7)</td>
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<tr>
<td>Intermediate</td>
<td>1142 (38.6)</td>
<td>7992 (37.7)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>804 (27.2)</td>
<td>6337 (29.9)</td>
<td></td>
</tr>
<tr>
<td>Institutionalized</td>
<td>160 (5.4)</td>
<td>1197 (5.6)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>I</td>
<td>332 (10.9)</td>
<td>4480 (20.5)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1168 (38.3)</td>
<td>6892 (31.5)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>861 (28.2)</td>
<td>5199 (23.8)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>625 (20.5)</td>
<td>4275 (19.6)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>66 (2.1)</td>
<td>999 (4.6)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant chemotherapy for stage III</td>
<td>304 (48.8)</td>
<td>1630 (50.9)</td>
<td>0.328</td>
</tr>
</tbody>
</table>
MCs were associated with a higher risk of death compared with rectal NMCs (HR 1.22, 95% CI 1.11-1.34, \( p < 0.0001 \)), while there was no difference in OS between MC and NMC located in the colon (Table 2).

![Figure 1](image1.png)

**Figure 1.** (A) Overall survival for mucinous carcinoma (MC) and non-mucinous carcinoma (NMC) patients with colon cancer \( (p = 0.386) \). (B) Overall survival for MC and NMC patients with rectal cancer \( (p < 0.0001) \).

### Adjuvant chemotherapy in stage III mucinous colon cancer patients

Stage III colon cancer patients showed higher survival in both MC and NMC groups when treated with chemotherapy (Figure 2). Median OS in all patients treated with chemotherapy was 133 months, compared with 33 months for patients who were not treated (on univariate analysis \( p < 0.0001 \)). The multivariate Cox proportional hazard model showed that MC was not an independent prognostic factor in the stage III subgroup when treated with adjuvant chemotherapy (Table 3).

![Figure 2](image2.png)

**Figure 2.** (A) Overall survival for stage III colon cancer patients with mucinous carcinoma, treated with or without adjuvant chemotherapy \( (p < 0.0001) \). (B) Overall survival for stage III colon cancer patients with non-mucinous carcinoma, treated with or without adjuvant chemotherapy \( (p < 0.0001) \).
Table 2. Multivariate survival analysis using the Cox model concerning all rectal and colon cancer patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rectal cancer</th>
<th></th>
<th>Colon cancer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% Confidence interval</td>
<td>Hazard ratio</td>
<td>95% Confidence interval</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 45</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>45-59</td>
<td>1.13</td>
<td>0.94-1.36</td>
<td>1.25</td>
<td>1.06-1.47</td>
</tr>
<tr>
<td>60-74</td>
<td>1.68</td>
<td>1.40-2.01</td>
<td>1.69</td>
<td>1.44-1.98</td>
</tr>
<tr>
<td>≥ 75</td>
<td>3.03</td>
<td>2.52-3.64</td>
<td>2.94</td>
<td>2.51-3.45</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
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<td></td>
</tr>
<tr>
<td>Female</td>
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<td>0.89-0.99</td>
<td>0.91</td>
<td>0.87-0.95</td>
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</tr>
<tr>
<td>I</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.81</td>
<td>1.66-1.97</td>
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<td>III</td>
<td>2.60</td>
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</tr>
<tr>
<td>Low</td>
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</tr>
<tr>
<td>Intermediate</td>
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<td>0.94</td>
<td>0.90-0.99</td>
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<td>1.17-1.38</td>
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<td>Adjuvant chemotherapy</td>
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<td>Yes</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No</td>
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<td>1.29-1.59</td>
<td>1.69</td>
<td>1.59-1.80</td>
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<td>1</td>
<td></td>
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<td>-</td>
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<tr>
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<tr>
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Chapter 3

Table 3. Multivariate survival analysis using the Cox model concerning all stage III colon cancer patients who were treated with adjuvant chemotherapy.

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<th>Hazard ratio</th>
<th>95% Confidence interval</th>
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Discussion

MC is a distinct CRC, with different clinical characteristics and prognosis compared with NMC. Due to reduced responsiveness to chemotherapy, MC has a poor prognosis in advanced disease. We showed that colon cancer patients with MC seem to benefit from adjuvant chemotherapy equally to patients with NMC.

In the present study over 24,000 prospectively registered CRC patients were analyzed. Approximately 12% of our population consisted of MC, which was constant over time and is consistent with previous reports. Many well-recognized MC-associated features were confirmed in the present study.

We found a statistically significant difference in OS between MC and NMC in stage I and III rectal cancer patients, consistent with the current literature. In contrast to previous reports, we could not...
demonstrate a difference in survival in stage IV.\textsuperscript{14, 23, 24} Many studies described a variable prognostic impact of MC.\textsuperscript{4, 6, 7, 10, 12, 16-18, 24} Unfortunately, these studies are generally relatively small and prospectively recorded information often does not include adjuvant treatment details. Furthermore, follow-up time usually is too short to reveal differences between groups adequately, leading to a possible statistical type II error. Moreover, most of the studies were hospital-based and not population-based, which could have led to a selection bias. Even though we are confident that the current study depicts an accurate image of MC, there are limitations due to its non-randomized nature. First of all, although definitions of MC have been standardized, variations in interpretation may result in misclassification. Due to the large population size, reviewing the individual pathological diagnosis was not feasible. However, we compared the percentage of MC and NMC per laboratory in order to exclude a possible bias, and there was no significant difference (data not shown). Also, frequencies were comparable with data available in the literature. Secondly, because this population-based registry does not include information regarding the duration of adjuvant treatment or treatment drugs given during adjuvant chemotherapy, we were not able to account for differences in therapy during the study period. However, when we compared survival in 5-year time periods we found improved survival following adjuvant chemotherapy to be significant over time (data not shown).

In the present, large population-based study, no differences in OS were demonstrated on multivariate analysis between MC and NMC, except when the tumor was located in the rectum. Consistent with these findings, a recent National Cancer Data Base (USA) study\textsuperscript{6} showed that MC of the rectum, but not of the colon is associated with an increased risk of death. Reasons for this difference in OS are not clear, but a possible explanation might be the poorer response to radiotherapy in MCs.\textsuperscript{11, 22, 27, 28} Other reasons could be the fact that MCs in the rectum are usually larger, more often have a positive circumferential margin after resection\textsuperscript{22} and might therefore have a worse outcome.\textsuperscript{12} Further studies are needed to evaluate these factors in rectal cancer.

The efficacy of chemotherapy in MC has mainly been studied in patients receiving palliative chemotherapeutic treatment.\textsuperscript{14, 23-25} In this setting, response is poorer in MC patients compared with NMC patients. The current study confirms that adjuvant chemotherapy for stage III carcinoma is associated with an improved OS. Although patients were not randomized and several biases cannot be accounted for, this finding is consistent with previous randomized trials regarding all CRC patients and current international treatment guidelines.\textsuperscript{15, 20, 21} To our knowledge this is the largest analysis in MC patients concerning adjuvant chemotherapy to date. Interestingly, the present analysis demonstrated a similar survival for NMC and MC patients who underwent adjuvant chemotherapeutic treatment for stage III colon cancer. A recent retrospective analysis\textsuperscript{19} with 178 MC patients also suggested a comparable benefit from adjuvant chemotherapy for both stage II and III MCs and NMCs after radical resection. Thus, MC seems clearly responsive to adjuvant chemotherapy and therefore results found in palliative treatment should not be extrapolated to the adjuvant setting.

The mechanisms behind the differences in response to chemotherapy in the adjuvant and palliative setting remain unclear. In general, MC patients have a larger number of metastatic sites and larger primary tumors,\textsuperscript{23, 29} with possibly a poorer vasculature, consequently leading to a decreased delivery
of therapy. In advanced disease, MC patients also more often suffer from extrahepatic localizations of metastases,\textsuperscript{14, 23, 24} such as distant lymph node metastases\textsuperscript{12, 24} or peritoneal metastases,\textsuperscript{12, 24, 25, 29, 30} which is associated with a poor outcome in CRC.\textsuperscript{31, 32}

Based on its histological appearance and its clinicopathological features MC may be considered a distinct entity with a predominant right-sided location. In consistency with recent literature we have shown that MC seems to have no impact on survival, except when located in the rectum. Despite lower response to palliative chemotherapy in MC, colon cancer patients treated with adjuvant chemotherapy seem to have similar benefits. Therefore, current adjuvant treatment recommendations should be adhered to regardless of tumor type.
References

Chapter 3


Supplemental material

Supplementary Figure S1. Consort diagram.

Supplementary Figure S2. (A) Overall survival for stage I mucinous carcinoma (MC) and non-mucinous carcinoma (NMC) patients with colon cancer \((p = 0.741)\). (B) Overall survival for stage I MC and NMC patients with rectal cancer \((p = 0.001)\).
Supplementary Figure S3. (A) Overall survival for stage II mucinous carcinoma (MC) and non-mucinous carcinoma (NMC) patients with colon cancer ($p = 0.512$). (B) Overall survival for stage II MC and NMC patients with rectal cancer ($p = 0.234$).

Supplementary Figure S4. (A) Overall survival for stage III mucinous carcinoma (MC) and non-mucinous carcinoma (NMC) patients with colon cancer ($p = 0.994$). (B) Overall survival for stage III MC and NMC patients with rectal cancer ($p < 0.0001$).

Supplementary Figure S5. (A) Overall survival for stage IV mucinous carcinoma (MC) and non-mucinous carcinoma (NMC) patients with colon cancer ($p = 0.498$). (B) Overall survival for stage IV MC and NMC patients with rectal cancer ($p = 0.066$).
COLORECTAL SIGNET-RING CELL CARCINOMA: BENEFIT FROM ADJUVANT CHEMOTHERAPY BUT A POOR PROGNOSTIC FACTOR


Abstract

Colorectal signet-ring cell carcinoma (SRCC) has been associated with poor survival compared with mucinous adenocarcinoma (MC) and the more common adenocarcinoma (AC). Efficacy of adjuvant chemotherapy in SRCC has never been assessed. This study analyzes the prognostic impact of SRCC and determines whether colonic SRCC patients benefit from adjuvant chemotherapy equally compared with MC and AC patients.

Data on 196,757 colorectal cancer (CRC) patients in the period 1989-2010 was included in this Dutch nationwide population-based study. Five-year relative survival estimates were calculated and multivariate relative survival analyses using a multiple regression model of relative excess risk (RER) were performed.

SRCC was found in 1972 (1.0%) patients. SRCC patients presented more frequently with stage III or IV disease than AC patients (75.2% versus 43.6%, \( p < 0.0001 \)) and SRCC was more frequently found in the proximal colon (57.7% versus 32.0%, \( p < 0.0001 \)). SRCC patients had a poor 5-year relative survival of 30.8% (95% CI 28.1-33.6) in the colon and 19.5% (95% CI 14.7-24.8) in the rectum compared with 56.8% (95% CI 56.4-57.1) and 58.5% (95% CI 57.9-59.1) for AC. This survival difference was found in stage II, but was most prominent in stage III. Compared with AC, there was no significant interaction between SRCC and adjuvant chemotherapy (RER 1.10, 95% CI 0.81-1.51), suggesting a comparable benefit from adjuvant chemotherapy in AC and SRCC.

In conclusion, the prognostic impact of SRCC is dismal in both colon and rectal cancer patients, but adjuvant chemotherapy is associated with improved survival in AC, MC and SRCC patients.
Prognosis and adjuvant chemotherapy in colorectal signet-ring cell carcinoma

Introduction

Colorectal cancer (CRC) is one of the most-common cancers worldwide, and several histological subtypes have been reported.\textsuperscript{1, 2} The common adenocarcinoma (AC) accounts for the majority of cases and in 10-15\% patients present with mucinous adenocarcinoma (MC).\textsuperscript{3-6} Signet-ring cell adenocarcinoma (SRCC) is a rare type of adenocarcinoma, found in approximately 1\% of CRC patients and contains abundant intracellular mucus in more than 50\% of its cells.\textsuperscript{3, 4, 7, 8} Due to the low incidence, SRCC has been evaluated in a limited amount of studies, with small numbers of patients. SRCC has been associated with a poor prognosis compared with AC.\textsuperscript{3, 7-11} However, it is unclear whether the prognostic impact is relevant for both colon and rectal cancer patients.

Adjuvant fluorouracil-based chemotherapy improves outcome in colon cancer patients, and is routinely administered to stage III patients following surgical removal of the tumor.\textsuperscript{12} The addition of oxaliplatin became standard of care around 2005 in the Netherlands. It is unclear whether different histological subtypes should influence treatment decisions, since it is often not addressed in clinical trials. Recently, we showed that colonic MC patients have a similar survival compared with AC patients and demonstrated that MC is not a prognostic factor in stage III colon cancer when treated with adjuvant chemotherapy.\textsuperscript{5} In the literature, there are no studies concerning outcome after adjuvant or palliative chemotherapy for SRCC. However, due to the aggressive behavior of SRCC it is important to gain insight in potential adjuvant treatment options in an effort to enhance survival in these patients.

In this population-based study we analyze clinicopathological characteristics of SRCC. Moreover, we establish the prognostic impact of SRCC for both colon and rectal cancer patients and determine whether colonic SRCC patients benefit equally from adjuvant chemotherapy following resection compared with MC and AC patients.

Patients and methods

Data on all CRC patients diagnosed between 1989 and 2010 in the Netherlands, were retrieved from the nationwide population-based Netherlands Cancer Registry (NCR). The NCR receives lists of newly diagnosed cancer patients on a regular basis from the pathology departments of all Dutch hospitals, who all participate in a nationwide network. In addition, the medical records departments of hospitals provide lists of diagnoses of outpatients and hospitalized cancer patients. Following these notifications, trained registrars of the NCR extract data on patient and tumor characteristics and primary treatment from the medical records. All tumors (n = 202,807) were classified according to the International Classification of Disease for Oncology (ICD-O). Patients were identified as SRCC (ICD-O morphology code: 8490), MC (ICD-O morphology codes: 8480, 8481) and AC (ICD-O morphology codes: 8000, 8010, 8020, 8021, 8140, 8141, 8143, 8144, 8210, 8211, 8220, 8221, 8260, 8261, 8262, 8263). Undifferentiated tumors (n = 547) and tumors that were classified as other than adenocarcinoma, such as GIST and carcinoids (n = 4,759) were excluded. Appendiceal neoplasms were not included in this study (n = 1,795), since they are considered a unique entity. Tumor stage was
determined according to the TNM classification of the WHO. In case (parts of) the pathological stage (pTNM) were unknown or missing, (parts of) the clinical stage (cTNM) were used to reconstruct the stage. The pathological T-stage was unknown or missing in 16.9% of cases. Other available variables for this study included: age, gender, year of incidence, primary tumor location, surgery performed, (neo)adjuvant therapy and survival time. Data on follow-up of vital status was retrieved by linkage to the nationwide municipal population registries network. Every patient who was included in this study was registered in the population registries network. Information concerning the cause of death was not available. Since a widely accepted standard for grading is lacking and grade assignment is highly subjective, this variable was not taken into account.

Tumors of the colon were classified as proximal if they were located in the cecum, ascending colon, or transverse colon up to the splenic flexure, and were classified as distal if they were found in the descending, sigmoid or rectosigmoid colon. If patients had two or more consecutive invasive CRCs, only the first tumor was included in the analyses. In the Netherlands stage III colon cancer patients are offered adjuvant chemotherapy in accordance with nationwide therapeutic guidelines applicable, which is 5-fluorouracil or capecitabine, with the addition of oxaliplatin since 2005. The study was approved by the supervisory committee of the NCR.

Statistical Analysis
The χ² test was used to compare proportions in demographic and clinical characteristics by histological subtype. Primary outcome was overall survival, which was defined as the interval between the date of diagnosis until the date of death or until last follow-up, December 31, 2011. Patients who were alive at the end of follow-up were censored in the survival analyses. To compare adjuvant chemotherapy in stage III we excluded patients who died within 30 days after surgery for these analyses (n = 828). Relative survival estimates were calculated as the ratio of observed survival of the cancer patients and the expected survival of an age and sex matched group of the general Dutch population. The data for the calculation of the expected survival in the general population was obtained from Statistics Netherlands and consisted of the gender-specific death rates per 1-year age group per calendar year. Survival between different histological subtypes was compared using a multiple relative survival regression model, in which age, gender, tumor localization, adjuvant chemotherapy and period of diagnosis were included to calculate variable specific relative excess risk (RER) of death estimates. All tests of significance were two-tailed and differences at P-values of < 0.05 were considered significant. Relative survival analyses were performed with SAS software (SAS system 9.2, SAS Institute, Cary, NC).

Results
Patient characteristics
A total of 196,757 patients was included in this study and most patients (85.4%) were diagnosed with AC (Table 1). MC accounted for 13.6% and SRCC was found in 1.0% of patients. In the group of SRCC patients 25.9% was younger than 60 years of age, compared with 18.7% and 19.6% in the MC and AC group (p < 0.0001). SRCC patients presented more frequently with stage III or IV tumors than AC patients (75.2% versus 43.6%, p < 0.0001) and SRCC was more commonly found in the proximal colon (57.7% versus 32.0%, p < 0.0001).
Subtype as a prognostic factor

SRCC patients had a statistically significant lower 5-year relative survival compared with MC and AC patients in both colon and rectal cancer patients (Figure 1). In SRCC 5-year relative survival rates were 31% (95% CI 28.1-33.6) for colon cancer patients and 20% (95% CI 14.7-24.8) for rectal cancer patients compared with 57% (95% CI 56.4-57.1) and 59% (95% CI 57.9-59.1) in AC. MC and AC had a similar survival in colon cancer patients, but a poorer survival was found in rectal MC patients. Five-year relative survival rates per stage and primary tumor location are presented in Table 2 and showed a poorer survival in SRCC patients in stage II and most prominently in stage III in colon and rectal cancer patients.

Adjuvant chemotherapy for stage III SRCC colon cancer patients

In SRCC 51.6% of stage III patients received adjuvant chemotherapy, compared with 51.0% and 54.0% in MC and AC (Table 1). Patients who were treated with adjuvant chemotherapy had a better survival
than patients who did not receive adjuvant chemotherapy (Figure 2). In patients who received adjuvant chemotherapy, 5-year relative survival rates for SRCC, MC and AC were 52% (95% CI 42.9-60.5), 69% (95% CI 66.6-72.1) and 74% (95% CI 72.8-75.1). In patients who did not receive adjuvant chemotherapy 5-year relative survival rates were 30% (95% CI 21.9-39.3), 52% (95% CI 47.9-54.9) and 50% (95% CI 48.5-51.5) in SRCC, MC and AC patients respectively. Results of the multivariate relative survival analysis are presented in Table 3. SRCC was found a poor prognostic factor in stage III colon cancer patients compared with AC with an RER of 2.16 (95% CI 1.84-2.52). However, in comparison with AC, there was no significant interaction between SRCC and adjuvant chemotherapy (RER 1.10, 95% CI 0.81-1.51), suggesting a comparable benefit from adjuvant chemotherapy in AC and SRCC. Compared with AC, MC and chemotherapy did show an interaction (RER 1.23, 95% CI 1.07-1.34), indicating a poorer benefit of adjuvant chemotherapy for MC. Eventually, this did not result in an overall poorer prognostic impact for MC compared with AC in stage III colon cancer patients (RER 1.04, 95% CI 0.97-1.12).

Figure 1. Relative survival in adenocarcinoma (AC), mucinous adenocarcinoma (MC) and signet-ring cell carcinoma (SRCC) patients with colon cancer (A) and rectal cancer (B).
Table 2. Five-year relative survival with 95% confidence intervals (CI) for patients with colorectal cancer in the Netherlands according to tumor location, stage of disease and histology (1989-2010).

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† Due to small number of patients (number at risk < 10), calculation could not be performed accurately
§ No patients alive at five years after diagnosis
AC, adenocarcinoma; MC; mucinous adenocarcinoma; SRCC, signet-ring cell carcinoma

Discussion

SRCC is an uncommon but unique histological subtype of CRC, with generally a poor prognosis compared with MC and AC. This population-based study demonstrated that prognosis of SRCC patients is impaired regardless of stage and location of the tumor. We also show that stage III colonic SRCC patients seem to benefit from adjuvant chemotherapy equally compared with AC in a national cohort study.

Due to the low frequency, SRCC has not been well studied in CRC and reports in the literature are uncommon. The present study analyzed over 196,000 CRC patients who were prospectively registered in the NCR. Our study population consisted of 1% (n = 1972) SRCC patients, which is in concordance with reported numbers in the literature. Several clinical characteristics of SRCC, such as localization in the proximal part of the colon, younger age at diagnosis and more advanced stage at presentation were confirmed in the present study.

Previous studies suggested a poor prognosis for SRCC compared with AC. In this study we confirmed this worse relative survival for SRCC in both colon and rectal cancer, which was most evident in stage III. In concordance with the literature, we demonstrated that MC was only associated with a worse survival when located in the rectum. Efficacy of adjuvant chemotherapy in CRC patients has mainly been studied in AC and to a limited extent in MC patients. In the present study
Figure 2. Relative survival in stage III colon cancer patients with adenocarcinoma (A), mucinous adenocarcinoma (B) and signet-ring cell carcinoma (C), treated with or without adjuvant chemotherapy.
MC showed an interaction with adjuvant chemotherapy in stage III colon cancer patients. Previous studies demonstrated that MC was not a prognostic factor in colon cancer patients treated with adjuvant chemotherapy. However, these studies did not define whether there was an interaction between MC and adjuvant chemotherapy. The current study used a different survival model and different variables were included in the multivariate analysis.
To our knowledge, the present study is the first that analyzed outcome after adjuvant chemotherapy in SRCC patients. Data presented in this large population-based study showed a longer survival in SRCC patients when treated with adjuvant chemotherapy. SRCC remained an independent prognostic marker for poor outcome in stage III colon cancer patients treated with adjuvant chemotherapy, but an interaction between chemotherapy and SRCC was not demonstrated. This indicates that adjuvant chemotherapy in SRCC patients is associated with improved survival, and that the poor outcome of SRCC patients is not related to a poor response to adjuvant chemotherapy.

Since adjuvant chemotherapy in SRCC patients was associated with an improved survival, there must be other factors leading to differences in outcome. The more advanced stage of disease in which SRCCs were found may be one of the reasons for the poorer outcome. Unfortunately, there are no studies available that have generated insight into this difference in tumor progression. Another explanation for the differences in survival may be the deviant metastatic pattern in SRCC. SRCC patients more often develop metastatic disease, are more likely to develop peritoneal metastases and SRCC metastasizes via the lymphatic route more frequently, whereas AC metastasizes primarily to the liver.\textsuperscript{7, 10, 14, 16} Peritoneal metastases are associated with a poor prognosis, and survival is even worse if metastases are present in other organs.\textsuperscript{17} Often, these metastases cannot be treated with curative intent. Curative surgery is an option mainly limited to liver and lung metastases, which are the most-common metastatic sites in AC patients. Moreover, chemotherapy for patients with peritoneal metastases may not yield the same results compared with patients with hematogenous metastases and this may lead to a poor outcome in advanced disease in SRCC patients.\textsuperscript{18}

The NCR covers nearly all cancer diagnoses in the Netherlands, thereby constituting an comprehensive population-based registry.\textsuperscript{19} The extensive amount of data offers the possibility to analyze relatively rare subtypes of cancer, such as SRCC and to study therapeutic interventions in these groups. However, this study has some limitations as well, because the non-randomized nature of this study could be a potential confounding factor. Compared with randomized controlled clinical trials that demonstrated efficacy of adjuvant chemotherapy, we found a much larger survival difference between patients who did and who did not receive adjuvant chemotherapy.\textsuperscript{20-23} Frail and elderly patients are more commonly ineligible for adjuvant chemotherapeutic treatment, thereby comprising a larger share in the patient group that did not receive chemotherapy in our study. This may have influenced outcome in this group, but this bias applies to all histological subgroups. Further, the NCR does not register detailed information concerning adjuvant treatment, therefore we were not able to account for differences in adjuvant therapy practice over the study period. Unfortunately, we were not able to analyze the benefit of adjuvant chemotherapy in high-risk stage II patients, due to the low number of patients who received chemotherapy in this group and because the motivation for administration of chemotherapy was not registered. Lastly, due to the population size, we have not been able to review the individual pathological diagnosis. Even though standard definitions for MC and SRCC as produced by the WHO are employed in the Netherlands, diagnostic heterogeneity may have led to misclassification. However, frequencies of subtypes were comparable with numbers reported in the literature.\textsuperscript{3}
In conclusion, SRCC is considered a distinct entity based on clinical presentation and pathological features. In this study we have shown that the prognostic significance of SRCC is dismal with a poor survival in both colon and rectal cancer patients. However, we found that adjuvant chemotherapy for SRCC stage III colon cancer patients is associated with improved survival.
References

Chapter 5

MODERN TREATMENT OF RECTAL CANCER CLOSES THE GAP BETWEEN COMMON ADENOCARCINOMA AND MUCINOUS CARCINOMA


Annals of Surgical Oncology, 2015;22:2669-76
Abstract

Mucinous carcinoma (MC) is a distinct form of rectal cancer (RC) comprising 10% of all cases, and has been associated with an impaired prognosis compared with non-mucinous adenocarcinoma (AC). The benefit of today’s modern treatment for MC patients is unknown, but a prospective randomized trial to answer this seems not feasible. This study provides an analysis of the modern treatment of rectal MC and efficacy of preoperative therapies for MC patients.

Data from three large (trial) cohorts was used. Data from the Netherlands Cancer Registry (NCR) was used to analyze the prognosis of RC patients over time (n = 38,035). To study the benefit of preoperative short-term radiotherapy patients from the TME trial (n = 1530) were selected and benefit from preoperative chemoradiotherapy was analyzed with data on 540 locally advanced RC (LARC) patients from two hospitals.

Data from the NCR confirmed that 5-year overall survival for MC was significantly worse from 1989-1998, but no longer different from AC from 1999 onwards. MC patients had a higher rate of positive circumferential resection margin (CRM) than AC (TME trial 27.2% versus 16.5%, \( p = 0.006 \); LARC cohort 34.5% versus 9.8%, \( p < 0.0001 \)), but there was no difference in outcome between MC and AC patients after preoperative short-term radiotherapy or chemoradiotherapy.

Modern treatment of RC has benefitted MC patients, leading to equal survival for MC and AC patients. Enhancements in the fields of imaging and quality of surgery have improved outcome and preoperative therapies should be recommended for both histological subtypes.
Introduction

Treatment of rectal cancer (RC) patients has improved rapidly over the last decades. At the end of the past century total mesorectal excision (TME), which encompasses resection of the tumor together with the fatty tissue surrounding the rectum was introduced and significantly improved outcome. Enhanced imaging by the means of MRI enabled multidisciplinary boards to make a better estimation of tumor invasion depth and preoperative therapies such as short-term radiotherapy and long-term chemoradiotherapy had a significant impact on RC treatment.

Many European oncologists consider patients with a cT2 to cT4 RC eligible for preoperative radiotherapy and recommend to add chemotherapy for patients with locally advanced rectal cancer (LARC) in which the mesorectal fascia (MRF) is threatened. In North America, conversely, short-term radiotherapy is not commonly used for treatment of RC patients.

RC can be classified according to the histological aspect of the tumor. The majority of RCs comprises non-mucinous adenocarcinoma (AC). Approximately 10% of RCs is characterized by extensive extracellular mucus that forms more than 50% of the tumor volume. These tumors are referred to as mucinous adenocarcinomas (MC) and form a distinct clinicopathological entity with an aberrant molecular background. MC is regarded as a cancer subtype with a poor prognosis when located in the rectum (Figure 1).

![Figure 1](image_url)

**Figure 1.** Seven publications were included in a meta-analysis comparing prognostic impact in mucinous (MC) and non-mucinous (AC) adenocarcinoma in a timed cohort. Patients who were included were accrued from 1968 to 2010. Five studies found a significantly worse survival in rectal MC patients. Hazard ratios (HRs) were combined in a meta-analysis which resulted in an overall HR of 1.23 (95% CI 1.18-1.28) for MC compared with AC in rectal cancer patients. Detailed information on literature search and analysis can be found in Supplementary Data S1. *, Multivariate HR; †, Univariate HR; CI, confidence interval.
Several studies have reported a poor response of rectal MC to preoperative therapies, leading to a higher rate of resections with a positive circumferential resection margin (CRM) and a poor outcome when compared with rectal AC. These findings, however, are based on small and foremost timed cohorts, that were analyzed prior to the introduction of TME surgery, preoperative therapies and MRI scanning.

In this study we investigate the overall survival (OS) in MC and AC patients over a long time period using data from a population-based cancer registry. Moreover, using data from large (trial) cohorts we analyze the impact for AC and MC patients of today’s modern RC treatment in which TME surgery, short-term radiotherapy and long-term chemoradiotherapy have become standard of care.

**Patients and methods**

**Patient populations**
For this study three independent study populations were selected to study the prognosis of RC over time (1) and the effects of short-term radiotherapy (2) and chemoradiotherapy (3).

1. **Patients from the national cancer registry**
Data on 38,035 RC patients diagnosed with AC \((n = 34,459)\) or MC \((n = 3576)\) between 1989 and 2006, were retrieved from the nationwide population-based Netherlands Cancer Registry (NCR) to study overall survival (OS) of RC patients over time. Tumors were classified according to the International Classification of Disease for Oncology (ICD-O). Patients were identified as MC (8480, 8481) and AC (8000, 8010, 8020, 8021, 8140, 8141, 8143, 8144, 8210, 8211, 8220, 8221, 8260, 8261, 8262, 8263). Follow-up of vital status was retrieved by linkage to the nationwide population registries network, until December 31, 2011.

2. **Patients from the TME trial**
To study benefit from short-term radiotherapy, patients from the randomized multicenter TME trial were selected. Between January 12, 1996 and December 31, 1999, 1861 patients with clinically resectable RC were assigned to either preoperative radiotherapy using 5x5 Gy followed by TME or TME alone. The design of the trial was reported previously. For the present study we only included Dutch patients \((n = 1530)\), who underwent a resection and did not have metastatic disease at the time of diagnosis. Previous research on the TME trial showed that short-term radiotherapy may lead to induction of a mucinous phenotype. Especially patients with a tumor containing more than 90% mucus were considered induced mucinous lesions when the preoperative biopsy was negative for mucus. However, prognosis and clinicopathological features of these induced lesions are comparable to AC. Therefore, all patients with a lesion with a mucinous component of 90% or more, but with a negative preoperative biopsy for MC were considered radiotherapy-induced MC and excluded from the analysis \((n = 29)\).

3. **Patients with locally advanced RC**
The LARC study group consisted of 540 patients without metastatic disease who underwent preoperative chemoradiotherapy followed by TME for LARC between April 1998 and January 2013.
Table 1. Patient and tumor characteristics according to histological subtype in patients from the TME trial and patients with locally advanced rectal cancer who received preoperative chemoradiotherapy.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TME trial</th>
<th>Locally advanced</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MC (%) n = 103</td>
<td>AC (%) n = 1157</td>
<td></td>
</tr>
<tr>
<td>Randomization TME trial</td>
<td>0.195</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Surgery alone</td>
<td>47 (7.2)</td>
<td>605 (92.8)</td>
<td>-</td>
</tr>
<tr>
<td>Radiotherapy and surgery</td>
<td>56 (9.2)</td>
<td>552 (90.8)</td>
<td>-</td>
</tr>
<tr>
<td>Chemoradiotherapy regime</td>
<td>-</td>
<td>-</td>
<td>0.326</td>
</tr>
<tr>
<td>CAP</td>
<td>21 (36.2)</td>
<td>210 (43.6)</td>
<td></td>
</tr>
<tr>
<td>CAP + OXA</td>
<td>15 (25.9)</td>
<td>140 (29.0)</td>
<td></td>
</tr>
<tr>
<td>CAP + BEV</td>
<td>1 (1.7)</td>
<td>9 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Bolus 5FU-Mayo</td>
<td>17 (29.3)</td>
<td>85 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (6.9)</td>
<td>38 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.686</td>
<td>0.605</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64 (62.1)</td>
<td>742 (64.1)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>39 (37.9)</td>
<td>415 (35.9)</td>
<td></td>
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<tr>
<td>Age</td>
<td>0.568</td>
<td>0.178</td>
<td></td>
</tr>
<tr>
<td>&lt; 45</td>
<td>5 (4.9)</td>
<td>52 (4.5)</td>
<td></td>
</tr>
<tr>
<td>45-59</td>
<td>31 (30.1)</td>
<td>323 (27.9)</td>
<td></td>
</tr>
<tr>
<td>60-75</td>
<td>52 (50.5)</td>
<td>546 (47.2)</td>
<td></td>
</tr>
<tr>
<td>&gt; 75</td>
<td>15 (14.6)</td>
<td>236 (20.6)</td>
<td></td>
</tr>
<tr>
<td>CRM</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Negative (&gt; 1mm)</td>
<td>75 (72.8)</td>
<td>966 (83.5)</td>
<td></td>
</tr>
<tr>
<td>Positive (≤ 1mm)</td>
<td>28 (27.2)</td>
<td>191 (16.5)</td>
<td></td>
</tr>
<tr>
<td>Pathological N-stage</td>
<td>0.001</td>
<td>0.293</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>48 (46.6)</td>
<td>728 (62.9)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>55 (53.4)</td>
<td>429 (37.1)</td>
<td></td>
</tr>
<tr>
<td>Type of resection</td>
<td>0.335</td>
<td>0.136</td>
<td></td>
</tr>
<tr>
<td>Low anterior</td>
<td>60 (58.3)</td>
<td>757 (65.4)</td>
<td></td>
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<tr>
<td>Abdominoperineal</td>
<td>38 (36.9)</td>
<td>349 (30.2)</td>
<td></td>
</tr>
<tr>
<td>Hartmann</td>
<td>5 (4.9)</td>
<td>51 (4.4)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

MC, mucinous adenocarcinoma; AC, non-mucinous adenocarcinoma; CRM, circumferential resection margin; CAP, capecitabine; OXA, oxaliplatin; BEV, bevacizumab; 5FU, 5-fluorouracil

This group was used to analyze benefit from preoperative chemoradiotherapy in LARC patients. Tumors were considered LARC if the MRF was endangered on imaging. Patients were treated in either Catharina Hospital Eindhoven (n = 486) or Radboud university medical center (n = 54), which are both tertiary referral centers and data was recorded prospectively. To rule out induction of the mucinous phenotype in irradiated patients, tumors with extensive mucus or designated as MC were assessed.
by a pathologist (IN) and evaluated on pre-operative MRI (when available for review) by a radiologist (JF). Evaluation of MRI was also performed for patients with a pathological complete response, leaving no vital tumor cells. Based on signal intensity measurements on T2-weighted fast spin echo images, MC was identified.\textsuperscript{26, 27} In accordance with Dutch radiotherapy guidelines, radiotherapy was administered five days per week at a daily dose of 1.8-2 Gy, with a total dose of 45-50.4 Gy in 25-28 fractions. Different concurrent regimens of systemic therapy were given during the study period. Tumors were operated with adherence to beyond TME principles, with the primary goal of obtaining a radical resection.\textsuperscript{28} Patients had regular follow-up after surgery.

\textbf{Statistical Analysis}

The $\chi^2$ test was used to compare clinicopathological characteristics by histological subtype. In survival analyses OS was defined as the interval between the date of surgery (date of diagnosis for NCR data) until the date of death or until last follow-up. Patients who were alive at the end of follow-up were censored in survival analyses. OS curves were generated according to the Kaplan-Meier method and equality of distributions was compared with log-rank testing. Multivariate analysis of OS was performed using the Cox proportional hazard model. Patients from the NCR were grouped into 1-year cohorts to analyze time related changes in 5-year OS. All tests of significance were two-tailed and differences at $P$ values of $< 0.05$ were considered significant.

\textbf{Results}

\textbf{Improved outcome of RC patients over time}

To analyze 5-year OS over time, data from the NCR on patients who were diagnosed with RC between 1989 and 2006 was used. Survival improved over time in both MC and AC patients (Figure 2). Five-year OS in AC patients improved from 40.3\% (95\% CI 37.8-42.8) in 1989 to 54.1\% (95\% CI 52.1-56.0) in 2006 ($p < 0.0001$), compared with 32.0\% (95\% CI 24.7-39.3) and 53.1\% (95\% CI 46.4-59.8) in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Five-year overall survival rates with 95\% confidence limits for mucinous (MC) and non-mucinous (AC) rectal cancer patients in a nationwide cohort from 1989 to 2006.}
\end{figure}
Modern treatment of mucinous rectal cancer

MC patients (p < 0.0001). This was most prominent in stage II and III patients. Five-year OS for MC was significantly worse from 1989-1998, but was no longer different from AC from 1999 onwards, indicating that modern treatment of RC using enhanced imaging, TME surgery and neo-adjuvant therapies may have had a significant impact on prognosis of MC patients.

**Contribution of short-term radiotherapy**

The benefit of short-term radiotherapy for MC and AC patients was analyzed using data from the TME trial. There were 103 (8.2%) MC patients and 1157 (91.8%) AC patients in the TME trial (Table 1). A positive CRM was more commonly found in MC patients than in AC patients (27.2% versus 16.5%, p = 0.006). AC patients less frequently developed a local recurrence (LR) after preoperative radiotherapy. The 10-year cumulative incidence of LR was 13% in the TME alone group and 5.6% in the group that underwent radiotherapy (p < 0.0001). A similar, though insignificant trend, was seen for MC patients, with a 10-year LR rate of 22.9% versus 15.6% (p = 0.218). The hazard ratio (HR) of developing LR was higher in MC patients than in AC patients (HR 2.06, 95% CI 1.23-4.46), independent of therapy. In accordance with previously published data from the TME trial, there was no improvement in survival for AC patients following radiotherapy (10-year OS 53.3% versus 50.5% after radiotherapy, p = 0.679; Figure 3). MC patients who underwent preoperative radiotherapy did not show an improved survival either (10-year OS 44.7% versus 49.4% after radiotherapy, p = 0.475).

![Figure 3](image)

**Figure 3.** Overall survival and local recurrence in (A) rectal non-mucinous adenocarcinoma and (B) rectal mucinous adenocarcinoma patients who were treated with total mesorectal excision (TME) with or without preoperative short-term radiotherapy (RT).

**Contribution of chemoradiotherapy**

A total of 58 (10.7%) MC patients and 482 (89.3%) AC patients with LARC were analyzed (Table 1). MC patients more commonly underwent a resection with a positive CRM (34.5% versus 9.8%, p < 0.0001) than AC patients. The higher rate of CRM positivity in MC patients was observed for ypT3 and ypT4 tumors only. The median follow-up period was 45 months (range 0-158 months). Long-
term chemoradiotherapy benefited MC and AC patients equally (Figure 4). At 5 years there was no difference in OS between MC and AC patients (64.3% versus 70.6%, \( p = 0.459 \)). The rate of LR was low and was not different between MC and AC patients.

Figure 4. (A) Overall survival and (B) local recurrence in mucinous adenocarcinoma (MC) and non-mucinous adenocarcinoma (AC) LARC patients who were treated with preoperative chemoradiotherapy.

The 5-year cumulative incidence rate of LR was 14.3% in MC patients and 9.5% in AC patients (\( p = 0.203 \)). Tumor downstaging, as indicated by a lower pT than cT, was more common in AC patients than in MC patients (68.7% versus 55.2%, \( p = 0.039 \)). Interestingly, pathological complete responses were not seen in MC patients, compared with 16.4% (\( n = 79 \)) in AC (\( p < 0.0001 \)).

Discussion

Consensus for treatment of RC patients is obtained in multidisciplinary team meetings, in which patient and tumor characteristics are taken into consideration. Herein, MC is commonly regarded as an unfavorable tumor subtype for preoperative therapies, with a poor prognosis. This study provided an analysis of the current treatment of rectal MC and showed that there is no longer a difference in overall survival between rectal MC and AC patients.

The generally accepted thought of a poorer prognosis for rectal MC came about from research that was performed with (small) patient groups from timed cohorts, as was demonstrated in our meta-analysis. Population-based data from the NCR was used to evaluate OS of RC patients from 1989 to 2006. Five-year OS analysis showed an improvement for both rectal MC and AC patients over time. Improved survival of RC patients as a consequence of modern treatment has previously been demonstrated, but was never assessed in MC patients.\(^{29,30}\) We found that survival of MC patients used to be worse than AC patients, but was comparable from 1999 onwards. The latter has not been described before and indicated that introduction of better preoperative imaging, TME surgery, and preoperative therapies may have led to equalization of survival in both subtypes.\(^{1,31-34}\) Surgery
according to TME principles or in advanced cases beyond TME principles improves chances of obtaining a radical resection, which is of particular importance in MCs, which are generally larger and have a less impressive response to chemoradiotherapy than ACs.21

The multicenter randomized TME trial and MRC CR07 trial demonstrated improved local control after preoperative short-term radiotherapy for patients with operable RC.7, 8 In the current study we showed that both MC and AC patients who were treated with preoperative short-term radiotherapy had a lower rate of LR than patients who were treated with surgery alone. These findings support the use of preoperative short-term radiotherapy for both subtypes.

In LARC patients the MRF is threatened and preoperative chemoradiotherapy is usually recommended. To study the benefit from chemoradiotherapy, a cohort of 540 patients with mucinous and non-mucinous LARCs from two experienced hospitals was assessed. There was no difference in OS between MC and AC, indicating equal benefit of preoperative chemoradiotherapy for both subtypes. Nevertheless, pathologic complete responses did not occur in MC patients, there was a lower rate of T-stage downstaging and MC patients more often had a positive CRM, as has been observed before.18, 20-22, 35 However, in our study this did not result in a worse outcome for MC patients. We hypothesize that other factors, such as mucus and tumor cell density, determine the impact of a positive CRM, but this has not been studied for MC and is an important issue for future research.36 It is unknown what mechanisms are responsible for the relative chemo and radio-resistance of MC. This is likely to be due to a combination of a different molecular signature and different physical properties of mucus containing tumors compared with AC. Mutated KRAS, which is more commonly found in MC, is one of the biomarkers that is strongly associated with a non-complete pathologic response to chemoradiotherapy.37 Furthermore, MC is associated with a higher rate of microsatellite instability (MSI).38 Tumors exhibiting MSI generally have a better prognosis than microsatellite stable tumors.39 Conflicting results have been published regarding the predictive impact of MSI on response to radiotherapy.39, 40 Unfortunately, KRAS and MSI status was not known for the patients in our study and these analyses could not be performed.

A complete response to chemoradiotherapy impedes the pathologist from determining the histological classification of a tumor. Since MC can be adequately recognized on MRI scanning, our radiologist reviewed MRIs of patients with a pathological complete response in the LARC group in the present study to address this problem.26, 27 A number of tumors showed at pathological evaluation extensive mucus pools without vital tumor cells (pathological complete response).41, 42 These tumors did not display the MC phenotype on preoperative MRI and thus were recognized as AC, emphasizing the role and importance of imaging in tumor characterization during preoperative work-up.

Based on findings from the present study we conclude that modern treatment of RC has benefited MC patients. Enhancements in the fields of imaging and quality of surgery most likely improved outcome. As a higher rate of CRM positive resections was demonstrated in the MC groups, there still should be a raised awareness for incomplete removal of the tumor. MRI may aid in early identification of rectal MC and recognition of this tumor characteristic may alter the therapeutic path.26, 27
To our knowledge, the present study is the largest study analyzing preoperative therapies for MC in RC patients. Data from different cohorts enabled us to study the impact of MC in different clinical settings. Although this study analyzed different treatment modalities in the largest group of rectal MC patients to date, it should be acknowledged that it is possible that observed trends were statistically insignificant as a consequence of lack of power. The TME trial and LARC cohort were not powered for the aim of this study, indicating once more the limitations of MC concerning accrual in clinical trials. Results from this study should be interpreted with caution since the non-randomized nature of this study could be a confounding factor. Patient from the LARC cohort underwent surgery after various waiting intervals, which may have influenced the degree of tumor regression. In that perspective, it seems interesting to study the effect of a longer time interval to surgery, since delaying surgery following chemoradiotherapy seems to result in the highest chance of a pathological (complete) response.43

In conclusion, our results indicate that there is no longer a difference in survival between MC and AC patients and that preoperative therapies should be recommended for both histological subtypes. However, the risk of a positive CRM was higher in MC patients. Therefore, more appreciation for MC in multidisciplinary team meetings is necessary and optimal preoperative staging remains a crucial factor in the clinical work-up of MC patients.
Modern treatment of mucinous rectal cancer

References


Supplemental material

Supplementary Data S1. Search strategy for meta-analysis.

To illustrate the prognostic impact of MC, a literature search was done in PubMed and EMBASE, last search performed on September 1, 2013, with a Boolean search term combination as shown below. Only cohort, case-control or cross-sectional studies in English or German, that used the WHO definition for MC, that compared survival between MC or AC in RC patients, included all stages of disease and contained data to calculate a hazard ratio (HR), were included in the meta-analysis (flow chart of study selection in Supplementary Figure S1). Reference lists of retrieved studies were searched for further relevant publications. The primary outcome was overall survival. HRs as reported in publications were used for analysis. If no HR was reported, it was calculated from the published data as described by Parmar et al. Heterogeneity was assessed by means of the I² statistic.

The National Library of Medicine (MEDLINE/PubMed)


The Intelligent Gateway to Biomedical & Pharmacoalogical Information EMBASE

1. (cancer or carcinoma* or tumor or tumors or tumour* or neoplasm* or adenocarcinoma*) and (rectum or rectal or colorectal).ti,ab.
2. rectum tumor/ or colorectal tumor/ or exp rectum cancer/
3. colloid carcinoma/
4. (mucin* or colloid).ti,ab.
5. 3 or 4
6. exp disease course/
7. (Survival or outcome or death or deaths or Prognosis).ti,ab.
8. 6 or 7
9. colon tumor/ or exp colon cancer/
10. 1 or 2 or 9
11. 10 and 5
12. 8 and 11
Supplementary Figure S1. Flow chart of study selection for meta-analysis.
Chapter 6

METASTATIC PATTERN IN COLORECTAL CANCER IS STRONGLY INFLUENCED BY HISTOLOGICAL SUBTYPE

N. Hugen, C.J.H. van de Velde, J.H.W. de Wilt and I.D. Nagtegaal

Annals of Oncology, 2014;25:651-7
Abstract

Clinical studies regarding colorectal cancer (CRC) have suggested differences in metastatic patterns between mucinous adenocarcinoma (MC), signet-ring cell carcinoma (SRCC) and the more common adenocarcinoma (AC). The current study systematically evaluates metastatic patterns of different histological subtypes in CRC patients and analyses metastatic disease upon primary tumor localization.

A nationwide retrospective review of pathological records of 5817 patients diagnosed with CRC who underwent an autopsy between 1991 and 2010 was performed. Patients were selected from the Dutch pathology registry (PALGA). To substantiate clinical relevance, metastatic patterns were compared with the prospective randomized multicenter Total Mesorectal Excision (TME) trial, which investigated efficacy of preoperative radiotherapy in rectal cancer patients.

In the autopsy study, 1675 patients had metastatic disease. MC and SRCC patients more frequently had metastatic disease (33.9% and 61.2% versus 27.6%, \( p < 0.0001 \)) and had metastases at multiple sites more often compared with AC patients (58.6% and 70.7% versus 49.9%, \( p = 0.001 \)). AC predominantly metastasized to the liver and MC and SRCC more frequently had peritoneal metastases. Metastatic patterns were also related to the primary tumor site, with a high rate of abdominal metastases in colon cancer patients, whereas rectal cancer patients more often had metastases at extra-abdominal sites. Results from the TME trial confirmed findings in rectal cancer patients from the autopsy study.

There are profound differences in metastatic patterns between histological subtypes and the localization of the primary tumor in CRC. Findings from this study encourage to take these factors into account for follow-up strategies and future studies.
Introduction

Despite the intensive follow-up for colorectal cancer (CRC) patients, metastatic disease still accounts for a high number of cancer-related deaths. At the time of presentation approximately 20% of patients have metastatic disease and 30-40% of patients treated for potentially curable CRC relapses. Large-scale autopsy studies have generated insight into metastatic patterns and demonstrated that different primary cancers metastasize to different sites with different frequencies. CRCs most commonly metastasize to the liver, lung and peritoneum, but various other metastatic sites such as bone, spleen, brain and distant lymph nodes have been described. Rare metastatic sites, such as pancreas and heart, are not well studied and generally only described in case reports.

Several clinical studies regarding CRC suggested that there are differences in metastatic patterns between histological subtypes. Mucinous adenocarcinoma (MC) represents 10-15% of CRC and is considered a distinct clinical entity, with a predominant right-sided location and a poor prognosis in metastatic disease. In follow-up of clinical trials, it was observed that MCs have a different distribution of metastatic disease, compared with the more common adenocarcinoma (AC). Signet-ring cell carcinoma (SRCC) is a relatively rare histological subtype of adenocarcinoma, present in 1% of CRC patients and is associated with a poor overall survival. Population- and institution-based studies found extensive lymphatic and peritoneal spread in SRCC suggesting a different biology.

Post-mortem studies offer a possibility to register both the extent and location of metastatic disease in different subtypes. Findings during autopsy may be considered the ultimate endpoint of disease. Most autopsy studies, however, have focused on metastatic patterns in one or more types of cancer, but have failed to address differentiating aspects such as histology within specific tumor subtypes.

This nationwide study evaluates the patterns of metastases in a large number of autopsies from patients with a history of CRC to generate insight into the relevance of histological subtype in the metastatic spread of CRC. To confirm the clinical relevance of our results, we also analyzed data from a prospective randomized multicenter trial.

Patients and methods

A nationwide retrospective review of pathological and autopsy records of 5930 patients diagnosed with CRC and eventually autopsied between 1991 and 2010 was performed. Patients were selected from the Dutch pathology registry (PALGA). In the Netherlands post-mortem examination is performed at the request of the family or treating doctor and is carried out by a pathologist. All autopsies included in this study were performed in order to obtain information on the medical status of the deceased or to determine the exact cause of death. No forensic autopsies were included. Undifferentiated tumors and tumors that were classified as carcinoids, neuro-endocrine tumors or other than adenocarcinoma (n = 87) were excluded. Furthermore, patients were excluded from the study if the location of the metastases could not be retrieved from the records (n = 27). Patient
demographics (gender and age) were available for all cases, but information on cause of death or other clinical information was lacking in this database.

A total of 1679 patients with metastatic colorectal disease was identified. Tumor histology had been assessed by different pathologist in all cases. For this study, only MC, AC and SRCC were included. Local staging according to the TNM classification (5th edition of the American Joint Committee on Cancer) was reconstructed from the tumor extension described in the pathology or autopsy record. Metastases that were found within six months after surgery were considered synchronous. Tumors were classified as proximal if they were found in the cecum, ascending colon or transverse colon, and were classified as distal if they were found in the descending or sigmoid colon.

To confirm the clinical relevance of data from the autopsy study, we selected patients from the Total Mesorectal Excision (TME) trial. The design of the TME trial was reported previously. This randomized multicenter study in the Netherlands included 1530 patients with primary resectable rectal cancer. Even though metastatic disease was an ineligibility criterion, there were 88 patients with synchronous metastases. Patients underwent clinical examination every three months during the first year after surgery and annually thereafter for at least two more years. Examination during follow-up included liver imaging and endoscopy. When metastatic disease was detected, only the metastatic lesions present at that moment were registered. Metastases that developed subsequently were not registered.

Statistical Analysis
The $\chi^2$-square test was used to compare demographics and tumor characteristics between the groups. All tests of significance were two-tailed: differences at $P$ values of < 0.05 were considered to be significant. Statistical analyses were performed with the statistical software package SPSS 20.0 (SPSS, Inc. Chicago, IL, USA).

Results
A total of 5817 autopsies was included in this study. AC was found in 4941 (84.9%) cases, compared with 809 MC (13.9%) and 67 SRCC (1.2%). The median age of all patients at death was 76 years (range 25-102). Metastatic disease was present in 1679 (28.9%) patients and was found in 27.6%, 33.9% and 61.2% of patients with AC, MC and SRCC, respectively ($p < 0.0001$). Clinicopathological data of metastatic CRC patients are presented in Table 1. The median time between surgery and autopsy was 28 months (range 7-246) in stage I, II and III patients and 1 month (range 0-222) in stage IV patients. Patients who developed metastatic disease were diagnosed with an initial stage I tumor in 3.2% and 1.1% of AC and MC cases. None of the SRCC patients had stage I disease. In more than half of all patients, metastatic disease was synchronous with the primary tumor.

Distribution of metastases according to histology
MC and SRCC patients more frequently had metastases at multiple sites (58.6% and 70.7%, versus 49.9% in AC, $p = 0.002$). Liver metastases were most frequent in both AC and MC patients (73.0%
and 52.2%). In SRCC patients, more than half of all patients developed metastases on the peritoneal surface. Uncommon sites of metastatic diseases were brain, kidney, adrenal gland, ovary, heart, omentum, bone, pleura, pancreas and spleen.

There were major differences in metastatic patterns between histological subtypes (Figure 1 and Supplementary Table S1). AC more frequently metastasized to the liver compared with MC and SRCC, 73.0% versus 52.2% and 31.7% ($p < 0.0001$). MC and SRCC metastases were more frequently found on the peritoneal surface, 48.2% and 51.2% respectively, compared with 20.1% in AC ($p < 0.0001$). Lung metastases were found in one-third of all cases, which was not different between the groups. SRCC markedly metastasized to distant lymph nodes more frequently, 43.9% compared with 22.3% and 19.9% in MC and AC, respectively ($p = 0.001$). These metastases were usually found adjacent to an organ with metastatic disease. Rare metastatic locations, such as heart, bone and pancreas, were

### Table 1. Clinicopathological features of patients with metastatic colorectal cancer diagnosed between 1991 and 2010.

<table>
<thead>
<tr>
<th>Features</th>
<th>AC</th>
<th>MC</th>
<th>SRCC</th>
<th>$P$ value</th>
</tr>
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<tr>
<td></td>
<td>$n = 1364$</td>
<td>$n = 274$</td>
<td>$n = 41$</td>
<td></td>
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<tr>
<td>Sex</td>
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<td>0.158</td>
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<tr>
<td>Female</td>
<td>539</td>
<td>124</td>
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<tr>
<td>Male</td>
<td>825</td>
<td>150</td>
<td>22</td>
<td></td>
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<tr>
<td>Age at diagnosis</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Median (range)</td>
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<td>72 (36-91)</td>
<td>73 (32-54)</td>
<td></td>
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<tr>
<td>&lt; 45</td>
<td>27</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>45-59</td>
<td>271</td>
<td>43</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>60-74</td>
<td>627</td>
<td>135</td>
<td>15</td>
<td></td>
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<td>≥ 75</td>
<td>439</td>
<td>89</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Location of primary</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>420</td>
<td>116</td>
<td>20</td>
<td>48.8</td>
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<tr>
<td>Distal colon</td>
<td>443</td>
<td>71</td>
<td>8</td>
<td>19.5</td>
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<td>Rectum</td>
<td>373</td>
<td>61</td>
<td>7</td>
<td>17.1</td>
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<tr>
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<td>26</td>
<td>6</td>
<td>14.6</td>
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<td>Initial Stage</td>
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<td>0.208</td>
</tr>
<tr>
<td>I</td>
<td>43</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>192</td>
<td>27</td>
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<td>7.3</td>
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<tr>
<td>III</td>
<td>272</td>
<td>59</td>
<td>10</td>
<td>24.4</td>
</tr>
<tr>
<td>IV</td>
<td>769</td>
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<td>26</td>
<td>63.4</td>
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<tr>
<td>Unknown</td>
<td>88</td>
<td>17</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>Number of metastases</td>
<td></td>
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<td>0.002</td>
</tr>
<tr>
<td>1</td>
<td>682</td>
<td>113</td>
<td>12</td>
<td>29.3</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>680</td>
<td>160</td>
<td>29</td>
<td>70.7</td>
</tr>
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</table>
found up to three times more frequently in SRCC than in MC or AC. Metastases to the ovary and skin or subcutaneous tissue were more common in SRCC and MC. Two autopsies on female patients reported a metastasis to the breast, both patients were diagnosed with SRCC.

**Distribution of metastases according to primary tumor site**

Supplementary Table S2 and Figure 2 show metastatic patterns in relation to the primary tumor site. The frequency of liver metastases did not differ between colon and rectal cancer patients (69.6% versus 67.4%). Colon cancer patients presented more frequently with intra-abdominal metastases, such as peritoneal metastases (28.8% versus 16.1%, \( p < 0.0001 \)), omental metastases (9.1% versus 2.9%, \( p < 0.0001 \)) and ovarian metastases (3.2% versus 1.1%, \( p = 0.019 \)). Rectal cancer patients, however, presented more frequently with extra-abdominal metastatic sites such as lung (42.0% versus 30.7%, \( p < 0.0001 \)) and brain (5.0% versus 2.6%, \( p = 0.014 \)). These findings were observed for both AC and MC patients, even though not all sites reached statistical significance. In SRCC patients, however, no differences in patterns between colon and rectum could be identified (data not shown).

**Combination of metastases**

Many patients developed metastatic disease at more than one site. Figure 3 summarizes the most frequent combinations. AC patients had the highest percentage of liver metastases, and suffered frequently from liver metastases as the only metastatic site. The occurrence of metastases exclusively to the liver was less common in MC and SRCC patients. Especially in SRCC patients, liver metastases...
Figure 2. Distribution of metastases according to tumor site.
LN, lymph node; *, $p \leq 0.05$; ****, $p \leq 0.0001$.

Figure 3. Relative frequencies of combinations with (A) liver metastases ($p < 0.0001$), (B) lung metastases ($p = 0.75$) and (C) peritoneal metastases ($p < 0.0001$) in adenocarcinoma (AC), mucinous adenocarcinoma (MC) and signet-ring cell carcinoma (SRCC).
were almost always observed in combination with other metastases. There were no differences in the frequencies of lung metastases, nor in the combinations of lung metastases with metastatic disease at other sites. Even though MC and SRCC patients suffered from peritoneal metastases more frequently, the proportion of peritoneal metastases only versus combinations with other metastases was equally distributed within all three groups.

**Clinical relevance: TME trial**
There were 403 (31.1%) AC patients and 50 (32.7%) MC patients in the TME trial who developed metastatic disease \( p = 0.398 \) during follow-up (median follow-up 11.6 years). There was only one SRCC patient in the study, who developed metastases in distant lymph nodes (both axillary and cervical) and on the peritoneal surface during follow-up. There were no differences in metastatic patterns between patients who were treated with or without preoperative radiotherapy (data not shown). In Supplementary Table S3 and Figure 4 the distribution of metastases in rectal cancer patients from the TME trial and autopsy study is summarized. In the TME trial, liver metastases occurred more frequently in AC patients (59.6% versus 36.0%, \( p = 0.002 \)), whereas peritoneal metastases were more common in MC patients (14.0% versus 4.5%, \( p = 0.005 \)). This was comparable with the findings from the autopsy study. Frequencies of metastases at other sites were not significantly different in the TME trial.

![Figure 4. Distribution of metastases in rectal cancer patients from the TME trial and autopsy study.](image)

*AC, adenocarcinoma; MC, mucinous adenocarcinoma; LN, lymph node; *, \( p \leq 0.05 \); **, \( p \leq 0.01 \); ****, \( p \leq 0.0001 \).
Discussion

This study is the first large-scale modern autopsy study for metastatic patterns in well-known subtypes of CRC. We show major differences in frequencies and combinations of metastatic sites between histological subtypes. These differences may have significant implications for clinical treatment, follow-up strategies and future clinical trials.

Compared with AC, the presence of metastatic disease in more than one location was more frequent in MC. Since curative surgery is an option mainly limited to liver metastases, this may be an explanation for the poor performance of MC patients in trials for metastatic disease.\textsuperscript{5, 12} In MC patients we found a high rate of peritoneal metastases. Several clinical studies already suggested differences in metastatic patterns between histological subtypes.\textsuperscript{5, 12, 13} These studies also described a high number of peritoneal metastases in MC, with percentages varying from 22\% to 45\%.\textsuperscript{12, 13} Peritoneal metastases are associated with a poor prognosis and survival is even worse if metastases in other organs are present.\textsuperscript{14} Moreover, palliative chemotherapy in patients with peritoneal metastases is not very successful.\textsuperscript{15} The high number of peritoneal metastases in MC is therefore another possible explanation for the poor survival in MC patients in advanced disease.

SRCC patients also presented more frequently with more than one metastatic site and an increased risk of peritoneal metastases. Interestingly, we found a high rate of distant lymph node metastases in SRCC. This enhanced lymphatic spread of SRCC has been noticed previously in small clinical studies.\textsuperscript{8} SRCC patients showed a divergent pattern of metastases, with involvement of rare metastatic sites, such as heart, bone, pancreas and skin. Therefore, attention should be paid to uncommon findings on imaging in SRCC patients during clinical follow-up, since these may reflect metastatic disease.

Underlying mechanisms for differences in metastatic patterns between histological subtypes are not clear. Several studies have described molecular and biological differences between AC, MC and SRCC, contributing to a more aggressive biological behavior.\textsuperscript{16} A theory behind the high number of peritoneal metastases in MC is that production of mucus under pressure allows cancers to gain access to the peritoneal cavity, through separation of tissue planes in the bowel wall and mucus producing tumors may spread throughout the peritoneal cavity more easily in the form of gelatinous ascites.\textsuperscript{17, 18} Moreover, fluid produced by MC tumors enhances uptake into regional lymph nodes, facilitating lymphatic spread throughout the body.\textsuperscript{17}

In this study, we also analyzed the differences in metastatic patterns between colon and rectal cancer. We show that both colon and rectal cancer predominantly metastasize to the liver. Moreover, colon cancer patients presented with abdominal metastases more often, whereas rectal cancer patients presented more frequently with extra-abdominal metastatic sites such as lung and brain. This was seen in both MC and AC patients, but not in SRCC patients. In the trial population, we confirmed the differences between rectal MC and AC regarding liver and peritoneal metastases, thus emphasizing the clinical relevance of our study data. The higher rate of metastatic lesions that was found in the autopsy study can be explained by the obvious reasons that only the first metastatic lesions were
registered in the TME trial and autopsy yields better detection of metastases. Studies that analyzed patterns of tumor recurrence and metastatic disease also found an increased risk of lung metastases in rectal cancer and found prognosis of lung metastases to be poor.\textsuperscript{19,21} The higher rate of distant metastases in rectal cancer can be explained by the venous drainage of the rectum bypassing the liver straight into the inferior vena cava.

Knowledge of differences in metastatic patterns is important and may induce changes in clinical practice. A high rate of peritoneal carcinomatosis was found in MC and it has been advocated that all MC patients should undergo resection accompanied by perioperative intraperitoneal chemotherapy to improve survival.\textsuperscript{17} The high number of peritoneal metastases in MC and SRCC should raise concern in case of tumor spillage and should possibly even result in adjuvant therapeutic measures. Insight into metastatic patterns may also impact the design of follow-up. Since liver and lung metastases are most-common, regular imaging of chest and liver should be maintained. However, in case of unusual or indefinable lesions, other imaging techniques such as PET-CT should be employed at an earlier stage, especially in MC and SRCC patients. Early detection of peritoneal metastases should be priority in these patient groups.

To our best knowledge this is the largest autopsy study focusing solely on metastatic disease from CRC. Furthermore, it is the first study that shows differences in metastatic pathways between histological subtypes in a large nationwide population. However, there are limitations due to the retrospective nature of this study. First of all, it is important to notice that an autopsy study unequivocally leads to a biased population, in which patients are included who have died postoperatively, had an unexpected clinical course, or died of other causes than CRC. Nevertheless, autopsy studies offer a unique opportunity to study the distribution of metastases and arguably can be seen as the gold standard in the study of cancer metastases. Secondly, it has not been possible to review the individual pathological diagnosis. Even though definitions of MC and SRCC have been standardized, variations in interpretation may have resulted in misclassification. However, the distribution of histological subtypes is similar to numbers reported in the literature.\textsuperscript{6,7} We also confirmed the clinical relevance of our findings with follow-up data of a phase III clinical trial. Even though the TME trial is a prospective trial, the numbers of metastatic lesions were lower, due to a more limited examination and registration compared with the autopsy study. This substantiates the importance of findings from autopsy studies. However, it may still be possible that there is an underestimation of the number of metastatic lesions in this autopsy study. Although we included only whole-body autopsies, metastatic lesions situated outside of the routinely examined regions may have been missed. Moreover, brain autopsy was not allowed for each patient. These limitations may have led to an underestimation of especially brain and bone metastases, but this bias potentially applies to all subgroups.

This study shows that histological subtype and primary tumor localization are important predictors of metastatic spread. MC and SRCC metastasize to different sites, in different combinations and are more likely to have a higher number of metastases. Furthermore, we show that colon and rectal cancers have different metastatic patterns as well. Based on profound differences in metastatic patterns between histological subtypes and localization of the primary tumor, we encourage to take
these factors into account during preoperative examination for metastases and during follow-up. Our results also indicate that these factors should be considered a stratification factor in future research initiatives focusing on advanced disease.

Acknowledgements

Collaborators in the PALGA-group, Dr L.I.H. Overbeek (PALGA), Dr S.H. Sastrowijoto (Orbis Medisch Centrum Sittard), Dr C. Jansen (Laboratorium Pathologie Oost-Nederland).
Chapter 6

References

## Supplemental material

**Supplementary Table S1.** Distribution of metastases according to histology.

<table>
<thead>
<tr>
<th>Metastatic site</th>
<th>AC</th>
<th>%</th>
<th>MC</th>
<th>%</th>
<th>SRCC</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>996</td>
<td>73.0</td>
<td>143</td>
<td>52.2</td>
<td>13</td>
<td>31.7</td>
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<td>Lung</td>
<td>460</td>
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<td>33.6</td>
<td>13</td>
<td>31.7</td>
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<td>132</td>
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<td>Distant lymph nodes</td>
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<td>18</td>
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<td>7.3</td>
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<td>17.1</td>
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<td>3</td>
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AC, adenocarcinoma; MC, mucinous adenocarcinoma; SRCC, signet-ring cell carcinoma
**Supplementary Table S2.** Distribution of metastases according to tumor location.

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<th>Rectum</th>
<th>P value</th>
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<td>n = 1238</td>
<td>%</td>
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<td>38</td>
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<tr>
<td>Ovary</td>
<td>40</td>
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<td>Heart</td>
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<td>Skin/subcutaneous tissue</td>
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<td>12</td>
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<tr>
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**Supplementary Table S3.** Distribution of metastases in rectal cancer patients from the TME trial and autopsy study.

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<tr>
<th>Metastatic site</th>
<th>TME trial</th>
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<td></td>
<td>AC n = 403 %</td>
<td>MC n = 50 %</td>
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<td>Liver</td>
<td>240 59.6</td>
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<td>Lung</td>
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<td>0.005</td>
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<td>32 7.9</td>
<td>4 8.0</td>
<td>0.988</td>
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AC, adenocarcinoma; MC, mucinous adenocarcinoma
Chapter 7

REDUCED RATE OF COPY NUMBER ABERRATIONS IN MUCINOUS COLONRECTAL CARCINOMA


Oncotarget, 2015; 6:25715-25
Abstract

Mucinous carcinoma (MC) is found in 10-15% of colorectal cancer (CRC) patients. It differs from the common adenocarcinoma (AC) in histopathological appearance and clinical behavior.

Genome-wide DNA copy number and survival data from MC and AC primary CRC samples from patients from two phase III trials (CAIRO and CAIRO2) was compared. Chromosomal copy number data from The Cancer Genome Atlas (TCGA) was used for validation. Altogether, 470 ACs were compared to 57 MCs.

MC showed a reduced amount of copy number aberrations (CNAs) compared with AC for the CAIRO/CAIRO2 cohort, with a median amount of CNAs that was 1.5-fold lower ($p = 0.002$). Data from TCGA also showed a reduced amount of CNAs for MC. MC samples in both cohorts displayed less gain at chromosome 20q and less loss of chromosome 18p. A high rate of chromosomal instability was a strong negative prognostic marker for survival in MC patients from the CAIRO cohorts (hazard ratio 15.60, 95% CI 3.24-75.05).

Results from this study indicate that the distinct MC phenotype is accompanied by a different genetic basis when compared with AC and show a strong association between the rate of chromosomal instability and survival in MC patients.
Introduction

Colorectal cancer (CRC) is categorized by histological subtype according to the WHO classification. The majority of patients (~ 85%) is diagnosed with adenocarcinoma not otherwise specified (AC). Mucinous adenocarcinoma (MC) is detected in 10-15% of patients and is characterized by abundant extracellular mucus lakes that comprise more than half of the tumor volume. MC differs from AC in both clinical and pathological presentation. MC is more frequently found in the proximal colon and at a higher stage at presentation than AC. Also, the response to therapies varies between MC and AC, as patients with MC show a poorer response to palliative chemotherapy compared with AC, resulting in a worse survival. These findings suggest a distinct genetic background of MC.

There are two major pathways through which genomic instability can occur in CRC, namely chromosomal instability (CIN) and microsatellite instability (MSI). CIN is found in the majority (~ 85%) of CRCs and is a type of genetic instability in which chromosomal aberrations accumulate, leading to an altered expression of tumor suppressor genes and oncogenes. MSI accounts for the remaining 15% of CRCs and is caused by a defective DNA mismatch repair mechanism that leads to a clonal change in the number of microsatellites. Tumors with MSI are more commonly found in MC patients compared with AC patients, and exhibit less copy number aberrations (CNAs).

Although MSI is more common in MC than in AC, the vast majority of MCs supposedly still develops through the CIN pathway. Unfortunately, most large-scale genomic studies of CRC did not address differences between histological subtypes and focused mainly on AC. Specific DNA CNAs that cause gene dosage effects in oncogenes and tumor suppressor genes, typically occur during adenoma to carcinoma progression, and thus are an integral part of the pathogenesis of CRC. Therefore, analysis of DNA CNAs between MC and AC in a sufficiently large collection of samples may generate more insight into an early and possibly diverging event in cancer development.

In this study we use DNA copy number data from primary tumor samples of patients with MC or AC who participated in two phase III clinical trials. These data were used to test whether the distinct MC and AC phenotypes relate to differences in genomic profiles. Molecular characterization of MC may improve our understanding of the reduced response rate to systemic therapies and therefore contributes to the development of targeted treatment modalities for MC.

Patients and methods

Patients and materials

For this study we used clinical and genome data of patients from two randomized controlled trials and validated our findings with data from The Cancer Genome Atlas (TCGA).
CAIRO and CAIRO2 cohorts

We used high resolution array comparative genomic hybridization (aCGH) data that were generated from DNA isolated from formalin-fixed and paraffin-embedded (FFPE) primary tumors, which was hybridized against paired germ line DNA samples. The processes of sample selection, DNA isolation and aCGH data have been described previously. Samples were derived from patients who participated in the CAIRO study (CKTO 2002-07, ClinTrials.gov; NCT00312000) or CAIRO2 study (CKTO 2005-02, ClinTrials.gov; NCT00208546) of the Dutch Colorectal Cancer Group (DCCG). These phase III trials had different systemic regimens as first-line treatment for CRC patients with metastatic disease. In the CAIRO study, patients were randomly assigned to either sequential or combination treatment with capecitabine and irinotecan, followed by oxaliplatin. In the CAIRO2 study patients were randomized between treatment with capecitabine, oxaliplatin and bevacizumab, with or without the addition of cetuximab. All patients had given written informed consent prior to study entry, which also included translational research on tumor tissue. aCGH was performed on a subset of the patients from these trials. In the CAIRO2 study the aCGH was only performed on material from the control arm since the addition of cetuximab in the experimental arm yielded a worse outcome. Furthermore, only tumors with paired germ-line tissue, and with areas of high tumor cell percentage available (> 70%) had been included. A total of 349 high quality DNA copy number profiles were generated. Tumors were classified according to the guidelines of the World Health Organization. If more than 50% of the tumor consisted of extracellular mucus it was classified as MC. AC was defined as a tumor without extracellular mucus. Only tumors that were categorized as MC or AC were included in our analyses. Tumors with MSI usually exhibit limited CNAs, and therefore form a separate entity among CRCs. These patients (n = 31) were excluded from the present study. In the current study DNA copy number profiles of 17 MC and 135 AC patients from the CAIRO study and 12 MC and 100 AC patients from the CAIRO2 study were compared.

The Cancer Genome Atlas cohort

To validate findings from the CAIRO cohorts, copy number information for MC and AC samples from the TCGA data portal was analyzed. On 27 January 2014 all available colon adenocarcinoma level 3 copy number data were downloaded from the TCGA Data Portal using the Data Matrix (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). TCGA copy number data had been generated with Affymetrix SNP 6.0 arrays (Santa Clara, USA). Only data obtained from primary tumors was used. The histopathological designation as provided by TCGA was used, and only tumors that were categorized as MC or AC were selected. Furthermore, MSI tumors were excluded from the analyses. In total, DNA copy number profiles of 28 MC and 235 AC patients were compared.

Clinicopathological data

For each patient, the following clinicopathological characteristics were available: age, gender, site of primary tumor, number of metastatic sites involved, invasion depth, lymph node status, MSI status and histological subtype. Tumors from the TCGA cohort were classified as proximal if they were found in the cecum, ascending colon or transverse colon, up to the splenic flexure, and were classified as distal if they were found in the descending colon or sigmoid colon. MSI status was determined by immunohistochemistry with antibodies against MLH1, MSH2, MSH6 and PMS2. MSI analysis was
performed on indication by PCR followed by GeneScan analysis for MSI markers (BAT25, BAT26, BAT40, D2S123, D5S346, D17S250). Differences in baseline characteristics between groups were determined using Fisher’s exact testing. Statistical analyses were two-sided and \( P \) values< 0.05 were considered significant.

**Processing of aCGH and SNP array data**

Array CGH was performed using customized Agilent oligonucleotide arrays. Methods of DNA extraction, labeling, hybridization and scanning were previously described\(^17\) and the exact array design can be found online in the Gene Expression Omnibus (GEO) (GPL8687 http://www.ncbi.nlm.nih.gov/geo). The probes were mapped to human reference sequence GRCh37/hg19 (February 2009). The statistical programming language R was used for data processing. The quality of the aCGH DNA copy number profiles was assessed by calculating the median absolute deviation (MAD) from the log2ratios of signal intensities from tumor and paired germ line DNA. A MAD value of 0.4 and smaller was used as a quality criterion, which all DNA copy number profiles passed. A wave-smoothing algorithm was applied on the profiles and the profiles were median normalized and corrected for tumor cell percentage using the R package ‘CGHcall’.\(^18\) For segmentation the R package ‘DNAcopy’ was used.\(^19\) Next, mode normalization was performed. Subsequently, the DNA copy number (deletion, loss, neutral, gain, or amplification) was determined for each segment using the R package ‘DNAcopy’.\(^12\) This data was used to generate genome-wide frequency plots and box plots with the number of aberrations. For further analyses the dimensions of the aCGH data set were reduced using the R package ‘CGHregions’ (averror = 0.01).\(^20\) This step reduced the calls into subregions. Each subregion consisted of a series of neighboring clones on the chromosome whose aCGH-signature was shared by all clones. With this step 2010 subregions were obtained with a median size of 0.5Mb (interdecile range = 110kb - 2Mb).

The TCGA level 3 SNP6 data consisted of copy number values (log2ratios) generated with ‘nocnv’ segmentation. For the genome wide frequency plots, information was extracted from the downloaded files with LINUX shell and BEDTools.\(^21\) By extracting the genomic positions of all segment ends, a file with unique genomic positions was made. This gave 52654 genomic positions distributed over all chromosomes. Next, for each TCGA sample the log2 ratio at these positions was collected. In R these copy number values were converted into calls. The threshold was set as previously described.\(^22\) Values lower than -0.23 were assigned copy number loss, and values higher than 0.2 were assigned gain, all other values were assigned neutral. These values correspond to 30% of the tumor cells with that CNA. This data was used to generate the frequency plots using functions of the R package ‘CGHbase’.

**Analysis of the level of chromosomal instability**

For each sample we counted the number of probes called as loss, neutral or gain and subsequently calculated the percentage of probes with an aberrant call. The distribution of this level of chromosomal instability was plotted in box-plots. To assess whether the distribution of MC and AC samples was different, the Wilcoxon rank sum test was used (also known as Mann-Whitney test).
Chapter 7

Identification of regions with differential copy number

DNA copy number information of the 2010 subregions was analyzed in a supervised way. MCs from both CAIRO studies were compared with ACs from the same studies. Per sample group, the frequencies of losses, neutrals and gains were determined for each region. To calculate the statistical significance of DNA copy number differences between MC and AC the Wilcoxon rank sum test was used, and a correction for multiple testing was performed with the Benjamini-Hochberg...
Genetic aspects of mucinous colorectal carcinoma

procedure. An adjusted $P$ value < 0.01 was considered statistically significant. The TCGA data were used for validation of the differential subregions identified with the CAIRO/CAIRO2 samples. Frequency plots were generated for subregions that showed differences in copy number and a Wilcoxon rank sum test was performed on the calls to determine significant DNA copy number differences between MC and AC, followed by correction for multiple testing with the Benjamini-Hochberg procedure.

![Figure 2](image)

Figure 2. Levels of chromosomal instability. For each sample the percentage of probes with an aberrant call was calculated. The box plots show per cohort the distribution of the percentage of probes with an aberrant call for the adenocarcinoma (AC) and mucinous adenocarcinoma (MC) samples. In both cohorts a lower median chromosomal instability for MC is observed.

Survival analysis
To determine the impact of CIN on survival in MC and AC patients, groups were divided into CIN high and CIN low. The threshold was set at the median level of CIN of all samples of the CAIRO cohorts which was 29.66%. Patients who demonstrated a CIN rate below the median were considered CIN low and consequently, patients who demonstrated a CIN rate that was above the median were considered CIN high. Overall survival (OS) was defined as the interval between the date of randomization until the date of death of any cause or until last follow-up. Patients who were alive at the end of follow-up were censored in the survival analyses. OS curves were estimated using the Kaplan-Meier method and compared with the log-rank test. Multivariable analysis of OS was performed using the Cox proportional hazard model. Statistical analyses were performed with the statistical software package SPSS 20.0 (SPSS Inc, Chicago, Illinois, USA).
Clinicopathological data of the CAIRO/CAIRO2 and TCGA cohort

The baseline characteristics on MC and AC patients from the CAIRO/CAIRO2 cohort are presented in Supplementary Table S1. MC patients were more commonly over 60 years of age than AC patients (86.3% versus 62.5%, \( p = 0.04 \)). There were no other significant differences in clinicopathological characteristics between MC and AC patients. Data on survival between AC and MC in advanced-stage disease were published previously on these series. Clinicopathological data on colon cancer patients from the TCGA cohort is presented in Supplementary Table S2. In MC patients from the TCGA cohort, tumors were more commonly located in the proximal colon than in AC patients (78.6% versus 49.4%, \( p = 0.004 \)). The distribution of tumors was not different in the CAIRO/CAIRO2 cohort. There were no further substantial differences in baseline characteristics.

Results

Figure 3. Distribution of differentiated regions. Genome plot depicting a linear view of the chromosomes and distribution of the regions with a significant differential DNA copy number frequency between mucinous adenocarcinoma (MC) and adenocarcinoma (AC) patients in the CAIRO/CAIRO2 cohort. The 234 regions are localized on 11 chromosomes.
Different copy number profiles between MC and AC

The frequency of CNAs in MC and AC patients from the CAIRO/CAIRO2 and TCGA cohorts are depicted in Figure 1. These genome-wide profiles of CNAs of MC and AC patients appeared rather similar in both cohorts, but overall MCs displayed a lower level of chromosomal instability (Figure 2, left). In the MCs from the CAIRO/CAIRO2 cohort a median of 21% of the genome showed either deletions, losses, gains or amplifications, compared with 31% for the ACs ($p = 0.002$). For the TCGA cohort this was 19% for MC versus 29% for AC, respectively ($p = 0.0002$). In the CAIRO/CAIRO2 cohort there were particularly differences in the overall frequencies of the gains between AC and MC (Supplementary Figure S1).

Next, analyses were performed to further identify chromosomal subregions with significant differential copy number. We identified 234 significantly differential subregions in the CAIRO/CAIRO2 cohort ($p = 0.01$; Figure 3). These subregions were located on chromosomes 5, 6, 7, 8, 10, 11, 13, 16, 17, 18 and 20.

Validation in TCGA data

To confirm the findings from the CAIRO/CAIRO2 cohort, data from TCGA were analyzed. The TCGA data not only represents another patient cohort, but also a different method to determine DNA CNAs, since single channel SNP arrays were used, rather than CGH arrays. In addition, no paired normal DNA was used and DNA was isolated from fresh frozen material. MC samples displayed a reduced rate of CNAs than AC samples (Figure 2, right). Furthermore, MC samples in this cohort showed a significantly differential copy number for chromosome 18 and 20 (Figure 4), but not for the other chromosomes with significant differential subregions for the CAIRO/CAIRO2 cohort (chromosomes 5, 6, 7, 8, 10, 11, 13, 16 and 17). This included 13q gain, which showed a highly significant difference between AC and MC in the CAIRO/CAIRO2 cohort, while in the TCGA cohort this could not be confirmed (Supplementary Figure S2).

Different copy numbers at chromosome 18 and 20

MC patients displayed significantly less losses at chromosome 18 compared with AC patients in both cohorts. In the TCGA cohort, this comprised nearly the entire chromosome, but for the CAIRO/CAIRO2 cohort, this was mainly restricted to regions of the p-arm (Figure 4). The significant loci on 18p for the CAIRO/CAIRO2 cohort were merged into one region of main interest of 14Mb, which involved almost the entire p-arm (18p11.32-18p11.21). For this region of interest, 34% of MC patients showed a loss in the CAIRO/CAIRO2 cohort, compared with 69% of AC patients. In the TCGA data these percentages were 14% and 64% respectively. At the q-arm of chromosome 20 both the CAIRO/CAIRO2 and TCGA cohorts showed less gains in MC patients compared with AC patients (Figure 4). This region of interest at chromosome 20 was 33Mb and comprised essentially the entire q-arm (20q11.21-20q13.33). A gain at this region was found in 52% MC patients in the CAIRO/CAIRO2 cohort, while AC patients showed 93% gain or amplification. The percentages of these gains were 46% and 84%, respectively for the TCGA data.

Survival is related to CIN status in MC patients

The relation between survival and either a high or low rate of CIN was explored. There were 112 AC and 20 MC patients in the CIN low group versus 123 AC and 9 MC patients in the CIN high group.
**Figure 4.** Frequency plots of DNA copy number aberrations (CNAs) in chromosome 18 and chromosome 20. Detailed view of chromosomes with the differential regions of the CAIRO/CAIRO2 cohort that are validated in the TCGA data set. The y-axis displays the percentage of tumors with gain (above zero) or loss (below zero). The chromosomes represent ideograms with chromosomal bands. The corrected $P$ values obtained with statistical significance testing and correction for multiple testing are depicted in boxes below the plots. Black represents adjusted $p < 0.01$ and indicated a significant difference in DNA copy number between adenocarcinoma (AC) and mucinous adenocarcinoma (MC); white represents adjusted $p > 0.01$ and no indication of a significant difference. The significant loci obtained from the CAIRO/CAIRO2 cohort on 18p and 20q can be merged into two regions of main interest: chr18:122131-13971462 and chr20:29833609-62880524. For the region of interest on p18 34% of MC patients showed a loss in the CAIRO/CAIRO2 cohort, compared with a loss in 69% in AC patients. For the region of interest on q20 52% of MC patients in the CAIRO/CAIRO2 cohort showed a gain, while AC patients showed 93% gain or amplification.
OS rates in MC patients were dependent on the rate of CIN. MC CIN high patient had a statistically significant poorer OS compared with MC CIN low patients. MC CIN high patients had a median OS of 6.6 months (95% CI 4.8-8.4) versus 19.4 months (95% CI 11.7-27.0) for MC CIN low patients (Figure 5a). An OS difference according to CIN status was not observed in AC patients, with a median OS of 19.7 months (95% CI 17.8-21.7) for AC CIN high patients compared with 21.2 months (95% CI 16.3-26.1) in AC CIN low patients (Figure 5b). Interestingly, MC CIN low patients had an OS that was comparable to that of AC patients. Also, in the multivariable Cox regression analysis a high rate of CIN was a strong negative prognostic marker for OS in MC patients from the CAIRO cohorts with a hazard ratio of 15.60 (95% CI 3.24-75.05; Supplementary Table S3).

**Discussion**

MC is considered a unique subtype of CRC based on its histopathological appearance and clinical behavior. This study investigated whether the distinct visual microscopic pathological characteristics of MC are associated with different genetic aberrations when compared with AC in MSS primary tumors.

In CRC, CIN and MSI are two well-defined genomic pathways that are involved in carcinogenesis. MSI is found in approximately 15% of CRCs, but is more commonly found in MCs than in ACs.\(^5\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^23\) Although MSI and CIN are not mutually exclusive, CNAs are far less common in MSI than MSS tumors.\(^24\)\(^,\)\(^25\) In the present series of MSS CRCs an overall lower level of genetic instability was observed in MC compared with AC. As to specific DNA CNAs, the frequency of gain of chromosome 20q and the frequency of loss of chromosome 18p was significantly lower in MC. Chromosomal gains of 20q and losses of 18p are among the most-common molecular aberrations in CIN induced CRCs and since their occurrence is associated with progression from adenoma to carcinoma they are considered early genetic events.\(^11\)\(^,\)\(^26\) It should therefore be noted that these regions are consequently most prone
to reach statistical significance if there are overall differences in genetic instability as we observed for AC versus MC. Notwithstanding, these regions could be less contributory in MC development.

Amplification of 20q is an early molecular event and considered one of the key events that may induce the malignant process.\textsuperscript{11, 27, 28} Gain of a chromosomal region at 20q is present in over 60% of CRCs.\textsuperscript{25, 28, 29} There are several common regions of overlap of the highest level of gains at the 20q arm, which makes it plausible that multiple genes are involved in CRC development. The exact mechanism through which this occurs has not yet been elucidated, but an increasing number of genes that would be responsible for this 20q amplicon-driven progression has been identified.\textsuperscript{27, 30, 31} Gain of 20q has also been associated with a poor prognosis in CRC patients.\textsuperscript{32} Previously, we found that prognosis of MC patients in advanced-stage disease was worse compared with AC patients, due to a decreased response to palliative chemotherapy.\textsuperscript{5} Our group identified chromosomal regions that were associated with a decreased responsiveness to the addition of irinotecan in advanced colorectal cancer.\textsuperscript{12} However, these specific regions did not differ between MC and AC patients in the current study. The poor response to chemotherapy may be related to the deviant pattern along which metastatic disease spreads in MC patients. Compared with AC, MC is less likely to present with liver metastases only, whereas intra-abdominal metastases are observed in more than half of all cases with advanced disease.\textsuperscript{33} One study that analyzed CNAs between patients with different metastatic patterns showed that gain of the 20q chromosomal arm was associated with liver-specific metastases, suggesting a role in the process of liver metastasis in CRC.\textsuperscript{34} Patients who did not develop metastatic disease and patients with peritoneal metastases showed a gain of 20q less frequently.\textsuperscript{34, 35} These findings fit very well with the aberrant metastatic pattern that has been observed in MC patients.

Losses of chromosome 18q and 18p are seen in two third of CRC patients.\textsuperscript{36} Especially loss of 18q is a well-known aberration that has been associated with adenoma to carcinoma progression. However, loss of 18p has also been found to be an early genetic change in primary CRC.\textsuperscript{26, 37} It is unknown what genes on 18p can be held accountable for the malignant progression. Gain of chromosome 13q is found in approximately half of CRCs.\textsuperscript{29, 36, 38} and is associated with adenoma to carcinoma progression as well.\textsuperscript{37} Interestingly, in the present study we found less gain for virtually the entire 13q-arm in the CAIRO/CAIRO2 cohort, but this could not be confirmed with data from the TCGA cohort.

We found that a high level of CIN was associated with a poor outcome in MC patients, but not in AC patients. Previously, CIN has been associated with a poor prognosis, mostly in stage II and III CRC.\textsuperscript{39} Apparently, this also accounts for MC stage IV patients. It has been suggested that abnormalities of the spindle checkpoint drive CIN.\textsuperscript{40} Overexpression of AURKA (located on Chr 20q13, encoding the kinase Aurora-A) or loss of CHEK2 (located on Chr 22q12, encoding the DNA damage checkpoint kinase Chk2) increase microtubule assembly, promoting CIN.\textsuperscript{41} The mitotic checkpoint may thus provide a novel therapeutic target to improve overall survival and/or to modify response to chemotherapy.\textsuperscript{42}

It is increasingly acknowledged that CRC is a heterogeneous disease and there is ample evidence that tumors differ on a molecular level. Previously, two studies that used allelotyping PCR for a few
loci showed that lower allelic imbalance is associated with MC. In the current study, based on data from two independent cohorts we conclude that the distinct phenotype of MC is accompanied by a different genome-wide genetic profile when compared with AC, marked by a reduced rate of DNA CNAs overall, as well as less frequent gain of 20q and less frequent loss of 18p. Therefore, it may be possible that CIN plays a less prominent role in MC development. The differences in CNAs were found in a metastatic cohort (CAIRO/CAIRO2 cohort) as well as in a cohort with more early stage tumors (TCGA cohort), supporting the view that there is a genetic distinction between MC and AC that transcends the stage of presentation. Evidently, the consequences of chromosomal aberrations on gene expression levels eventually determine the functional phenotype. This study did not provide an insight into the specific pathway along which MCs developed, but was able to demonstrate that MC differed from AC on a molecular level. Since the DNA copy number effect was seen for large chromosomal regions and throughout the genome, the effects are probably due to genomic imbalance and alterations at multiple genes rather than specific genes. Further studies that can assess molecular differences between MC and AC with a higher resolution (e.g. next-generation sequencing) are therefore needed. Previously, it has been reported that mutation rates in the therapeutically important RAS/RAF/MAPK and PI3K/AKT pathways are significantly higher in MC than in AC. Moreover, MC is more frequently associated with MSI and the CpG island methylator (CIMP) phenotype. Although these features are not exclusive for MC patients, they do suggest that MC may develop through an alternative genetic instability pathway than CIN, which may explain the distinct tumor behavior and response to therapies. Due to low number it was not possible to compare differences in copy number changes between MSI MC and MSS MC patients in this study. It will be important to further investigate the molecular background of MC to increase knowledge on tumor behavior and to explore opportunities for targeting therapies. These data will enable clinicians to improve prediction of the course of disease and response to systemic treatment.

Acknowledgement

The European Community’s Framework Programme Seven (FP7) under contract no. 278981 “AngioPredict”. We would like to thank Oscar Krijgsman and Martijn Cordes from the Department of Pathology of the VU University Medical Centre for their insightful suggestions concerning the data analysis.
Chapter 7

References


Supplemental material

**Supplementary Table S1.** Clinicopathological characteristics of metastatic colorectal cancer patients who were included in the CAIRO/CAIRO2 cohort.

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Fisher’s exact test was applied.
AC, adenocarcinoma; MC, mucinous adenocarcinoma
## Supplementary Table S2. Clinicopathological characteristics of patients from the TCGA cohort.

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Fisher’s exact test was applied.
AC, adenocarcinoma; MC mucinous adenocarcinoma; Proximal colon, ascending colon from cecum up to the splenic flexure; Distal colon, descending colon, sigmoid and rectosigmoid junction
**Supplementary Table S3.** Multivariate analysis with 95% confidence interval (CI) on overall survival in mucinous adenocarcinoma patients from the CAIRO/CAIRO2 cohort.

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<td>CIN high</td>
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Supplementary Figure S1. Levels of chromosomal instability. The box plots show the number of calls per cohort that have been categorized as loss or deletion, neutral, or gain or amplification for the adenocarcinoma (AC) and mucinous adenocarcinoma (MC) samples. In the CAIRO/CAIRO2 cohort there were particularly differences in the overall frequencies of gains between AC and MC. In the TCGA cohort the overall frequencies of both gains and losses were lower in MC compared with AC.
Chapter 7

Supplementary Figure S2. Frequency plots of DNA copy number aberrations (CNAs) in chromosome 13 determined in mucinous adenocarcinoma (MC) and adenocarcinoma (AC) patients from the CAIRO/CAIRO2 and TCGA cohorts. Probes on the array are ordered along the x-axis by their genomic position and the y-axis represents the frequency as the percentage of tumors with the respective gains (above zero) or losses (below zero). The chromosomes represent ideograms with chromosomal bands. The corrected $P$ values obtained with statistical significance testing and correction for multiple testing are depicted in boxes below the plots. Black represents adjusted $p < 0.01$ and indicated a significant difference in DNA copy number between AC and MC; white represents adjusted $p > 0.01$ and no indication of a significant difference.
Part III

DISCUSSION AND FUTURE PERSPECTIVES
Chapter 8

ADVANCES IN THE CARE OF PATIENTS WITH MUCINOUS COLORECTAL CANCER


Nature Reviews Clinical Oncology, 2015; Epub ahead of print
Abstract

The majority of colorectal cancers (CRCs) are classified as adenocarcinoma not otherwise specified (AC). Mucinous carcinoma (MC) is a distinct form of CRC and is found in 10-15% of patients with CRC. MC differs from AC in terms of both clinical and histopathological characteristics and has long been associated with an inferior response to treatment compared with AC. The debate concerning the prognostic implications of MC in patients with CRC is ongoing and MC is still considered an unfavorable and unfamiliar subtype of the disease. Nevertheless, in the past few years epidemiological and clinical studies have shed a new light on the treatment and management of patients with MC. Use of a multidisciplinary approach, including input from surgeons, pathologists, oncologists and radiologists is beginning to lead to more tailored approaches to patient management, on an individualized basis. In this review, the authors provide insight into advances that have been made in care the of patients with MC. The prognostic implications for patients with colon or rectal MC are described separately; moreover, the predictive implications of MC regarding responses to commonly used therapies for CRC, such as chemotherapy, radiotherapy and chemoradiotherapy, and the potential for, and severity of metastasis are also described.
Introduction

Colorectal cancer (CRC) is a major health burden: an estimated 1.2 million people develop CRC worldwide every year and it is the fourth most-frequent cause of cancer-related mortality. CRCs are clinically divided into those of the colon and of the rectum, and these subgroups require distinctly different treatment regimens. CRCs can also be classified on the basis of findings from histological assessment of the tumor specimen. Adenocarcinoma not otherwise specified (AC) is the most-common form of CRC (observed in ~ 85% of patients with CRC), whereas 10-15% of patients with CRCs have mucinous adenocarcinoma (MC), which is characterized by abundant mucus secretion comprising at least 50% of the tumor volume. MC is more commonly found in patients with colon cancer than in those with rectal cancer (15% versus 9% of patients, respectively). The prognostic and predictive implications of MC, as well as the clinical implications for treatment and follow-up of patients with this form of CRC are currently subject to debate. Researchers are currently attempting to identify prognostic and predictive factors that might improve the individualized management of patients with MC. The prospect of personalized medicine, which enables targeting of specific tumor phenotypes in patients with CRC on the basis of tumor histology, has attracted both substantial and increasing interest from the oncology community. Histological classification of the tumor subtype is part of routine histopathological assessment and results of these analyses are used by clinicians to guide decision-making regarding multimodality treatment of patients with CRC. The infrequent occurrence of MC relative to AC renders results from randomized controlled trials using unselected populations of patients with CRC inappropriate, and limits the availability of convincing evidence regarding the prognostic or predictive relevance of this subtype. Thus, factors involved in development of MC are not well understood and results from small studies have been generalized to the entire population of these patients. Developments in this area in the past 10 years, however, have shed a new light on the MC subtype of CRC. This review aims to provide insight into the advances that have been made in care of patients with MC. Important details include the prognostic and predictive implications of MC regarding responses to commonly used therapies for CRC, and how responses to treatment differ from those of patients with AC. Other important issues are also discussed, including the potential to tailor management strategies to best suit the needs of individual patients, the value of early recognition of the MC subtype using MRI and the implications of the aberrant metastatic pattern of MC observed in most patients.

Pathology of mucinous adenocarcinoma

MC is distinguished as a subtype of CRC that is more frequently found in female patients and is predominantly, but not exclusively, located in the proximal colon. The aetiology of MC is not well understood. Observations from clinical studies have shown that MC is less common in patients in Asian countries, and higher rates of MC have been reported in Europe, North-America and Australia. These geographical variations in prevalence suggest that several factors, including lifestyle and dietary variations might have a role in the development of MC. Furthermore, MC is more commonly diagnosed in patients with inflammatory bowel disease (IBD), such as Crohn’s disease or ulcerative
colitis, and patients with a history of pelvic or abdominal radiotherapy are also more likely to be diagnosed with MC.\textsuperscript{8} The chronic inflammation observed in patients with both IBD and radiation enteritis is possibly responsible for the higher rates of MC observed in these patients, although exact mechanisms of development are unclear.\textsuperscript{8}

Despite the limited understanding of MC development, MC is recognized to constitute a distinct pathological entity within the spectrum of CRCs. By definition, MC is a subtype of CRC in which more than 50\% of the tumor consists of extracellular mucus. The designation of a tumor as mucinous is, therefore, arbitrary and is often dependent on the individual pathologist’s subjective assessment and level of experience. Histological tumor grading of patients with MC is also challenging. According to the WHO criteria,\textsuperscript{3} tumor grade should be assigned based on the extent of glandular formation. By convention MC is considered poorly differentiated (grade 3).\textsuperscript{3} Nevertheless, grade assignment is largely subjective, with no or few defined criteria, and this prognostic factor is considered inappropriate for classification of patients with MC.\textsuperscript{9} MC is thus considered to be a poorly differentiated tumor type, although no clinical or molecular arguments exist that might substantiate this dogma. The extent to which histopathological characteristics, such as growth pattern, tumor border aspect, location of mucus and tumor cell:mucus ratio, influence outcomes is currently unknown. Furthermore, the presence of a signet-ring cell component in patients with MC has been associated with poor outcome, but the exact clinical importance of this factor needs to be further investigated.\textsuperscript{10} Standardized histological assessment of a large series of patients with MCs might enable greater insight into the defining characteristics of MC. In addition, research on molecular variations might help to identify groups of patients that have more benign or malignant tumor behavior in terms of survival and/or response to therapies.

Compared with AC, MC is more commonly diagnosed at an advanced stage of disease.\textsuperscript{4, 6} A few possible explanations for this phenomenon exist. Firstly, MC has a tendency to be located in the proximal colon and MCs are of a less firm consistency than ACs, causing detectable symptoms to arise only when the disease reaches a more advanced stage.\textsuperscript{5, 6} In addition, MC has a different molecular signature to AC, which might cause faster disease progression.\textsuperscript{11} The exact molecular aberrations that are responsible for this pattern of disease progression, and the sequence of any alterations, are currently unknown. Various molecular aberrations have been described, although a common mucinous pathway has not yet been identified. For example, compared with AC, MCs more often have microsatellite instability (MSI), which is also observed in patients with Lynch syndrome, suggesting that MC might arise from an alternative oncogenic pathway.\textsuperscript{12} Whenever MCs are microsatellite stable, however, they are characterized by a markedly reduced rate of copy-number aberrations when compared with AC.\textsuperscript{13} \textit{BRAF} mutations are also commonly found in patients with MC and are associated with an infiltrative pattern of tumor growth.\textsuperscript{12, 14} The \textit{MUC2} gene, which encodes mucin-2 (MUC2), a protein that coats the epithelia of the intestines, airways and other mucous-membrane-containing organs is frequently overexpressed in patients with MC, although this molecular feature is not exclusive to this form of disease.\textsuperscript{15} By contrast, expression of MUC2 is generally decreased in patients with other forms of CRC.\textsuperscript{16} Overexpression of MUC2 might protect against antitumor immune effectors by forming a mucus layer, thus promoting tumor development.\textsuperscript{17} Interestingly, a study in which investigators
classified cellular phenotypes and compared responses to therapy among patients with different MC phenotypes showed that patients with high MUC2 expression had a better prognosis than those with other molecular subtypes. This finding suggests that mucus-producing tumors are not per se associated with a worse outcome. An increased rate of mutations is observed in the RAF/RAF/MAPK (BRAF and KRAS) and PI3K/AKT (PIK3CA) pathways in patients with MC.

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<td>Hazard ratio (95% CI)</td>
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<td>1.22 (1.11-1.34)*</td>
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Indicates relative survival; * Indicates overall survival; CI, confidence interval

### Prognosis of mucinous carcinoma

#### Early stage colonic mucinous carcinoma
In a study published in 2012, the authors reviewed the prognostic significance of MC, compared with AC in patients with CRC. A total of 34 publications reporting the outcomes of patients with MC and patients with AC were evaluated and survival was compared using a meta-analysis. An overall hazard ratio (HR) of 1.05 (95% confidence interval [CI] 1.02-1.08) in patients with MC versus those with AC was calculated after correction for tumor stage at presentation. The treatment approaches for patients with colon cancer versus those with rectal cancer differ substantially and patient survival is also related to the location and stage of the primary tumor, meaning that patients with CRC are by no means a homogeneous population. Thus, two independent population-based studies were conducted to analyze the effects of MC versus AC in patients with either colon or rectal cancer, using multivariable analyses to minimize the influence of confounding factors. Findings of both studies demonstrated that the MC histological phenotype, compared with AC histology, was not a negative prognostic factor in patients with colon cancer (Table 1). In fact, in another independent study in a cohort of patients with non-metastatic colon cancer, the investigators demonstrated better overall survival in patients with MC compared with those of patients with AC (HR 0.33, 95% CI 0.14-0.79) and fewer systemic recurrences. The results of this study, which were published in 2014, suggest that advances in patient care apparently resulted in improved outcomes for patients with colonic MC compared with those of patients with colonic AC.

#### Early stage rectal mucinous carcinoma
In contrast to colonic MC, rectal MC was associated with a poor prognosis, relative to rectal AC, in both aforementioned population-based studies (Table 1). This difference in survival was most prominent in patients with stage III rectal cancer. However, the approaches to diagnosis and treatment of rectal cancer have both changed substantially over the past 30 years and the survival of patients with rectal cancer has, as a result of these changes, improved over time. Modern treatment of rectal cancer has...
also benefited patients with rectal MC, resulting in equal overall survival outcomes of patients with MC and AC, which were reported in January 2015. This analysis of 5-year overall survival data from patients in the Netherlands Cancer Registry between 1989–2007 demonstrated that overall survival was initially worse for patients with MC, but was not significantly worse, statistically, than that of patients with AC from 1999 onwards.\(^\text{22}\) This improvement in outcomes coincides with the introduction of both total mesorectal excision (TME) and neo-adjuvant radiotherapy and chemoradiotherapy for the treatment of CRC in the Netherlands. Tumor size in patients with rectal cancer is a limiting factor for complete surgical removal. Patients with MC generally have tumors of a higher T-stage than those with ACs, and large rectal tumors are less likely to be removed radically. For patients with MCs this leads to a higher incidence of positive circumferential resection margins (CRM), which is considered a strong indicator of a negative prognosis (HR 1.7, 95% CI 1.3-2.3).\(^\text{23, 24}\) The introduction of adequate rectal cancer imaging and TME surgery has lowered the number of CRM positive resections and thereby improved the outcomes of patients with MC relative to those of patients with AC. Over the past 15 years, surgeons have become more experienced with TME surgery and because the quality of surgery is an important factor that influences the frequency of local recurrences, the improved prognosis of patients with MC is most likely a result of this development.\(^\text{25}\)

**Advanced disease**

When MC is diagnosed in the metastatic setting, the prognosis of the patients is generally worse than that of patients diagnosed with metastatic AC. Several studies, including two randomized trials investigating patients with metastatic CRC (mCRC), have demonstrated that the MC disease subtype, versus other types of CRC, is a poor prognostic factor: median overall survival rates varied between 8.0-14.0 months for patients with metastatic MC, compared with 17.9-23.4 months for those with metastatic AC (Figure 1).\(^\text{26-28}\) The controversies reported in the literature regarding the prognostic relevance of MC versus AC in patients with CRC are probably caused by the use of cohorts from different points in time, merging of data from patients with colon cancer and rectal cancer and inclusion of patients with advanced disease.

**Predictive relevance of mucinous carcinoma**

**Advanced-stage colonic MC**

Findings from clinical studies of systemic treatment of metastatic forms of MC provide the best available evidence on the predictive relevance of this disease versus AC regarding benefit from therapies. Treatment with chemotherapy can, in some patients, add to disease morbidity and, in patients who have severe toxicity, can even result in premature death. Thus, adverse effects and outcomes of chemotherapy should be carefully weighed against any expected benefits. As mentioned previously, several study reports indicated a poor outcome in patients with MC who were treated with palliative systemic therapy for advanced-stage disease.\(^\text{26-28}\) This poor outcome was independent of \textit{BRAF} mutational status, which is a strong negative prognostic indicator in patients with mCRC and is commonly found in patients with MC (Figure 1).\(^\text{26, 28}\) Moreover, the MC phenotype is an adverse prognostic factor in patients with CRC who underwent resection of liver metastases.\(^\text{30, 31}\) In these studies, patients with mucinous metastases had a poorer response to preoperative chemotherapy as well as inferior disease-free and overall survival.
Advances in the care of patients with mucinous colorectal cancer

Adjuvant treatment of colonic MC

Adjuvant chemotherapy is recommended for patients with high-risk or node positive colon cancer. Histological subtype is generally not taken into consideration in the decision-making process concerning use of adjuvant chemotherapy. In two studies, investigators analyzed the effects of adjuvant chemotherapy in patients with stage III disease, using multivariable analysis to correct for confounding factors; no difference in overall survival was found in patients with MC compared with patients with AC in either study (Figure 1).5, 32 The extent of heterogeneity in treatment responses among patients with MC, however, suggests that patients with CRC should not be considered one entity. Firstly, MSI is common in patients with MC, and this tumor characteristic has been associated with a better prognosis than having a microsatellite stable (MSS) tumor, but has also been related to less benefit from 5-fluorouracil (5-FU) chemotherapy.33 Within the subgroup of patients with MC, one hypothesis is that the presence of MSI might determine whether a patient has a good or bad response to chemotherapy.34, 35 Secondly, patients with MC more frequently have high levels of DNA topoisomerase 1 (TOP1) expression than those with AC; high TOP1 expression is also correlated with a poor response to 5-FU treatment in patients with mCRC.36 A search for markers that predict treatment responsiveness should, therefore, be

**Figure 1.** Forrest plot depicting outcomes from studies of palliative and adjuvant chemotherapy for patients with mucinous adenocarcinoma (MC) versus those with adenocarcinoma (AC). Differences between categorical outcomes were calculated by the author based on primary data reported in the relevant manuscript using the hazard ratio and corresponding 95% confidence interval (CI).
performed and subgroup analyses could be used to distinguish between patients who might, or not benefit from 5-FU treatment.

These theories, however, do not explain the differences in responses to chemotherapy in the adjuvant and/or palliative setting. The mechanisms behind these differences in response to chemotherapy are unclear, but the pathway that leads to development of advanced-stage disease in patients with MC might account for differences in outcomes in the palliative setting. 

Patients with MC more frequently have metastases at more than one site, and the distribution and the combination of metastases is often different to that of patients with AC. Patients with AC predominantly have metastases in the liver, whereas those with MC more frequently have extrahepatic metastases, such as distant lymph nodes metastases or metastases on the peritoneal surface. 

The presence of extrahepatic metastases, especially within the peritoneum, is associated with a very poor prognosis. Also, chances of successful delivery of chemotherapy might be altered in patients with CRC of the MC subtype. In these patients mucus, which surrounds the tumor cells, might function as a physical barrier to the delivery of chemotherapy. A difference in pharmacokinetics between the solid metastases observed in patients with advanced-stage disease, which are protected by a layer of extracellular mucus, and micrometastases without such a barrier, which are the key target of adjuvant therapy, might explain the differences in response. Furthermore, a poorly developed microvasculature owing to the large mucus volume surrounding the tumor might reduce delivery of chemotherapeutics in patients with larger solid metastases. 

Also, compressing forces (solid stress) exerted by the bulky mucinous tumor on the penetrating vascular system might reduce the extent of drug delivery to the tumor (Figure 2). 

In conclusion, MCs are not per se resistant to chemotherapeutic treatment compared with AC, but responses of patients with MC might vary owing to variations in stage of disease and molecular tumor characteristics.

(Neo)adjuvant treatment of rectal MC

The use of adjuvant chemotherapy in patients with rectal cancer is highly controversial and is not universally recommended. In one study, investigators analyzed the effects of adjuvant chemotherapy in patients with MC of the rectum following TME surgery. Improved overall survival was detected in patients with MC who received adjuvant chemotherapy, compared with those who did not (5-year overall survival 66.1% versus 32.5%, \( p < 0.0001 \)). This finding was irrespective of preoperative therapies, but the results might have been biased by selective patient inclusion.

Preoperative treatment strategies used to treat patients with rectal cancer vary between nations. In Europe, results of the Dutch TME and MRC CR07 trials resulted in the recommendation of short-term radiotherapy (5x5 Gy) for treatment of patients with early stage (T3) and/or node positive tumors to reduce the risk of local recurrence. Downstaging of the tumor using long-course chemoradiotherapy (CRT) is recommended for patients with a T4 or T3 tumor with threatened CRM (locally advanced rectal tumors). In North-America, short-term radiotherapy is not commonly used, and preoperative therapy mainly consists of CRT for locally advanced rectal tumors (Figure 3). The poor prognosis of patients with rectal MC gave rise to the idea that responses to preoperative therapies
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Figure 2. Differences between mucinous carcinoma (MC) and adenocarcinoma (AC) not otherwise specified (NOS). MCs have tumor characteristics that might explain the resistance to systemic therapy, especially in the setting of advanced disease, including microsatellite instability, less favorable intrinsic tumor characteristics and the formation on multiple metastases when compared with AC. The effects of differences in tumor micro-environment (mucus layer, vasculature, architecture) are currently hypothetical.

Figure 3. Schematic axial view of rectal tumors. The mesorectal fascia forms the outer lining of the mesorectum, which surrounds the rectal canal. A total mesorectal excision is the current surgical standard of care and involves resection of the tumor together with the fatty tissue surrounding the tumor. The circumferential resection margin is defined as the shortest distance from an affected region to the mesorectal fascia and is an important prognostic marker. Primary resection of a locally advanced rectal tumor has a high risk of incomplete resection (resulting in tumor positive circumferential resection margins). Thus, these tumors are generally treated with preoperative chemoradiotherapy in order to reduce tumor size.
Tumor downstaging is an important objective of CRT in patients with locally advanced rectal cancer. An inferior rate of tumor downsizing has been observed in patients with MC compared with those with AC following CRT (Figure 4). Measuring tumor response to CRT on MRI remains challenging in patients with MC, as differentiation between tumors and inactive mucus lakes is often difficult. Patients with rectal MC have a higher risk of having positive CRM irrespective of use of preoperative therapy than patients with AC. A pathological complete response (pCR), which means that no vital tumor cells are found after preoperative treatment, is seldom observed in patients with MC. This finding is suggestive of differences in susceptibility or inherent resistance to preoperative CRT in patients with MC and raises questions regarding the benefit of this treatment in these patients. This poor tumor response to CRT is not well understood, but it has been hypothesized that a reduced vascular density, with a decreased blood supply and corresponding hypoxic state, might reduce the effectiveness of CRT. Patients with MCs are also more likely to have a KRAS mutation than those with AC, which is considered a biomarker of an incomplete response to therapy in patients with rectal cancer. Patients with KRAS mutant rectal cancers are less likely to develop a pCR to 5-FU based CRT than those with wild-type KRAS rectal cancers. The higher rate of CRM positivity in patients with MC, however, does not unequivocally lead to a worse overall survival than that of patients with AC. This finding suggests that having positive CRM has different implications in patients with MC. These patients have tumors with a lower cellular density than those with ACs and mucus is usually found in the periphery of the tumor, which might lead to more tumor residues being present in patients with positive CRM. Previous studies have already demonstrated, however, that improved local control is not equivalent to prolonged survival and that development of distant metastases mainly determines survival. Further studies on the effects of CRT on tumor cell characteristics should be performed to analyze whether invasiveness is altered after CRT. Also, a more targeted approach towards treatment of patients with MC, possibly with increased use of preoperative CRT, or longer waiting intervals between neo-adjuvant treatment and surgery, with the aim of improving tumor downsizing, should be investigated.

Besides the establishment of preoperative therapies for treatment of patients with rectal cancer, both the introduction of TME surgery and better imaging techniques has contributed to an improvement in overall survival in patients with rectal cancer over the past decades. Introduction of TME surgery to routine clinical practice has resulted in a decrease in CRM positive resections and is, therefore, an appropriate procedure for obtaining better resections in patients with MC, who generally have larger tumors. Technological advances in imaging have improved preoperative staging and selection of the most-appropriate preoperative therapies. Improved early recognition of MCs offers clinicians an opportunity to attempt personalized treatments with greater awareness of the risks of incomplete removal of the tumor.
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Figure 4. Forrest plot depicting outcomes from studies on the frequency of downstaging in response to chemoradiotherapy in patients with mucinous carcinoma (MC) versus adenocarcinoma (AC). *Indicates author’s own risk ratio and 95% confidence interval (CI) calculations based upon primary data reported in the relevant manuscript.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>AC</th>
<th>MC</th>
<th>Weight</th>
<th>Risk ratio</th>
<th>M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grillo-Ruggieri</td>
<td>2007</td>
<td>66</td>
<td>81</td>
<td>13</td>
<td>22</td>
<td>47.6% 1.38 [0.96 - 1.98]</td>
</tr>
<tr>
<td>Shin</td>
<td>2011</td>
<td>176</td>
<td>324</td>
<td>6</td>
<td>21</td>
<td>26.2% 1.90 [0.96 - 3.77]</td>
</tr>
<tr>
<td>Yu</td>
<td>2014</td>
<td>111</td>
<td>270</td>
<td>14</td>
<td>60</td>
<td>32.6% 1.76 [1.09 - 2.85]</td>
</tr>
<tr>
<td>Qiu</td>
<td>2011</td>
<td>42</td>
<td>79</td>
<td>4</td>
<td>16</td>
<td>15.5% 2.13 [0.89 - 5.09]</td>
</tr>
<tr>
<td>Oberholzer</td>
<td>2012</td>
<td>37</td>
<td>67</td>
<td>1</td>
<td>21</td>
<td>10.6% 3.87 [1.33 - 11.27]</td>
</tr>
<tr>
<td>Sengul</td>
<td>2006</td>
<td>20</td>
<td>35</td>
<td>1</td>
<td>11</td>
<td>2.2% 6.29 [0.95 - 41.63]</td>
</tr>
</tbody>
</table>

Total: 482 AC, 907 MC, 42 events, 150 patients
Risk ratio: 1.87 [1.44 - 2.43]
Heterogeneity: Chi² = 4.25, df = 5 (P = 0.51); I² = 0%
Test of overall effect: Z = 4.72 (P < 0.0001)

Figure 5. Identification of mucinous adenocarcinoma (MC). (A) Sagittal and (B) axial T2-weighted MRI scan of a patient with MC, which can be easily recognized owing to the distinctive mucinous high signal intensity on high-resolution MRI images. (C,D) Haematoxylin and eosin stained tumor (magnification 1x). This figure emphasizes the need for careful mapping of tumor biopsy samples to preoperative imaging findings, in order to enable personalized medicine, based on initial biopsy. Arrows indicate the presence of mucinous regions of the tumor.
Chapter 8

Imaging

Radiologists can recognize a tumor as a MC during preoperative work-up, owing to the distinctive high signal intensity of these tumors on high-resolution T2-weighted MRI scans, which is caused by the presence of mucus (Figure 5). This finding implies that, before obtaining histopathological confirmation of MC (after resection of the tumor), information concerning tumor type is available and could potentially influence the choice of therapy. Analysis of biopsy samples from a suspect lesion usually provides the multidisciplinary team with information regarding the tumor subtype and, thus, enables a limited set of tests for tumor typing to be performed. However, in patients with MC the chance of obtaining a false-negative histological diagnosis based on findings of biopsy analysis (such as an MC tumor being identified as AC) is five times as high when compared with MRI assessment. Mucus commonly coats the tumor at the extraluminal side, however, tissue in a biopsy is usually taken from the more superficial aspect of the tumor and might not contain a representative amount of mucus.

Therefore, preoperative MRI is a more accurate method for detection of MC than biopsy sampling. Moreover, preoperative therapies, such as radiotherapy and CRT might alter tumor characteristics, thus hampering accurate histological assessments. For example, increased mucus production and mucus pools are observed in patients with tumors treated with short-term radiotherapy and these tumors might display a colloid response following CRT. Indeed, radiation seems to drive mucinous differentiation; therefore, these phenomena should be regarded as a treatment response and should not be mistaken for tumors of the mucinous phenotype. This radiation-induced response is of particular importance for future research initiatives, otherwise all tumors which appear mucinous might be considered to be MC. Imaging is not only a very useful addition to biopsy sample analysis in the preoperative setting, but might also be used to determine whether a tumor with a mucinous phenotype was induced or not. Thus, radiologists must document tumor phenotype at baseline and pathologists must be aware of the imaging findings when reviewing the histology of these patients.

Future directions

The histological subtype of CRC is an easily accessible factor that, in the near future, is likely to be important in tailoring treatment to the characteristics of the individual patient. Discussions aimed at raising awareness of MC, and how it differs from AC, in multidisciplinary team meetings might help to prevent incorrect clinical decision-making in managing patients with CRC of this subtype. Despite the clear definition of MC, a common terminology whereby all pathologists can describe MCs is currently lacking. More insight into histopathological characteristics of MCs, such as tumor differentiation, growth pattern, tumor border aspect, location of mucus and tumor cell:mucus ratio is required. From a research perspective, further investigations of the molecular background of MCs, in order to increase understanding of tumor behavior and resistance to therapies should be considered a priority. In vivo and in vitro studies that assess the role of the mucus layer in chemotherapy delivery might also improve insight into chemoresistance. Investigations in this area might assist in the
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development of a chemotherapy delivery system that overcomes the possible penetration difficulties associated with MC. The engineering of a coated nanoparticle drug-delivery vehicle that can cross the mucus barrier, therefore, offers great promise for improving the effectiveness of chemotherapeutic treatment in patients with advanced-stage disease. Agents that target the mucus layer itself, although not currently readily available, might also provide an additional therapeutic option that should be explored in an attempt to improve the effectiveness of systemic therapies. Furthermore, studies designed to assess differences between MC and AC at a high-resolution molecular level, using technologies such as next-generation sequencing, are needed. These data will enable clinicians to improve prediction of the course of disease and responses to systemic treatment, thereby aiding in selection of patients for additional treatment.

Figure 6. Potential contributions from different medical specialties. A multimodality approach has become the standard of care for management of patients with colorectal cancer and multidisciplinary team meetings should aim to raise awareness of the importance of histological subtypes where patients are managed using such an approach. AC, adenocarcinoma; MC, mucinous adenocarcinoma; CRM, circumferential resection margin.

Conclusions

A multimodality approach to management of patients with CRC has become the standard of care, and exchange of information between different disciplines has influenced patient management (Figure 6). Cancer therapy is increasingly being tailored to the individual patient, depending upon both patient and tumor characteristics. Tumor histology is one of the most easily accessible clinical features, although this information is not yet widely used in clinical practice. This lack of utilization most probably reflects conflicting results reported in the literature and uncertainty regarding exact clinical implications. Consensus on how to classify MCs accurately is also needed.

MCs can be diagnosed preoperatively and high-resolution MRI, being more accurate than analysis of initial biopsy samples, has an important role in this regard. Documenting MC using findings of
both imaging and analysis of pathological specimens is important and has direct clinical implications. Established mechanisms exist that might explain the relative resistance to chemotherapy and radiotherapy of patients with MC compared with those with AC. This resistance to treatment is probably caused by a combination of a different molecular signature and the markedly different physical properties of mucus containing tumors compared with ACs, which gives rise to unique patterns of spread, and substantially different patterns of vascularity and tumor cellularity. Despite a relatively poor prognosis, advances have been made in survival rates by careful en bloc removal of such tumors through improved standards of TME surgery. This removal strategy avoids intra-operative spillage and rupture of gelatinous MCs into the abdominal cavity. Awareness of the diagnosis of this subgroup, which has a poor prognosis, is important for surgical planning and follow-up surveillance of these patients. Future improvements in adjuvant and neo-adjuvant therapy might be achievable by using different approaches that take into account both the unique physical properties as well as molecular profiles of these tumors. Advances in tumor characterization will also have an important role in future, and will possibly enable further tailoring of treatment.

**Review criteria**

A search for relevant articles was performed using the PubMed database on February 1, 2015, using the following Boolean search term combination: (((“Colorectal Neoplasms”[Mesh]) OR (((cancer[tiab] OR carcinoma*[tiab] OR tumour[tiab] OR tumours[tiab] OR tumour*[tiab] OR adenocarcinoma*[tiab])) AND (rectum[tiab] OR rectal[tiab] OR colorectal[tiab] OR colon[tiab]))) AND (((“Adenocarcinoma, Mucinous”[Mesh]) OR (mucinous[tiab]) OR (colloid[tiab])))). A modified search was performed in the EMBASE database. Reference lists of selected articles were assessed for additional studies. Only studies that used the WHO definition of MC were selected. Inclusion or exclusion of a study was based on quality and relevance of the article.
References


Advances in the care of patients with mucinous colorectal cancer


Future perspectives
Future perspectives

In colorectal cancer (CRC) patient care personalized medicine is a promising concept. It is moving us to more precise medicine, which is rationally customized for the individual patient. Current treatment concepts, therefore no longer solely focus on stage of disease and the patient’s general health status, but also take specific tumor characteristics into account.

Recent studies, among those within this thesis, have demanded more appreciation for the histological subtype of CRC in multidisciplinary team meetings. However, the distinction between merely mucinous adenocarcinoma (MC), signet-ring cell carcinoma (SRCC), and adenocarcinoma not otherwise specified (AC) may no longer be sufficient. There is an urge for better prediction of prognosis and therapeutic planning for the individual patient, based on individual tumor characteristics. In this regard, very little is known about the impact of tumor features that are specifically seen in MC and SRCC. MC exhibits distinct molecular and epigenetic characteristics that can be associated with outcome and response to therapies. These characteristics may be relevant for development of future targeted therapies. Identification of tumor characteristics that are specific for MC or SRCC patients may improve prediction of prognosis and can be used to determine efficacy of (neo)adjuvant treatment regimens in these patients. Research initiatives that aim to improve knowledge in this regard will be performed in the near future and form an important extension of the studies from this thesis.

A common histopathological language

Although MC has a clear-cut definition, a common language to describe MCs is lacking. It appears that MC cannot be considered a single entity when visualized under the microscope. There is a variety of presentations regarding the growth pattern, wall composition and contents of the lumina of mucinous lakes in MC (Figure 1). MCs can present with either circumscribed or a diffuse infiltrative growth pattern. The wall composition varies from acellular, single layer or multi layer of cells to irregular. The contents of the mucinous lakes also shows diversity, ranging from single cells, clumps of cells to signet-ring cells. The frequency of these features has not been well described, nor has the prognostic impact been evaluated. Nonetheless, these features could present valuable tools in further classification of MCs. A future goal is to develop a system for MC that enables grading and classification of these tumors, which can be linked to outcome. Large-scale histological assessment of MC will generate more insight into the various pathological forms in which it can be found. In addition, the occurrence of other well-known histological and immunohistochemical features that are relevant for prognosis and selection of adjuvant treatment options, can be analyzed including lymphatic invasion, extramural vascular invasion, perineural growth, loss of mismatch repair proteins and mutations in \textit{BRAF} and \textit{KRAS}.

Analyzing aberrations of a higher resolution

Besides the interest in a more accurate histological assessment of MCs and SRCCs there is a growing trend towards molecular characterization of tumors. Our analysis on copy number data as presented in Chapter 7 in this thesis already showed that MC was associated with less chromosomal instability.
than AC. Analyzing DNA aberrations of a much higher resolution using next-generation sequencing will be the next step forward in determining the frequency of mutations in MC compared with AC. Using a panel of several hundred cancer related genes will enable us to determine the frequency of mutations in these genes in MC compared with AC. These findings may be linked to outcome and response to therapies.

**Figure 1.** Histopathological features in mucinous carcinoma. Modified and reproduced with permission.¹

**Optimizing current treatment strategies**

Prior to the broad implementation of targeting therapies, it remains necessary to optimize current treatment modalities. Findings from our studies have shown that MCs show a less impressive tumor down staging to neo-adjuvant chemoradiotherapy when compared with AC. It is common practice to evaluate the effect of neo-adjuvant chemoradiotherapy using repeat MRI prior to surgery. Especially in locally advanced rectal tumors the restaging MRI is used to evaluate the rate of downsizing and the involvement of the mesorectal fascia in the tumor process. Moreover, it aids in preoperative planning to determine the extent of surgery that is needed for cure. Since regression and downsizing of mucinous lakes in rectal MC patients is not expected, the restaging accuracy of repeat MRI is
Future perspectives

not clear for MC patients. It is an important future goal to validate the use of restaging MRI in MC patients.

Timing of surgery after neo-adjuvant chemoradiotherapy for rectal cancer is another controversy. Surgery is generally performed six to eight weeks following completion of neo-adjuvant chemoradiotherapy. During the waiting interval the tumor regresses, which improves chances of a radical resection of the tumor. Recent studies have suggested a time-related response of the tumor to chemoradiotherapy and a prolonged waiting interval may increase chances of a radical resection. It would be interesting to determine the optimal waiting time to surgery for rectal MCs.

![Graph showing survival for adenocarcinoma (AC), mucinous adenocarcinoma (MC) and signet-ring cell carcinoma (SRCC) patients from the autopsy study who developed metastatic disease during follow-up.](image)

**Figure 2.** Survival for adenocarcinoma (AC), mucinous adenocarcinoma (MC) and signet-ring cell carcinoma (SRCC) patients from the autopsy study who developed metastatic disease during follow-up.

The finding that the metastatic pattern of CRC is strongly influenced by the histological subtype may alter the attitude of multidisciplinary teams towards MC and SRCC patients during follow-up. We have shown major differences in metastatic patterns with a high rate of peritoneal metastases for MC patients, and especially for SRCC patients. Peritoneal metastases are considered a poor prognostic factor. Survival data that was extracted from the autopsy study in Chapter 6 shows that SRCC patients with metachronous metastases are associated with a very poor survival (Figure 2) compared with MC and AC patients. Although complete cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy (HIPEC) has shown promising results for patients with peritoneal metastases from colorectal origin, survival for SRCC patients remains poor. It has even been suggested that a surgeon should refrain from HIPEC in SRCC patients in the presence of relative other contraindications. Therefore, histological subtype should be taken into consideration during multidisciplinary team meetings in which follow-up strategies are discussed. From a research perspective, understanding the
Future perspectives

resistance to systemic therapies is another main challenge. Studies that further define the role of the mucus layer in delivery of chemotherapy may improve insight into chemoresistance. Development of a chemotherapy delivery system that overcomes the possible penetration difficulties associated with MC, especially in patients with advanced-stage disease, should therefore be explored. On the other hand, it is not inconceivable that a more aggressive approach to prevent peritoneal metastases in high-risk patients such as SRCC patients will be introduced. Results of the recently started COLOPEC trial, in which patients with a cT4 stage colon cancer are treated with resection of the primary tumor and HIPEC will provide further insight in this matter.
Future perspectives

References

Summary
Summary

Mucinous colorectal carcinoma

The rapid development of individualized therapy for cancer patients has led to an increased interest of the oncology community in tumor subtypes. Improved identification of subtypes may lead to a better prediction of outcome and response to targeted therapies. In this perspective, the impact of the mucinous subtype in colorectal cancer (CRC) has been a matter of debate. Mucinous adenocarcinoma (MC) is a CRC subtype in which extracellular mucus contributes at least 50% of the tumor volume. However, neglecting this subtype in randomized clinical trials and inaccurate CRC registries have posed problems in determining clinical impact. Moreover, despite the deviant phenotypic presentation, tumor development and the molecular background of MC are poorly understood. For the signet-ring cell carcinoma (SRCC), another distinct CRC subtype, a poor prognostic impact has been established previously. However, insight into potential adjuvant treatment options is lacking, as well as understanding of tumor biology.

In Chapter 1 we reviewed factors that are involved in MC development in an effort to elucidate different etiological aspects of MC. We demonstrate that there are worldwide differences in the prevalence of MC as low rates have been found in studies from Asian countries and higher rates have been reported in the Western world. Moreover, we show that MC is more commonly diagnosed in patients suffering from inflammatory bowel diseases such as Crohn’s disease or ulcerative colitis. Based on merged data from the literature we noticed that MC is found in approximately 23% of Crohn’s disease patients and in 21% of patients with ulcerative colitis that are diagnosed with CRC. MC is also more common in Lynch syndrome patients, with approximately 22-40% of Lynch syndrome-associated CRCs being MC. Finally, an increased rate of MC is observed in patients who develop CRC following radiotherapy. Our literature search revealed 180 cases of radiation-associated CRCs, of which 38% was MC. In rectal cancer patients even 52% of radiation-associated tumors were MC. These findings are suggestive of a different oncogenic development for MC in which there may be a predominant role for inflammation and may generate more insight into the etiological pathways leading to the development of MC.

In Chapter 2 we describe a review of the literature on the molecular background of MC. The distinct clinical presentation and pathological appearance of MC suggest a deviant development and molecular background for this subtype. MC is characterized by a high rate of MUC2 expression, which accounts for the mucinous phenotype. In the review we merged data from a large number of studies and demonstrate that mutations in the therapeutically important RAS/RAF/MAPK and PI3K/AKT pathways are significantly higher in MC compared with non-mucinous adenocarcinoma (AC). Furthermore, MC shows higher rates of microsatellite instability (MSI) and MC is more frequently of the CpG island methylator phenotype (CIMP). Although the majority of MCs arises in the large intestine, this subtype also develops in other organs, such as the stomach, pancreas, biliary tract, ovary, breast and lung. We compared findings from the colorectal MC with molecular characteristics of MCs from other organs. In these organs, MCs show alterations in the RAS/RAF/MAPK and PI3K/AKT pathways as well, but a common “mucinous pathway” cannot be identified. Interestingly, the genetic instability pathway of MSI, which is a predominant characteristic of mucinous CRC, cannot be
linked to MC in all other organs. Further identification of molecular aberrations may improve therapy development, but could also explain resistance of tumors to such therapies.

In Chapter 3 we used population-based data from the Eindhoven Cancer Registry on 27,251 unselected CRC patients who were diagnosed between 1990 and 2010 to study the prognostic impact of MC in CRC. Colorectal MC has been associated with an impaired prognosis compared with AC. It has been demonstrated that response to palliative chemotherapy is poor in metastatic disease, but benefit of adjuvant chemotherapeutic treatment had never been assessed in large patient groups. In this study, MC was found in 12% ($n = 3052$) of CRCs and MC had a distinct distribution of tumor location compared with AC. Only 24% of MCs was located in the rectum and 54% in the proximal colon, compared with 38% of ACs in the rectum and 31% in the proximal colon. Also, AC was more often classified as stage I disease than MC (20.5% versus 10.9%). Survival analysis demonstrated that MC was a poor prognostic factor for rectal cancer patients (hazard ratio 1.22, 95% confidence interval [CI] 1.11-1.34). However, there was no difference in overall survival between MC and AC when the tumor was located in the colon. Additionally, we studied the benefit of adjuvant chemotherapy for stage III colon cancer patients following radical resection between MC and AC patients. Multivariate regression analysis showed a similar survival after adjuvant chemotherapy for stage III MC and AC patients, supporting current adjuvant treatment recommendations for both subtypes.

The relatively rare signet-ring cell carcinoma (SRCC) is a poorly studied subtype of CRC, but has been associated with a poor survival compared with MC and AC. In Chapter 4 we performed a nationwide study on 196,757 CRC patients who were diagnosed between 1989 and 2010 and were registered in the Netherlands Cancer Registry (NCR). SRCC was found in 1.0% of CRC patients and SRCC was more commonly found at stage III or IV, compared with AC (75% versus 44%). SRCC was also more frequently found in the proximal colon than AC (58% versus 32%). We assessed prognostic impact of SRCC according to the primary site of the tumor and found that SRCC was a poor prognostic factor at both colonic and rectal localization. Five-year relative survival estimates demonstrated a poorer survival in both colon and rectal cancer patients in stage II, III and IV. Due to the aggressive behavior of SRCC we aimed to gain insight into potential adjuvant treatment options in an effort to enhance survival in these patients. Therefore, we analyzed benefit from adjuvant chemotherapy for stage III colon cancer patients for SRCC and AC. Although stage III colonic SRCC patients had a poorer relative survival compared with AC after receiving adjuvant chemotherapy, there was no significant interaction between SRCC and adjuvant chemotherapy (relative excess risk 1.10, 95% CI 0.81-1.51). This suggested a comparable benefit from adjuvant chemotherapy in AC and SRCC; therefore adjuvant treatment recommendations should be adhered to, regardless of histological subtype.

Treatment of rectal cancer patients has changed rapidly over the past 20 years. Introduction of TME surgery in combination with preoperative radiotherapy with or without the addition of chemotherapy has improved local control rates and overall survival. Nevertheless, MC is generally regarded an unfavorable prognostic subtype with a decreased response to preoperative therapies. In Chapter 5 we studied the benefit from modern rectal cancer treatment for MC. In this study we analyzed the prognostic impact of rectal MC over time, using data from the Netherlands Cancer Registry. We found
Summary

A worse 5-year overall survival for rectal MC patients compared with rectal AC patients from 1989-1998 (32% versus 40%), but demonstrated that survival was no longer different from 1999 onwards (54% versus 53%). Furthermore, we analyzed the benefit of preoperative therapies for rectal cancer patients, using two other cohorts. To study the benefit of preoperative short-term radiotherapy, we used patients from the prospective randomized multicenter TME trial (n = 1530), who were randomly assigned to either short-term radiotherapy followed by TME surgery or to TME surgery alone. A higher rate of resections with a tumor positive circumferential resection margin (CRM) was found in MC patients, but there was no difference in overall survival between both subtypes (10-year overall survival 49% versus 51%). Both MC and AC patients developed less local recurrences if they were treated with preoperative short-term radiotherapy. Efficacy of long-term chemoradiotherapy was analyzed in a cohort with patients who underwent preoperative chemoradiotherapy for locally advanced rectal cancer (LARC). It was found that MC patients demonstrated less tumor downsizing and no pathological complete responses occurred in MC patients. Moreover, MC patients more often had a positive CRM, but this did not result in a poorer survival. In conclusion, in the era of modern rectal cancer treatment, survival between MC and AC is no longer different. Most probably enhancements in the fields of imaging and quality of surgery have improved outcome. Given the higher rate of positive CRM in MC, achieving a radical resection should remain the main focus during treatment of rectal MC patients and warrants more attention for MC during preoperative multidisciplinary team meetings.

In Chapter 6 we systematically evaluated metastatic patterns of different histological subtypes in CRC patients and analyzed metastatic disease upon primary tumor localization. We conducted a nationwide retrospective review of pathological records of 5817 patients who were diagnosed with CRC and who finally underwent an autopsy between 1991 and 2010. Patients were selected from the Dutch pathology registry (PALGA). A total of 1675 patients had metastatic disease and this was more often found in MC and SRCC patients, than in AC patients (34% and 61% versus 28%). Moreover, MC and SRCC patients had metastases at multiple site more often compared with AC patients (59% and 71% versus 50%). Liver metastases were most frequent in both AC and MC patients (73% and 52%). More than half of all SRCC patients developed metastases on the peritoneal surface. Major differences between metastatic patterns were also found between subtypes. AC more frequently metastasized to the liver compared with MC and SRCC. The latter two were more frequently found on the peritoneal surface compared with AC. There was no difference in lung metastases between the subtypes. Metastatic patterns were also related to the primary tumor site, with a high rate of abdominal metastases in colon cancer patients, whereas rectal cancer patients more often had metastases at extra-abdominal sites. To substantiate the clinical relevance of our autopsy study, we compared metastatic patterns with data from the TME trial. These results confirmed our findings in rectal cancer patients from the autopsy study. Results from this study demonstrated that there are profound differences in metastatic patterns between histological subtypes and the localization of the primary tumor in CRC. This may account for differences in response to chemotherapy for metastatic disease. Findings from this study encourage to take these factors into account during preoperative examination and follow-up. Moreover, they indicate that these factors should be considered stratification factors in future research initiatives focusing on advanced disease.
Although the differences in tumor behavior and response to therapies are indicative of a distinct molecular background of MC, most large-scale genetic studies on CRC did not address differences between histological subtypes. In Chapter 7 we conducted an in-depth analysis of the genetic background of MC using high quality array comparative hybridization data from microsatellite stable patients who participated in the CAIRO and CAIRO2 studies. For validation purposes we used publicly available data from The Cancer Genome Atlas (TCGA). We compared copy number profiles of 527 patients among which were 57 MC patients. Genome-wide we found that MC showed a reduced amount of copy number aberrations (1.5 fold lower) compared with ACs. Especially less gain of chromosome 20q and loss of chromosome 18p was observed. Moreover, a high rate of chromosomal instability was a strong negative prognostic marker for survival in MC patients from the CAIRO cohorts (hazard ratio 15.60, 95% CI 3.24-75.05). These results indicated that the distinct clinicopathological entity of MC is also accompanied by a different genetic basis.

Progressive insight into MC and the clinical relevance of this subtype are reflected in Chapter 8, in which we have put the findings from this thesis in a broader (clinical) context. The multidisciplinary team approach has become standard of care for colorectal cancer patients and demands appreciation of histological subtypes from each involved medical specialty. The review describes how individual management could be tailored and summarizes the pitfalls when managing individuals with MC. Future improvements in adjuvant and neo-adjuvant therapy might be achievable by using different approaches that take factors into account such as tumor location, tumor stage and the unique physical properties as well as molecular profiles of these tumors. Advances in tumor characterization will have an important role in future, and may possibly enable further targeting of treatment.
Dutch summary
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**Mucineus colorectaal carcinoom**

Darmkanker is een van de meest voorkomende vormen van kanker en is verantwoordelijk voor een groot deel van de kanker gerelateerde sterfte wereldwijd. In Nederland worden jaarlijks meer dan 15.000 patiënten met darmkanker gediagnosticeerd en ontwikkelt ongeveer 5% van alle mensen een colorectaal carcinoom (CRC) gedurende zijn of haar leven. Omdat niet iedere soort kanker dezelfde is, is vanuit de medisch oncologische wereld een toenemende interesse in op maat gesneden therapie voor de individuele patiënt. Hierbij wordt rekening gehouden met patiëntfactoren, zoals leeftijd en geslacht, maar ook met kankerspecifieke factoren, zoals het stadium van de tumor en het histologische subtype. Onderzoek heeft aangetoond dat deze factoren in meer of mindere mate kunnen bijdragen aan het voorspellen van de overlevingskans en het effect dat te verwachten is op therapie. De voorspellende waarde van het histologische subtype hierin is echter controversieel. Het meest voorkomende subtype CRC is het reguliere adenocarcinoom (AC), dat in 85% van de gevallen voorkomt. In ongeveer 15% van de gevallen wordt een mucineus adenocarcinoom gediagnosticeerd (MC) en in 1% van de patiënten is er sprake van een zegelringcelcarcinoom (ZRCC). Het MC kenmerkt zich door een tumor die voor meer dan 50% uit slijm bestaat. Dit slijm bevindt zich buiten de tumorcellen. Bij ZRCC is er ook sprake van uitgebreide slijmvorming, maar dit bevindt zich in de tumorcel. Doordat MC en ZRCC minder vaak voorkomen dan AC, zijn deze subtypen beperkt bestudeerd. Er is weinig kennis over de ontwikkeling van deze subtypen en hun moleculaire achtergrond. Bovendien is de behandeling van CRC gebaseerd op uitkomsten van grote klinische studies die zich voornamelijk richtten op AC. In dit proefschrift wordt het MC nader gekarakteriseerd op basis van klinische, epidemiologische en moleculaire bevindingen.

**Hoofdstuk 1** gaat in op de etiologische achtergrond van MC en geeft een overzicht van de literatuur. Er is een wereldwijd verschil in de prevalentie van MC, waarbij MC minder vaak voorkomt in Azië dan in de Westerse wereld. MC blijkt vaker voor te komen bij patiënten die darmkanker ontwikkelen op basis van inflammatoire darmziekten zoals Morbus Crohn en colitis ulcerosa. Data uit verschillende studies laten zien dat ongeveer 23% van de Morbus Crohn patiënten en 21% van de colitis ulcerosa patiënten die darmkanker ontwikkelden een tumor van het mucineuze type hebben. MC wordt bovendien vaker gevonden in patiënten met het erfelijke Lynch syndroom. In 22-40% van de patiënten met een Lynch syndroom geassocieerde tumor blijkt het om een MC te gaan. Behandeling met radiotherapie lijkt ook van invloed te zijn op de ontwikkeling van het mucineuze subtype. In de literatuur werden 180 gevallen gevonden van darmkanker na bestraling, waarvan het in 38% een MC betrof. Dit percentage lag zelfs nog hoger bij de rectumcarcinomen die na radiotherapie ontstonden (52%). Het herkennen van omstandigheden waaronder MC zich ontwikkelt, zoals ontsteking, biedt meer inzicht in het ontstaan van MC en in factoren die een rol kunnen spelen bij de ontwikkeling hiervan.

Het bepalen van moleculaire kenmerken van tumoren is een recente ontwikkeling binnen de oncologie die leidt tot een verbetering van diagnostiek en therapie. Moleculaire verschillen tussen tumoren kunnen leiden tot een variatie in tumoredrag en kunnen de werkzaamheid van een behandeling verminderen. Een inleiding in de moleculaire achtergrond van MC wordt gegeven aan de hand van een overzicht van de literatuur in **Hoofdstuk 2**. De afwijkende klinische en pathologische presentatie van MC doet vermoeden dat er ook op moleculair niveau verschillen zijn tussen MC en AC. Overexpressie
van MUC2 is een kenmerkende moleculaire eigenschap van MC en verklaart het slijmvormende aspect van deze tumoren. Ten aanzien van MC is er verder echter beperkt inzicht in de mutatiefrequentie van therapeutisch relevante moleculaire kenmerken zoals de RAS/RAF/MAPK en PI3K/AKT cascades. Door het combineren van gegevens uit diverse studies die zich richtten op deze cascades blijkt dat BRAF, KRAS en PIK3CA mutaties vaker voorkomen in MC dan in AC. Bovendien komt bij MC vaker microsatellietinstabiliteit en het CpG island methylator phenotype (CIMP) voor. Ook buiten de darm worden tumoren van het mucineuze subtype gevonden, zoals in de maag, pancreas, galwegen, ovarium, borst en long. De betrokkenheid van mutaties in de RAS/RAF/MAPK en PI3K/AKT cascades in deze tumoren wordt verder uiteengezet in dit hoofdstuk en een vergelijking met het colorectale MC wordt gemaakt. Hoewel de informatie in de literatuur beperkt is, zijn ook voor de andere mucineuze tumoren afwijkingen beschreven in de RAS/RAF/MAPK en PI3K/AKT cascades. Er kan echter geen eenduidige “mucineuze cascade” worden gevonden die in MCs van alle organen aanwezig is.

Het MC wordt beschouwd als een prognostisch ongunstige tumor, maar wordt op eenzelfde manier behandeld als het AC. In Hoofdstuk 3 beschrijven we de prognostische waarde van MC aan de hand van gegevens van het Integraal Kankercentrum Zuid. Voor deze studie werden 27.251 patiënten geselecteerd die tussen 1990 en 2010 waren gediagnosticeerd met CRC. In 12% (n = 3052) van de patiënten was er sprake van een MC. Er waren verschillen tussen MC en AC patiënten op een aantal punten. MC werd vaker in het proximale deel van het colon gevonden en minder vaak in het rectum dan AC. Daarnaast werd MC vaker in een hoger stadium gediagnosticeerd dan AC. Bij overlevingsanalyses bleek dat de overleving van MC afhankelijk was van de locatie van de tumor. Zo werd er geen verschil in overleving gevonden tussen MC en AC wanneer het een coloncarcinoom betrof, terwijl de overleving van MC patiënten slechter was wanneer ze waren gediagnosticeerd met een rectumcarcinoom (hazard ratio 1,22; 95% betrouwbaarheidsinterval 1,11-1,34). Verder werd in deze studie de overleving na adjuvante chemotherapie onderzocht voor coloncarcinoom patiënten die een resectie van de primaire tumor hadden ondergaan. De uitkomsten van een multivariate analyse bij stadium III coloncarcinoom patiënten lieten zien dat er geen verschil in overleving was tussen MC en AC patiënten die aanvullend met chemotherapie waren behandeld. Op basis van de resultaten van deze studie kan behandeling met adjuvante chemotherapie worden aanbevolen voor zowel MC als AC stadium III coloncarcinoom patiënten.

Het ZRCC wordt over het algemeen gezien als een agressief subtype met een slechte prognose. Het is een zeldzame tumorsoort en er is niet veel bekend over de effectiviteit van adjuvante behandelingen voor patiënten met een ZRCC. In Hoofdstuk 4 beschrijven we de resultaten van een landelijke studie met gegevens van 196.757 CRC patiënten die werden gediagnosticeerd tussen 1989 en 2010. In deze studie had 1% van de patiënten een ZRCC. In vergelijking met AC werd ZRCC vaker geclassificeerd als een stadium III of IV tumor. Ook was er tussen de tumortypen een verschil in verdeling over het colorectum. Bij ZRCC patiënten bevond 58% van tumoren zich in het proximale colon, terwijl dit slechts het geval was bij 32% van de AC patiënten. Ongeacht of de tumor zich in het colon of rectum bevond, hadden ZRCC patiënten een slechtere overleving dan AC patiënten. Vanwege de sombere prognose van het ZRCC is het belangrijk om inzicht te krijgen in de effectiviteit van aanvullende behandelingen, om de prognose van deze patiënten te kunnen verbeteren. Ondanks de slechtere overleving van ZRCC patiënten werd er geen verschil gevonden in overlevingswinst tussen AC en
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ZRCC na adjuvante chemotherapie voor stadium III coloncarcinoom. Deze uitkomst geeft aan dat er een vergelijkbaar voordeel is van adjuvante chemotherapie voor ZRCC en AC patiënten en patiënten met een ZRCC geen aanvullende behandeling mag worden onthouden op basis van het subtype.

De behandeling van rectumcarcinoom patiënten is de afgelopen twintig jaar sterk verbeterd. Zowel de introductie van TME chirurgie, waarbij de tumor in het rectum wordt verwijderd samen met het omliggende vetweefsel, de nauwkeurige beeldvorming met MRI, als de behandeling met neoadjuvante therapieën hebben geleid tot een verbetering van locale controle en een betere overleving. In Nederland worden rectumcarcinoom patiënten, afhankelijk van het stadium van de tumor, voorafgaand aan de operatie behandeld met radiotherapie of chemoradiotherapie. Zoals beschreven in hoofdstuk 3 wordt het rectale MC gezien als een prognostisch ongunstige tumor. In Hoofdstuk 5 beoordelden we de effectiviteit van de moderne behandeling van het mucineuze rectumcarcinoom. De prognose van het rectale MC werd in verschillende tijdsperioden geanalyseerd. Overeenkomstig de literatuur bleek op basis van data van het Integraal Kankercentrum Nederland dat de 5-jaarsoverleving in de periode van 1989 tot 1998 van MC patiënten slechter was dan van AC patiënten (32% versus 40%). Echter was deze overleving niet langer verschillend vanaf 1999 (54% versus 53%), wat verklaard kan worden door verbetering van de behandeling van rectumcarcinoom patiënten op diverse gebieden, zoals betere chirurgie en betere beeldvorming. De effectiviteit van kortdurende neoadjuvante radiotherapie voor rectumcarcinoom werd onderzocht aan de hand van data uit de TME trial. Patiënten in deze multicenter trial ondergingen TME chirurgie en werden gerandomiseerd tussen het wel of niet ontvangen van kortdurende neoadjuvante radiotherapie. Patiënten met een MC hadden vaker een positieve circumferentiële resectiemarge (CRM) dan AC patiënten. Hierdoor ontwikkelden MC patiënten vaker een lokaal recidief. Er was echter in beide groepen sprake van een afname van de lokaal recidiefkans na preoperatieve radiotherapie en er was geen verschil in overleving tussen MC en AC patiënten. Het effect van neoadjuvante chemoradiotherapie werd onderzocht met behulp van een cohort van 540 patiënten met een lokaal uitgebreid rectumcarcinoom (locally advanced rectumcarcinoom). Vergeleken met AC patiënten reageerden MC patiënten minder sterk op de chemoradiotherapie. De volumereductie van de tumor was minder en een pathologisch complete respons, waarbij geen vitale tumorcellen meer werden aangetroffen in het resectiepreparaat kwam niet voor bij MC. Bovendien hadden MC patiënten vaker een incomplete resectie. Omdat dit niet leidde tot een slechtere overleving, is het mogelijk dat een positieve CRM na chemoradiotherapie bij MC patiënten een andere betekenis heeft dan bij AC patiënten. Concluderend heeft de moderne behandeling van het rectumcarcinoom, waarin de beeldvorming en kwaliteit van chirurgie zijn verbeterd, geleid tot een sterke verbetering van de prognose van het rectale MC. Voor zowel MC als AC zijn preoperatieve therapiën effectief. Echter dient gezien het vaker voorkomen van een incomplete resectie bij MC patiënten er onverminderd aandacht te zijn voor de preoperatieve beoordeling van het rectumcarcinoom en de herkenning van een MC. Obductiestudies worden nog zelden verricht, hoewel ze een belangrijke bron van informatie over het eindstadium van ziekte kunnen vormen en hierdoor bij uitstek geschikt zijn voor het bestuderen van metastasering van tumoren. Hoofdstuk 6 beschrijft de uitkomsten van een obductiestudie waarin de metastaseringspatronen van verschillende histologische subtypen systematisch zijn geanalyseerd. In deze studie werden na een landelijke zoekvraag in de PALGA registratie de pathologievragen beoordeeld van 5.817 CRC patiënten die tussen 1991 en 2010 werden geobduceerd. Er waren 1.675 patiënten met gemetastaseerde ziekte. Metastasen kwam vaker voor bij MC of ZRCC patiënten dan bij AC patiënten.
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(34% en 61% versus 28%). Bovendien was er bij MC en ZRCC vaker sprake van multipele metastasen dan bij AC (59% en 71% versus 50%). De meest voorkomende locatie voor metastasering van MC en AC was de lever (52% en 73%), maar MC metastaseerde ook vaak naar het peritoneum. Bij het ZRCC was het peritoneum zelfs de meest voorkomende locatie van metastasering. Naast het histologisch subtype bleek ook de primaire locatie van de tumor van belang voor het metastaseringspatroon. Het coloncarcinoom had in vergelijking met het rectumcarcinoom vaker intra-abdominale metastasen, zoals het peritoneum. Het rectumcarcinoom daarentegen metastaseerde vaker naar extra-abdominale locaties, zoals de longen en hersenen. Ter validatie werden gegevens over metastasering gebruikt uit de TME trial. Hiermee werd de klinische relevantie van de obductiestudie bevestigd. De bevindingen uit deze studie laten zien dat zowel het histologische subtype als de tumorlocatie dienen te worden meegenomen in de preoperatieve screening voor gemetastaseerde ziekte en ondersteunen het invoeren van deze factoren als stratificatiefactoren voor toekomstige onderzoeken die zich richten op gemetastaseerd CRC.

Omdat het MC verschilt van het AC in zowel uiterlijke verschijning als in klinisch gedrag is het aannemelijk dat er ook in het genetische profiel variaties zijn tussen beide typen. In Hoofdstuk 7 analyseren we de genetische achtergrond van het MC aan de hand van array comparative genomic hybridization (aCGH). De datasets met gegevens over het aantal kopieën van een bepaald chromosomaal gebied (copy number profielen) waren afkomstig van twee klinische trials (CAIRO en CAIRO2) die verschillende systeemtherapieën voor patiënten met gemetastaseerd CRC onderzochten. Daarnaast werd gebruikgemaakt van de data van The Cancer Genome Atlas (TCGA) voor validatie. Data van 527 patiënten (470 AC en 57 MC) werd geanalyseerd. Er werden profielen gemaakt, op basis waarvan het genoom van AC en MC tumoren werd vergeleken. Het was opvallend dat MC minder chromosomale instabiliteit liet zien dan AC. Dit was significant voor chromosoom 20q (minder toename) en chromosoom 18p (minder verlies). Tevens werd in deze studie de overleving voor MC patiënten geanalyseerd op basis van de chromosomale instabiliteit. Hierbij bleek dat MC patiënten die meer chromosomale instabiliteit lieten zien een slechtere prognose hadden dan patiënten met een minder instabiel profiel (hazard ratio 15.60, 95% betrouwbaarheidsinterval 3.24-75.05). Voor de laatste groep was de overleving vergelijkbaar met die van AC patiënten. De resultaten van deze studie laten zien dat MC niet alleen een tumor subtype is dat een afwijkende klinische presentatie heeft, maar dat ook de genetische basis van deze tumoren anders is dan van AC. Voor de ontwikkeling van toekomstige “targeted” therapieën is dat een bevinding die van belang zou kunnen zijn.

Ten aanzien van het mucineuze subtype is er sprake van een voortschrijdend inzicht in de moleculaire achtergrond en de klinische relevantie. Enkele studies uit dit proefschrift hebben hieraan bijgedragen. In Hoofdstuk 8 wordt dit in de context van de huidige literatuur besproken. Doordat de zorg voor CRC patiënten een multidisciplinair karakter heeft, is het voor alle disciplines die betrokken zijn bij deze keten essentieel om de relevantie van het histologische subtype op waarde te kunnen schatten voor prognostische en therapeutische doeleinden. In de review wordt ingegaan op de verschillen tussen MC en AC vanuit een klinisch perspectief en worden handvatten geboden voor een op maat gesneden therapie. Verdere verbetering van adjuvante en neoadjuvante therapie zou verkregen kunnen worden door zowel de unieke fysieke eigenschappen als het moleculaire profiel te betrekken in de keuze voor therapie. Grootschalige analyses die gericht zijn op tumorkarakterisering kunnen op deze manier bijdragen aan een potentieel vergrote doelmatigheid van therapie.
Curriculum vitae
Niek Hugen was born on March 25, 1989, in Doetinchem, the Netherlands. After graduating from St.-Ludgercollege in Doetinchem (Gymnasium), he started medical school in 2006 at Radboud University Nijmegen. In his fourth year of medical school he went to the United States for eight months to perform a research project in the Cancer Genomics Core Lab of Prof. W. Zhang at The University of Texas MD Anderson Cancer Center in Houston, Texas.

Upon returning to the Netherlands, Niek started a research project during his clinical rotations in the Department of Surgery (Prof. dr. J.H.W. de Wilt) and the Department of Pathology (Prof. dr. I.D. Nagtegaal) in 2012. His research initiative was later turned into a PhD research project resulting in the present doctoral thesis. His senior and elective internships were performed at Radboud university medical center in the Department of Surgical Oncology and at Steve Biko Academic Hospital in Pretoria, South-Africa in the Department of Trauma Surgery. Niek obtained his medical degree cum laude in December 2012. Subsequently, he devoted a year to full-time research. Results of the studies performed during his PhD research project were presented at national and international conferences.

In March 2014, Niek started working in clinical practice in the Department of Surgery at Jeroen Bosch Hospital in 's-Hertogenbosch. Later that year he and Prof. dr. I.D. Nagtegaal received an MLDS Focus project grant from the Dutch Maag Lever Darm Stichting for further research on mucinous carcinoma. In January 2015, Niek started his surgical training at Rijnstate Hospital in Arnhem under the supervision of Dr. M.M.P.J. Reijnen.
Publications
List of publications


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