Chemotherapeutic drug delivery by tumoral extracellular matrix targeting

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Abstract

Systemic chemotherapy is a primary strategy in the treatment of cancer, but comes with a number of limitations such as toxicity and unfavorable biodistribution. To overcome these issues, numerous targeting systems for specific delivery of chemotherapeutics to tumor cells have been designed and evaluated. Such strategies generally address subsets of tumor cells, still allowing the progressive growth of tumor cells not expressing the target. Moreover, tumor stem cells and tumor supportive cells, such as cancer associated fibroblasts and cancer associated macrophages, are left unaffected by this approach. In this review, we discuss an alternative targeting strategy aimed at delivery of anti-tumor drugs to the tumoral extracellular matrix with the potential to eliminate all cell types. The extracellular matrix of tumors is vastly different from that of healthy tissue and offers hooks for targeted drug delivery. It is concluded that matrix targeting is promising, but that clinical studies are required to evaluate translation.

1. Introduction

Targeted drug delivery of chemotherapeutics is an increasingly important area in the field of cancer treatment research. Although conventional chemotherapy remains one of the most important treatment modalities, significant side effects may be induced that can result in premature cessation of chemotherapy [1–3]. Moreover, chemotherapeutics have an unfavorable biodistribution and are generally rapidly removed from the body. Advanced drug delivery systems may overcome these hurdles. By entrapment chemotherapeutics in a drug delivery system, exposure to healthy cells may be decreased, which, together with an increased concentration of chemotherapeutics specifically at the tumor site, can result in enhanced treatment efficacy with reduced side effects [4]. However, to achieve this, drug delivery systems should be designed to deliver chemotherapeutics to tumors only and not to surrounding healthy tissue. Several approaches, including passive targeting and ligand mediated targeting, are currently being evaluated to achieve local delivery to tumor cells. While the majority of the field is focusing on targeting the tumor cells itself, we here discuss an alternative approach i.e. targeting the tumor’s extracellular matrix (ECM). This strategy may result in a higher treatment efficacy by affecting not only tumor cells, but also tumor supportive cells. Tumor supportive cells are considered to have major roles in supporting tumor growth. The cancer associated fibroblast (CAF), for instance, is a tumor-distinctive cell type responsible for excretion of proliferating, pro-angiogenic, and anti-immunogenic factors, creating an ideal environment for tumor growth and subsequent metastasis [5]. Further, the cancer associated macrophage shares many of the tumor supportive characteristics of CAFs. Once derived from monocytes to its specific subtype and located in the ECM of tumors the cancer associated macrophage is thought to produce and secrete tumor enhancing factors [6,7]. Endothelial cells are another cell type considered as key players in tumor growth. By facilitating the supply of nutrients (e.g. oxygen, glucose, etc.) through the generation and support of novel blood vessels, tumors continue to proliferate [8]. Finally, the tumor stem cell is a major player in tumor progression. Tumor stem cells are considered responsible for self-renewal of tumor cells thereby driving tumor growth [9,10]. A strategy that simultaneously affects tumor cells and tumor supportive cells may be beneficial in improving treatment efficacies. We will present an overview of the possibilities and limitations of strategies to deliver chemotherapeutics to tumor cells in the extracellular matrix.

2. Conventional tumor targeting strategies

Passive targeting is one of the main strategies to guide drug delivery systems to cancer cells making use of the enhanced permeability and retention effect (EPR) [11]. This phenomenon is based on newly formed leaky vessels in tumor areas (permeability) with decreased lymphatic drainage resulting in an increased retention [12]. As a result, accumulation of drug delivery systems at the tumor site may occur. Despite extensive evaluation over the last 30 years and initial promising
preclinical results of passively targeted chemotherapeutic drug delivery through the EPR effect, serious questions have been raised about the existence and clinical application of the EPR effect in humans [13,14]. The opposite of passive targeting, active targeting, is therefore under growing attention. Active targeting is based on the concept that drug delivery systems can actively bind and subsequently internalize into tumor cells using tumor cell specific antibodies or ligands [11,15]. The ideal target is highly overexpressed on tumor cells, and absent or expressed to a limited extent on healthy cells. Examples are membrane bound receptors such as the human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor-1 (EGFR), transferrin receptor and folate receptor-α. However, there are several limitations associated with targeted delivery to tumors that need to be resolved in order to further improve the treatment outcome.

3. Limitations of current targeting strategies

Conventional drug delivery systems that target tumor cells through binding membrane bound molecules have several pitfalls. First, tumors show a high intratumoral heterogeneity resulting in heterogeneous expression of targets for drug delivery [16]. This may implicate that in practice targeted therapy may result in removal of only the subset of tumor cells expressing the target, while tumor cells lacking the target are left unaffected. As a result, these cells may proceed proliferating and finally result in a tumor lacking expression of the initial target (Fig. 1).

A second aspect of the current tumor targeting is the disregard of tumor supportive cells being present in the tumoral extracellular matrix (ECM). Tumor supportive cells are responsible for important cues for tumor proliferation and involved in the maintenance of a tumor supportive environment. Drug delivery strategies aimed at a specific tumor cell can leave other tumor cells and, perhaps even more importantly, tumor stem cells, tumor supportive cells and their supportive environment intact and may therefore not be sufficient to eradicate the whole tumor and prevent relapse.

Thirdly, delivery of entrapped drugs to their location of action by a targeted drug delivery system has proven more complex than initially anticipated. The majority of the current targeting drug delivery systems are designed to deliver their payload to their site of action (e.g. the nucleus). For most chemotherapeutics, this implicates that once a drug delivery system is bound to its target (e.g. membrane receptor) rapid internalization should occur. Thereafter, the drug should be released and subsequently move to its site of action, and not diffuse back into circulation. Although targeting the tumor cell membrane with subsequent internalization can be accomplished using antibodies or ligands, the steps to deliver chemotherapeutics to its site of action are more complicated. Once internalized into lysosomal compartments in the cytoplasm, the drug should be released from its carrier. A wide range of drug delivery systems struggle to release their payload after internalization because of failure to escape from lysosomal compartments in which they end up after internalization [17,18]. For example, the majority of injected PEGylated liposomal doxorubicin, a clinically approved passive targeting drug delivery system for doxorubicin, is found to be entrapped in lysosomes after internalization. In vivo experiments have shown that as a consequence of this lysosomal entrapment, less than 1% of the administered doxorubicin from liposomes reaches the nucleus, its actual target [19]. Lysosomal sequestering of drug delivery systems following internalization can thus prevent chemotherapeutics from reaching their site of action.

Overall, the majority of the tumor targeting drug delivery strategies for chemotherapeutic delivery focus on targeting tumor cells and may result in eradication of only a specific subset of tumor cells. Importantly, tumor supportive cells and tumor stem cells are left unaffected. Impaired release of chemotherapeutics and lysosomal entrapment may further limit the treatment efficacy. Therefore, an alternative targeting strategy that tackles these issues is desired.

4. Alternative tumor targeting strategy

Targeting chemotherapeutics to the extracellular matrix (ECM) of tumors may be a promising alternative strategy that can offer advantages over conventional targeting. The strategy is not aimed to target membrane bound receptors on specific tumor cells, but is aimed to target the unique tumoral ECM. Upon binding, depots of chemotherapeutic carriers in the tumor ECM are formed. Finally, when chemotherapeutics are released in the ECM they are able to diffuse to and affect all surrounding tumor cells including the heterogeneous tumor cell subsets, tumor supportive cells and tumor stem cells (Fig. 2). Here we will discuss this emerging field and define a number of conditions necessary to use drug delivery systems or antibody-drug conjugates as local extracellular chemotherapeutic depots.

4.1. The unique tumoral extracellular matrix

The normal ECM has many important functions including support

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**Fig. 1.** Limitations of the conventional tumor targeting strategy. By addressing a specific tumor marker, tumor cells lacking the marker may survive and continue to grow progressively.
and strength for tissues. It consists of various components such as (glyco)proteins (e.g. tenascin, collagen, elastin, laminin, fibronectin, and proteoglycans) and glycosaminoglycans (e.g. heparan sulfate and chondroitin sulfate) [21,22]. The tumoral ECM is considered distinct from normal tissue ECM in various aspects. For instance, several types of collagen are abundantly deposited during tumor formation and chondroitin sulfate and heparan sulfate are also more abundantly present in the ECM of tumors, both having the capacity to bind tumor promoting growth factors. Moreover, ECM remodeling enzymes are overexpressed in tumors [23]. It is believed that these factors contribute to tumor progression and invasion. Consequently, the deregulated tumoral ECM may also provide targeting possibilities as the tumor ECM may be enriched in certain molecules that are almost absent in normal ECM.

4.2. Targets in the tumoral extracellular matrix

As mentioned, the tumoral ECM is distinct from the normal ECM and may offer targeting possibilities. To ensure specific tumor delivery, the target should be expressed specifically in the ECM of tumors. Several ECM targets that may be used for drug delivery have been described as suitable. An overview of tumor ECM targeting strategies is presented in Table 1, and some examples will be discussed here.

Tenascin-C is a large glycoprotein of about 300 kDa which is highly expressed in the ECM of several tumors including breast, colon, lung, and ovarian tumors. It supports several aspects of tumor growth, such as tumor proliferation, angiogenesis and metastasis [24]. Moreover, its expression in normal ECM is almost absent [24], making it suitable for ECM targeting. Dal Corso et al. used a non-internalizing antibody directed against tenascin-C to deliver a chemotherapeutic compound (the anthracycline PNU159682) to the ECM of tumors [25]. Upon intravenous injection, the antibody-drug conjugate bound to tenascin-C (Fig. 3) and the drug was released through cleavage of the protease-sensitive linker between the drug and antibody. Significant tumor growth inhibition was observed in epidermoid carcinoma mouse xenografts. Chen et al. developed a strategy targeting tenascin-C using liposomes functionalized with a tenascin-C binding peptide and loaded with navitoclax, a small molecule inducing apoptosis primarily in CAFs. These liposomes modulated the ECM of tumors through efficient removal of CAFs, making the ECM more accessible for subsequently administered doxorubicin loaded nanoparticles [26,27]. Because only CAFs were affected by the initial ECM targeting strategy, they still had to apply a subsequent tumor cell specific targeting method. Using nanoparticles containing doxorubicin and targeting the human transferrin receptor, significant tumor growth inhibition was observed in liver tumor-bearing mice. Kang et al. targeted both, tumor cells using neuropilin-1 and tumor ECM tenascin-C with nanoparticles for glioma therapy. When loaded with paclitaxel, they tripled the median survival of intracranial glioma tumor bearing mice [28]. Lin et al. evaluated another tenascin-C targeting strategy. Doxorubicin loaded liposomes functionalized with sulfatide, a tenascin-C binding glycosphingolipid, were evaluated in mice bearing subcutaneous colorectal tumors and subcutaneous glioma tumors [29–32]. Although prolonged survival and decreased side effects were observed, the strategy was still dependent on endocytic cellular uptake of liposomes by glioma cells, which may limit the full potential of this strategy due to lysosomal entrapment of the liposomes. Another tenascin-C targeting approach was evaluated by Li et al. in a breast cancer mouse model. Mice were treated with paclitaxel loaded sulfatide-containing lipid nanoparticles. Again, despite increased efficacy over non-targeted delivery and free drug, the
molecules, which could result in potential o...

introducing drug release in the ECM to overcome potential lysosomal...

Therefore, the use of a tenascin-C binding peptide or a selected aptamer...

It should be noted, however, that sulfatide, while suitable for...

Another molecule to target in the ECM of tumors is...

Fibronectin-fibrin complex

CLT-1 peptide-FITC conjugation

FITC

Imaging only

[42]

CLT-1 peptide nanoparticles

Paclitaxel

[43]

CLT-1 imaging complex

Gadolinium

Imaging only

[44,45]

CREKA-nanoparticles

Iron oxide

In vitro targeting

[46]

CREKA-thermosensitive-liposomes

Doxorubicin

ECM release by external heat

[47]

Antibody-drug conjugate

SN-38

ECM release

[48]

Collagen

Antibody-drug conjugate to type IV collagen

SN-38

ECM release

[49]

Collagen-binding domain peptide fused with Fab fragment of an antibody against EGFR (type of collagen not specified)

None

Membrane receptor binding of specific tumor cell subset required

[50]

Galectin-1

Anginex galectin-1 binding peptide-liposomes

Cisplatin and arsenic trioxide

[51,52]

Aggrecan

Quaternary ammonium-drug-conjugate

Melphalan

Therapeutic mechanism not clear

[53-55]

Heparan sulfate

CGKRE peptide nanoparticles

Paclitaxel

Dual targeting to heparan sulfate and endothelial cells

[56]

Chondroitin sulfate

TRX-20 modified liposomes

Cisplatin

ECM release, in vitro study

[59]

Single chain variable fragment (GD3G7)-lyophilisomes (against CS type E)

Doxorubicin

[20]

Table 1
Overview of drug delivery strategies targeting the extracellular matrix in tumors.

<table>
<thead>
<tr>
<th>Target</th>
<th>Targeting system</th>
<th>Payload</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenascin-C</td>
<td>Antibody drug conjugate</td>
<td>Anthracycline</td>
<td>ECM release</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>FHKRSPALSVPVGGG peptide-liposomes</td>
<td>PNU159682</td>
<td>Removal of CAFs, subsequent tumor cell targeting of liposomes required</td>
<td>[26,27]</td>
</tr>
<tr>
<td></td>
<td>FHKRSPALSVP peptide- lLyp-1-peptide nanoparticles</td>
<td>Paclitaxel</td>
<td></td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Sulfatide-liposomes</td>
<td>Doxorubicin</td>
<td></td>
<td>[29-32]</td>
</tr>
<tr>
<td></td>
<td>Sulfatide-nanoparticles</td>
<td>Paclitaxel</td>
<td></td>
<td>[33]</td>
</tr>
<tr>
<td>Fibronectin extra domain A or B</td>
<td>Antibody (SIP-F8) drug conjugate (extra domain A)</td>
<td>Maytansinoid derivative MD1</td>
<td>Extracellular release strategy</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>Single chain variable fragment (CGS-1)-liposomes (extra domain B)</td>
<td>Iron oxide</td>
<td>Imaging only</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>Single chain variable fragment (L19)-interleukin 2 fusion protein (extra domain B)</td>
<td>Interleukin 2</td>
<td>Stimulation of immune response</td>
<td>[41]</td>
</tr>
<tr>
<td>Fibronectin-fibrin complex</td>
<td>CLT-1 peptide-FITC conjugation</td>
<td>FITC</td>
<td>Imaging only</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>CLT-1 peptide-nanoparticles</td>
<td>Paclitaxel</td>
<td></td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td>CLT-1 imaging complex</td>
<td>Gadolinium</td>
<td>Imaging only</td>
<td>[44,45]</td>
</tr>
<tr>
<td></td>
<td>CREKA-nanoparticles</td>
<td>Iron oxide</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>CREKA-thermosensitive-liposomes</td>
<td>Doxorubicin</td>
<td></td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>Antibody-drug conjugate</td>
<td>SN-38</td>
<td></td>
<td>[48]</td>
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<tr>
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<td>[49]</td>
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<td></td>
<td>Collagen-binding domain peptide fused with Fab fragment of an antibody against EGFR (type of collagen not specified)</td>
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</tr>
<tr>
<td>Galectin-1</td>
<td>Antibody-drug conjugate</td>
<td>Cisplatin and arsenic trioxide</td>
<td></td>
<td>[51,52]</td>
</tr>
<tr>
<td>Aggrecan</td>
<td>Quaternary ammonium-drug-conjugate</td>
<td>Melphalan</td>
<td>Therapeutic mechanism not clear</td>
<td>[53-55]</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>CGKRE peptide nanoparticles</td>
<td>Paclitaxel</td>
<td>Dual targeting to heparan sulfate and endothelial cells</td>
<td>[56]</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>TRX-20 modified liposomes</td>
<td>Cisplatin</td>
<td>ECM release, in vitro study</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>Single chain variable fragment (GD3G7)-lyophilisomes (against CS type E)</td>
<td>Doxorubicin</td>
<td></td>
<td>[20]</td>
</tr>
</tbody>
</table>

* Internalization of carrier ± drug required for therapeutic activity, endosomal escape necessary.
Next, Kruse and co-workers used CREKA, a peptide that also binds fibrin-fibronectin complexes [46]. Although the iron oxide nanoparticles were able to specifically target fibrin-fibronectin in vitro, no in vivo tumor targeting or therapeutic studies were reported. CREKA was also used for tumoral ECM targeting by others. Wang et al. prepared doxorubicin loaded CREKA-functionalized liposomes with thermosensitive release characteristics. Mice bearing subcutaneous multidrug resistance adenocarcinomas showed significant inhibition in tumor growth when treated with doxorubicin loaded CREKA liposomes [47]. Moreover, when tumors were heated to induce doxorubicin release, tumor growth was even more inhibited, indicating that upon binding in the ECM of tumors, the released doxorubicin was able to reach its site-of-action and affected tumor growth. In a slightly different approach, Yasunage et al. targeted fibrin clots in the tumoral ECM using an antibody that was conjugated with the cytotoxic compound SN-38, the active metabolite of irinotecan, that was modified to be only cytotoxic upon release from the antibody due to an alkaline labile ester bond [48]. An in vivo therapeutic study in a chemically induced skin carcinoma mouse model showed a significant tumor growth inhibition in mice treated with the antibody-drug conjugate. Further analyses showed a significantly higher concentration of the drug in tumors of mice treated with the antibody drug conjugate, indicating that fibrin-fibronectin targeting in the tumor ECM may be useful for targeted chemotherapeutic drug delivery.

Although targeted less frequently, collagen may also be a potential target in the ECM of tumors. Collagen is a structural protein abundantly present in the ECM of most tissues. Despite the presence of collagen throughout the body and risk of off-targeting with toxicity as a result, several attempts have been made to target collagen in the tumoral ECM for the delivery of chemotherapeutics. Yasunaga et al. developed an antibody drug conjugate against type IV collagen that released the antineoplastic drug SN-38 through the labile ester bond linker in the tumor ECM [49]. Evaluation in mice with two types of subcutaneous pancreatic tumors (stroma poor and stroma rich tumors) showed almost complete tumor growth inhibition of stroma rich tumors treated with the anti-collagen drug conjugate. Interestingly, growth of stroma poor tumors was less affected by the antibody-drug conjugate suggesting specific targeting of stroma rich tumors. In spite of the abundant expression of collagen in the body, body weight was not affected and no toxicity in the liver, kidney and bone-marrow was observed, suggesting that distribution to other organs may be limited. Liang et al. also targeted collagen in the ECM of tumors. They designed an antibody drug conjugate by combining a collagen (collagen type not specified) binding domain peptide with the Fab fragment of a clinically approved antibody (cetuximab) directed against the epidermal growth factor receptor (EGFR) which has antitumor activity itself [50]. In a therapeutic in vivo study with mice bearing subcutaneous EGFR positive tumors, tumor growth was significantly inhibited in mice treated with the collagen binding domain-anti EGFR conjugate compared to cetuximab only. In general, the full potential of tumoral ECM targeting was not utilized because the therapeutic molecule required binding of a specific receptor (EGFR) on a tumor cell subset.

Next to collagen, galectin-1 has been used for targeting to the tumoral ECM. Galectin-1 is a carbohydrate binding protein that plays a role in cellular interactions. Underlining the limitations of cellular targeted therapies due to the tumor heterogeneity of triple negative breast cancer (i.e. breast tumors not overexpressing the estrogen receptor, progesterone receptor and epidermal growth factor receptor-2), Upreti and colleagues developed a tumor ECM targeting strategy directed at galectin-1 [51,52]. Cisplatin and arsenic trioxide loaded liposomes functionalized with anginex, a small galectin-1 binding peptide, were evaluated for their therapeutic efficacy in an orthotopic triple negative breast cancer mouse model. They showed significant tumor growth reduction compared to treatment with non-targeting drug loaded liposomes. Although initial results were promising, an increased efficacy may be reached by applying extracellular release of the
cytotoxic agents instead of using liposomes requiring receptor mediated 
endocytosis and release inside cells to be therapeutically active. 

As proteoglycans are abundantly present in the ECM, they may be 
used for targeting chemotherapeutics to the tumor ECM as well. For 
instance, aggrecan, a proteoglycan expressed in the ECM of cartilage 
but also abundantly present in the ECM of chondrosarcomas was 
targeted by Peyrode et al. [53–55]. Using a conjugate of quaternary am-
onium with the chemotherapeutic compound melphanal, aggrecan 
was targeted in an orthotopic Swarm rat chondrosarcoma model. Re-
results showed a reduction of tumor volume for the drug conjugate. The 
reduction, however, was not significantly different from non-targeted 
melphanal, although more toxicity was observed for this group indi-
cating an improved toxicity profile for the aggrecan targeting drug 
conjugate. Despite promising results, care should be taken with possible 
off-targeting to aggrecan rich tissues such as cartilage, a tissue that 
was not included in the toxicity evaluations, even though toxicity may be 
limited due to the limited blood supply to cartilage. Another pro-
teoglycan targeted in the ECM of tumors is heparan sulfate, which is 
found highly upregulated in ECM of tumors making it an attractive 
target for tumoral ECM chemotherapeutic drug delivery [56]. Hu et al. 
used a CGKRK peptide with high affinity to heparan sulfate and con-
jugated it with an endothelial cell binding peptide to paclitaxel loaded 
nanoparticles [57]. This strategy was evaluated in mice bearing in-
tracranial glioblastoma tumors and showed that mice treated with pa-
clitaxel loaded nanoparticles targeted against heparan sulfate and en-
dothelial cells significantly improved survival. It is not clear whether 
the effect is through extracellular release with potential removal of 
tumor supportive cells or by internalization in tumor cells only. Finally, 
chondroitin sulfate can be a target in the tumoral ECM because of its 
high expression in the ECM of various tumor types [58]. Lee et al. used 
cisplatin loaded liposomes modified with the chondroitin sulfate 
binding molecule TRX20 (3,5-dipentadecylbenzamidine hydro-
chloride) which showed tumor growth inhibition in a subcutaneous 
mouse tumor model [59]. While the strategy was designed to target 
chondroitin sulfate at tumor cell membranes, it may also be applied as 
ECM targeting to tumors with chondroitin sulfate in the ECM. Our 
group developed a drug delivery system that targets chondroitin sulfate 
subtype-E (CS-E), which was found to be highly upregulated in the ECM 
of ovarian cancer [60]. Although currently only evaluated in vitro, 
doxorubicin loaded albumin particles functionalized with a scFv anti-
body against CS-E were indeed able to target CS-E and efficiently 
eliminate ovarian cancer cells by extracellular drug release [20]. 
Overall, proteoglycans and glycosaminoglycans in the tumoral ECM 
may offer several opportunities, but care should be taken with off target 
effects to healthy tissue due to expression of these molecules 
throughout the body.

### 4.3. Extracellular drug release

Next to the presence of promising targets in the ECM, a tumor ECM 
drug delivery strategy is also highly dependent on the type of drug 
carrier. Many chemotherapeutic drug delivery systems have been de-
veloped over the last decades. Each system has unique characteristics 
that can be important for tumoral ECM drug delivery, such as size, drug 
content, charge, base material, modifications, etc. An important char-
acteristic of drug delivery systems for tumoral ECM drug delivery is the 
drug release mechanism. Upon release, most drug molecules will retain 
in the tumor area because of the enhanced retention effect and will pass 
cell membranes due to the hydrophobic properties of the majority of 
chemotherapeutics. However, if not rapidly taken up by tumor or tumor 
supportive cells, there is a risk of diffusion back into the circulation, 
which may result in off-target effects. Unfortunately, preclinical studies 
generally do not assess reuptake of released drugs into the circulation, 
but such analyses should be included in future studies. Without suffi-
cient release of chemotherapeutics once a drug delivery system is 
bound to its target, no therapeutic effect will be induced. Therefore, 
extracellular release once bound to the ECM of tumors is required. Next 
to simple diffusion, with possible unwanted preliminary drug release, 
several innovative release mechanisms have been developed. Examples 
are triggered release by enzymes, pH, magnetism, heat, light, and 
ultrasound. For example, by exploiting the lower pH in the tumor ex-
tracellular matrix (6.2–6.9) caused by accumulation of lactic acid pro-
duced by highly proliferating tumor cells [61], Chiang et al. designed 
tumor ECM targeting doxorubicin-loaded liposomes in which the imi-
dazole ring of histidine was protonated in an acetic environment re-
sulting in increased uptake of the doxorubicin liposomes [62]. Dong 
et al. synthesized a pH and enzyme responsive doxorubicin delivery 
system [63]. The acetic tumor environment exposed the gelatin-DNA-
doxorubicin complex to subsequently release doxorubicin by enzymatic 
degradation of gelatin due to matrix metalloproteinases upregulated in 
the tumoral ECM. In antibody-drug conjugates, triggered release is 
applied as well. For example, Rossin et al. developed a non-inter-
nalizing antibody-drug conjugate with a click-to-release mechanism 
[64]. Upon binding to the tumor specific membrane bound target, the 
non-internalizing antibody-drug conjugate released its payload after 
reaction of an administered activator compound. This strategy enables 
release of the drug specifically at the tumor site as non-bound antibody-
drug complexes are allowed to be excreted from the body before ad-
ministration of the activator compound. Next to these examples, a 
manifold of other release mechanisms have been developed. As thor-
ough discussion of these external/internal stimuli driven response is 
beyond the scope of this review, we refer to excellent reviews on this 
topic [61,65,66]. The combination of stimuli triggered release and 
binding to a tumor ECM target seems a promising idea, but more studies 
should be performed to indicate its full potential.

### 5. Future outlook

The therapeutic effect of conventional tumor targeting chemother-
apapeutic delivery systems that addresses molecules on cancer cells may 
be limited by intratumoral heterogeneity and inadequate drug release 
due to lysosomal entrapment. Combining the knowledge of tumor 
heterogeneity and the importance of the tumor extracellular matrix 
with its tumor supportive cells, delivery of chemotherapeutics to the 
tumoral ECM may be a promising alternative. Various studies have 
identified unique tumoral ECM targets. In vivo studies indicate that 
targeting these unique tumoral ECM targets combined with extrac-
ellular release of chemotherapeutics can improve treatment outcome. 
Tumoral ECM targets should be critically selected. Potential expression 
in healthy tissue may cause off-targeting with possibilities of inducing 
toxicity and side effects. Moreover, care should be taken when selecting 
a drug delivery system. The effect caused by extracellular drug release 
and diffusion of the drug to tumor supportive cells in the tumor area 
may be limited when the drug as such is not released extracellularly, 
but instead is contained in a carrier that is taken up into the cell 
through endocytosis. To overcome these limitations, stimuli driven 
extracellular drug release may offer promising opportunities. By ex-
ternal or internal triggered drug release, chemotherapeutic agents will 
only be released in the tumor area and will be able to diffuse into tumor 
cell and tumor supportive cells. Moreover, it may prevent early drug 
release that results in exposure to healthy tissue. Next to chemother-
apapeutic delivery, the emerging field of immunotherapy may greatly 
benefit from tumor ECM drug delivery. In a study from Zegers et al. 
[67], the chemokine IL2 was targeted to the tumoral ECM fibronectin 
extra domain B. Upon radiation, the cytotoxic effect of infiltrating CD8 
cytotoxic T lymphocytes was enhanced by the extracellular presence of 
IL2, illustrating the possibilities to include tumor ECM targeted drug 
delivery in immunotherapy.

Despite a number of promising in vivo results, no clinical studies 
using tumor ECM targeted chemotherapeutic delivery were identified. 
Therefore, to understand the full potential of this strategy, the step to 
clinical studies should be taken once the most potential tumoral ECM
targeting strategy has been identified.

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