

## Short Communication

# Melanoma-associated Cellular Adhesion Molecules (MACAM)

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VARIOUS monoclonal antibodies (Mabs) have been described which react with antigens expressed on the cell surface of murine and human melanoma cells. Recently, several of these antigens have been shown to be involved in adhesion-associated processes. Among these are the melanoma-associated proteoglycan and the ganglioside GD3. Recently we raised a set of Mabs which all affect the adhesive properties of human melanoma cells. In the present study we report on the functional properties of two antigens recognized by these Mabs.

The Mab NKI-M6 (IgG1) recognizes a melanoma-associated proteoglycan as mentioned above, a heavily glycosylated molecule with a protein backbone of 250 kD. This antibody reacts with all cultured melanomas (>30) and all melanomas in frozen tissue sections (>50) tested so far.

A second antibody, NKI-M7 (IgG1), reacts with a molecule, designated MACAM-1, consisting of a bimolecular complex with two chains of 150 and 90 kD, respectively, and under reducing conditions four chains of 120, 95, 29 and 25 kD. Similar to NKI-M6, NKI-M7 reacts with all cultured melanoma cell lines and primary cultures of resected melanoma metastases tested. In addition the antibody reacted with 100% of the melanoma cells in frozen tissue sections. Both antibodies stained nevi but did not react with normal melanocytes. Furthermore, we observed significant cross-reactivity of NKI-M7 with endothelial cells both in culture and in frozen tissue sections (small vessels). NKI-M6 has been shown to cross-react with peri-

neurium. These data indicate that the antigens recognized by those antibodies are almost exclusively expressed by melanoma cells and are not found on a large series of other tumors.

To investigate modulation of the antigens recognized by NKI-M6/7, melanoma cells were cultured for various periods of time in the presence of interferon- $\gamma$  (IFN- $\gamma$ ) or retinoic acid. Even after a culture period of up to 6 days we were unable to measure significant differences in the expression of both antigens as measured by FACS analysis. This in contrast to the expression of the MHC class II molecule HLA-Dr, which was rapidly induced (24-28 h) by retinoic acid and IFN- $\gamma$ . In addition, cultivation of melanoma cells in the presence of the antibodies did not result in modulation of the antigens.

In another series of experiments we examined the effects of NKI-M6 and NKI-M7 on the adhesive properties of melanoma cells. It was found that NKI-M7 efficiently inhibited the adhesion of 16 different melanoma cell cultures to both plastic surfaces and monolayers of cultured vascular endothelial cells derived from umbilical cords. The percentage of inhibition varied between 35 and 85% with an average inhibition of 59%. Although we observed considerable differences in the capacity of NKI-M7 to inhibit adhesion of different melanoma cell cultures, we were unable to correlate this effect with the quantity of molecules expressed on the cell surface of the various melanoma cells as measured by immunofluorescence. The results indicate that in addition to NKI-M7 other melanoma surface antigens with adhesive properties contribute to the adhesion of melanoma cells. In contrast, the Mab NKI-M6 was not able to affect the adhesion to plastic and endothelial

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cell surfaces. Furthermore, we observed that NKI-M7, and to a much lesser extent NKI-M6, delayed the cytoplasmic spreading of melanoma cells during the adhesion process. After an incubation period of 4 h a relatively large portion of the adherent cells were still rounded up. In addition to the adhesive properties of the antigens recognized by these antibodies, we investigated whether these polypeptides were part of the extracellular matrix formed by cultured melanoma cells. Therefore melanoma cells were grown on glass coverslips for a period of 48 h and analyzed for the presence of so called adhesion plaques deposited by the melanoma cells. The results demonstrated that both the antigen detected by NKI-M6 and the antigen recognized by NKI-M7 were abundantly present in these adhesion plaques.

Finally we studied whether the molecules were involved in migratory processes, since after adhesion, migration is an important step in the metastatic process. Migration was studied in a microchemotaxis chamber, consisting of an upper compartment which contained the cells divided from a lower compartment by a 10  $\mu\text{m}$  thick poly-

carbonate filter (8  $\mu\text{m}$  pore size), which contained 300  $\mu\text{g}$  fibronectin/ml. A small percentage of the melanoma cells migrate along the gradient of fibronectin formed, for a period of 2 h. Subsequently the number of melanoma cells that migrated to the reverse side of the filter was determined. If the Mab NKI-M7 was added to the cells, a strong inhibition of the chemokinetic and the chemotactic response was observed (60–95%). A much lower, but significant, inhibition was observed after the addition of NKI-M6 (34–58%).

### CONCLUSION

The results presented in this study are summarized in Table 1 and indicate that MACAM-1, recognized by NKI-M7, and the proteoglycan, detected by NKI-M6, are associated with biological functions of human melanoma cells and may play a general role in hematogenic dissemination of malignant melanoma. Identification of other MACAMs, and particularly their 'counter-structures' present on endothelial cells and other tissues, are relevant for gaining a better insight in human melanoma.

Table 1. Summary of the properties of two melanoma associated cellular adhesion molecules

	MACAM-1	Proteoglycan
Antibodies	NKI-M7	NKI-M6
MW (kD)	150, 90	>450–250
Distribution	Melanoma Nevi Capillary endothelium	Melanoma Nevi Perineurium
Modulation by IFN- $\gamma$ retinoic acid	–	–
Involved in:		
adhesion	+	–
migration/motility	+	+
Present in adhesion plaques	+	+

### REFERENCE

1. De Vries JE, Keizer GD, Te Velde AA *et al.* Characterization of melanoma-associated surface antigens involved in the adhesion and motility of human melanoma cells. *Int J Cancer* 1986, **38**, 465–473.