Cancer and Metastasis Reviews **10**: 11–22, 1991.

Cell adhesion molecules of the immunoglobulin supergene family and their role in malignant transformation and progression to metastatic disease

Judith P. Johnson
Institute for Immunology, Goethestrasse 31, 8000 Munich 2, Germany

Key words: MHC antigens, ICAM-1, NCAM, MUC18, CEA, DCC

Summary

Cell adhesion molecules (CAMs) of the immunoglobulin supergene family may play important roles in tumorigenesis and the development of metastatic disease. In a variety of human malignancies, tumor progression has been observed to be associated with changes in CAM expression. An early event in colorectal tumorigenesis appears to be the down regulation of a normally expressed CAM, DCC. Overexpression of a second CAM, carcinoembryonic antigen, is associated with colorectal tumors which have a high risk for metastasis development. Several tumors, including Wilms tumors and neuroblastoma, have been found to express a developmentally regulated form of NCAM which inhibits a variety of cell-cell interactions. Malignant cells not only show aberrations in the expression of their CAMS and thus their normal cell-cell interactions, but establish new adhesive interactions. The development of metastatic potential in cutaneous melanoma is associated with the de novo expression of two CAMs, one of which is ICAM-1, a molecule mediating adhesion between the tumor cells and leukocytes.

Introduction

The interaction of cells with their environment is mediated in large part by cell surface molecules with specific affinities for molecules on the surface of other cells and which are known collectively as cell adhesion molecules (CAMs). Adhesive interactions mediated by CAMs allow cells to sort into groups and form complex structures and as such are of critical importance in organogenesis and tissue reconstruction [1]. CAMs do not, however, simply glue cells together; most CAM interactions are accompanied by the transmission of signals regulating differentiation or proliferation to one or both partners [1, 2]. Perhaps even more importantly, by maintaining cells in close contact, CAMs allow the exchange of information mediated by other cellular components [2, 3]. In this context they support and provide direction for essentially all intercellular communication in multicellular organisms.

While CAMs are found in several different molecular families, the majority identified to date belong to the immunoglobulin (Ig) supergene family (Fig. 1; Ref. [4]). Common to all these molecules is the immunoglobulin fold, a structure resembling a sandwich of β pleated sheets stabilized by a disulfide bond. Although the members of this supergene family are functionally diverse, most are cell surface molecules and are involved in recognition of other molecules, either soluble or cell associated. This recognition can be homophilic (between like molecules) or heterophilic (between two different molecules) and frequently occurs between members of the Ig supergene family. Since most members are involved in molecular recognition, it is speculated that this supergene family evolved

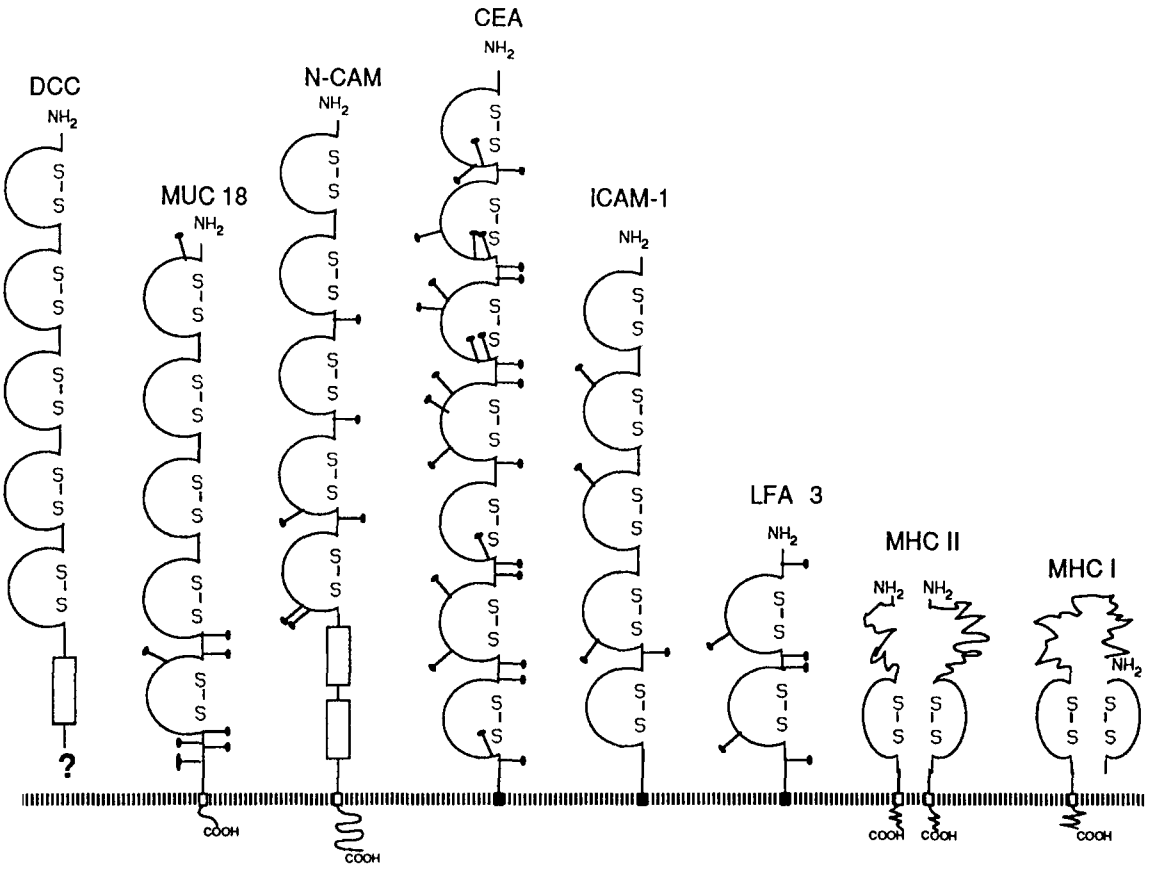

Fig. 1. Schematic diagram of the Ig supergene family CAMs. All molecules are characterized by the Ig domain which is stabilized by disulfide bonds. Rectangles indicate fibronectin homology units which are present in some of the molecules. ● indicate sites of potential N-linked glycosylation.

from a common precursor which functioned in self recognition. While all members of this family share conserved amino acid residues, these are limited to positions within the core of the Ig fold and probably are important for its formation. In contrast, the functional regions of the various members are highly diverse. Sequence comparisons, however, indicate that within the Ig supergene family some molecules are more closely related to each other, suggesting that they may have evolved from a common ancestor. One of these groups consists of neural cell adhesion molecules which have been shown to mediate intercellular adhesion during the development of the nervous system and which include the neural cell adhesion molecule (NCAM), myelin associated glycoprotein (MAG), L1, amalgam, contactin and fascicilin II [5].

Given the role of CAMs in controlling cellular interactions, it seems likely that disturbances in the function of these molecules will be intimately involved in malignant transformation and tumor progression. The malignant cell demonstrates a severely altered relationship with its environment. Connections to neighboring cells are disturbed and the tumor cells migrate away from their normal location, invade the surrounding connective tissue, enter the vascular and lymphatic systems and exit to take up residence in foreign environments. There are many steps in this process where alterations in CAM function could be important (Fig. 2). The loss of normal intercellular adhesion which can free cells from a variety of contact mediated regulatory controls, and also lead to the dissemination of cells from the primary tumor, could occur

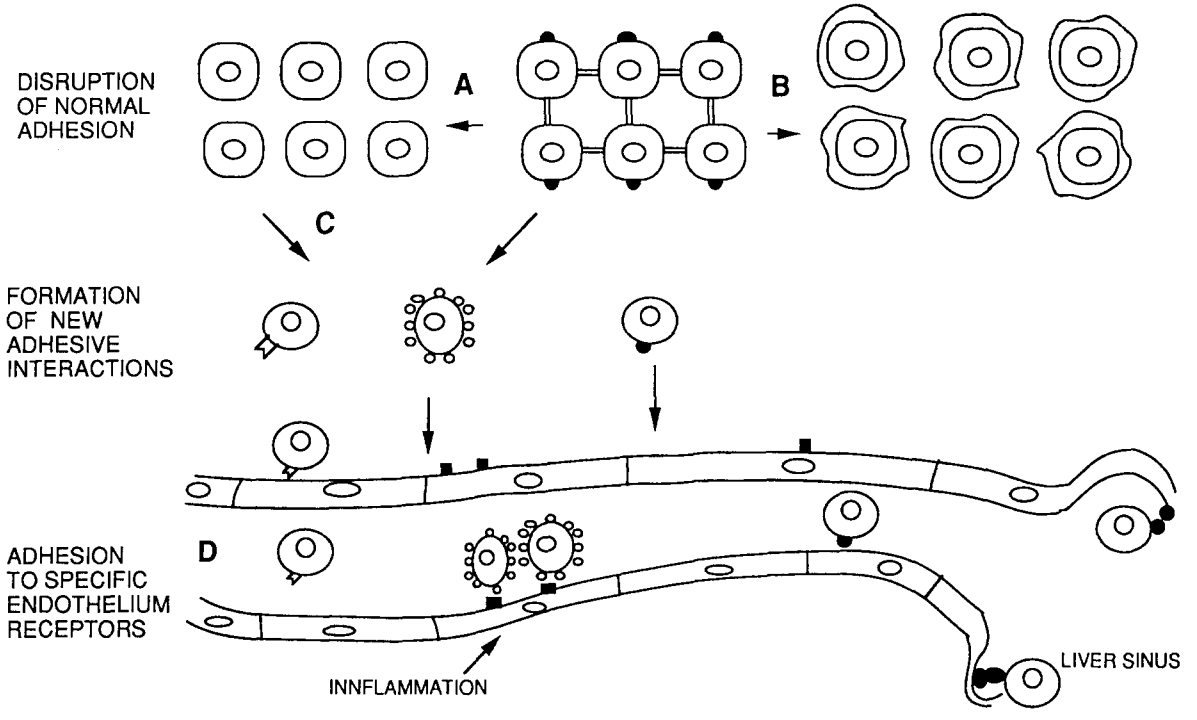

Fig. 2. Diagram of the steps involved in metastases formation. Disruption of normal adhesive interactions between cells can occur through the down regulation of CAM expression (A) or through expression of molecules blocking adhesion (B). Cells may then acquire new adhesive interactions (C) which aid in invasion and entry of the cells into capillaries (D). Extravasation may be enhanced by adhesive interactions with endothelium or liver sinusoid (D). ◎ = tumor cell; ○ = leukocytes; ● = CAM expressed by normal cell; ◘ = new CAM expressed by tumor; ■ = endothelial cell CAM.

through the down regulation of CAMs (Fig. 2A) or the expression of an altered CAM (B). The expression of new CAMs (C) could endow the tumor cells with new adhesive protentials and contribute to migration, invasion and extravasation (D). Recently a number of Ig superfamily cell adhesion molecules have indeed been implicated in these various steps. A survey of these molecules and their postulated roles in tumor progression emphasizes not only that CAMs may be important in many different stages in this process but also indicates that different CAMs are involved in different types of tumors.

Malignant cells may express a 'cell repulsion' form of the neural cell adhesion molecule NCAM

The neural cell adhesion molecule, NCAM, was the first intercellular adhesion molecule to be isolated, characterized and cloned [5, 6]. Although it was originally identified in neural tissues, NCAM is now known to be broadly expressed during embryonic development and probably plays an important role in directing cell-cell interactions in many different tissues. NCAM is encoded by a single gene, but due to differential mRNA splicing exists in a variety of isoforms ranging in apparent molecular weight from 115 kD to 140 kD. Analyses of transfectants as well as studies using purified NCAM molecules in liposomes have shown that this molecule mediates cell adhesion in a homophilic fashion, that is, the NCAM molecules located on apposing cell surfaces interact with each other. NCAM remains the best characterized of the CAMs and the only one where the diverse regulatory levels are beginning to be defined. Many of the levels at which NCAM function can be regulated, including gene expression, mRNA splicing and glycosylation, appear to be common to the other Ig superfamily CAMs. In this respect NCAM serves as a prototype for understanding CAM regulation.

The level of expression of NCAM at the cell surface has been shown to severely affect adhesion, a 2 fold difference altering the rate by more than 30 fold [7]. The various NCAM isoforms, which are created through alternative mRNA splicing, differ from each other primarily in their transmembrane and cytoplasmic regions. Since the expression of these isoforms is regulated in a tissue and stage specific manner, it seems probable that they are functionally distinct. Support for this has recently been obtained in studies using transfection of chimeric cDNA constructs [8]. These studies demonstrated that, while not affecting the specificity of adhesion, the cytoplasmic region of the CAM is critical for the functional consequences of adhesive interactions, such as sorting out of cells.

A striking variability in the degree of N-linked glycosylation is a prominent characteristic of CAMs examined in different tissues and at different stages of development. In the case of NCAM this has been shown to have a striking functional significance [6]. The amount of sialic acid can comprise up to 30% of the weight of NCAM. Most of this is present as polysialic acid (PSA), an α-2-8-linked linear homopolymer of sialic acid which is attached to N-linked high mannose core oligosaccharides. PSA is an unusual form of sialic acid and has not been detected on other molecules in higher vertebrates. However, it is commonly found in the protective outer coat of certain bacteria and in the perivitelline zone of some fish eggs. NCAM exists in high PSA (h-PSA, 30% sialic acid) and low PSA (l-PSA, 10% sialic acid) forms. Comparisons of h-PSA and l-PSA NCAM molecules in liposomes demonstrate that the amount of PSA affects the adhesion, h-PSA molecules showing a 4–6 fold reduction in rate of adhesion. However, in intact cells, the expression of the h-PSA form of NCAM has a much more dramatic effect. In addition to the reduction in NCAM-NCAM interactions, adhesive interactions mediated by unrelated CAMs are also disrupted [9]. This has led to the proposal that the expression of the h-PSA form of NCAM creates a carbohydrate barrier around the cell which effectively reduces its ability to participate in most contact mediated phenomena. The expression of the h-PSA form of NCAM is tightly regulated, being limited to particular cells in certain stages of development, and it is thought to be important in temporarily freeing these cells from adhesive interactions with their neighbors.

In the adult, the h-PSA form of NCAM is not found in epithelial and neural tissues. However, the use of antibodies specific for PSA indicate that it is expressed by some types of tumors [10, 11]. This was first demonstrated for Wilms tumor, a rapidly growing highly malignant tumor of the kidney. Immunohistochemical analyses revealed that the h-PSA form of NCAM characterized the tumor cells while the l-PSA form characterized the surrounding normal epithelial cells [11]. NCAM is expressed by a wide variety of tumors, but it is not yet known how frequently it is in the h-PSA form. However, whenever h-PSA NCAM is expressed (Fig. 2B), the experimental data suggest that these cells will be freed from a variety of contact mediated restraints including positional control and growth inhibition.

Overexpression of carcinoembryonic antigen in colorectal carcinoma may promote tumor cell homing to the liver

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein of colon mucosal cells. In a number of epithelial malignancies CEA appears in high levels in the serum and this characteristic has led to its use as a marker to monitor tumor burden. CEA is a member of a multigene family whose members cross react serologically and cross hybridize at the nucleic acid level [12]. CEA has a protein core with an apparent molecular weight of 80 kD, but is highly glycosylated and migrates with an apparent mw of 180 kd. Like NCAM, diversity of CEA molecules can be generated by differential mRNA splicing and by post translational modification. Recent studies using purified CEA and cells transfected with CEA encoding cDNA clones have shown that this molecule functions as a CAM [13, 14]. It can mediate intercellular adhesion both through homophilic interactions (i.e., CEA-CEA) and heterophilic interactions as demonstrated by the ability of CEA expressing transfectants to form adhesive

contacts with transfectants expressing a second member of the CEA family, nonspecific-crossreacting-antigen (NCA).

Malignant colon epithelial cells frequently express 10–100 fold higher levels of CEA than their normal counterparts, the cellular distribution of the molecule is altered and large amounts are secreted into the circulation. The preoperative level of serum CEA in colorectal carcinoma patients has been shown to be an important prognostic parameter [15]. Patients with Duke's C and D stage tumors with CEA serum levels greater than 5 ng/ml have a shorter disease free interval and lower survival rate than patients with tumors of the same stage but with normal CEA serum levels. These results have led to the speculation that the overexpression of CEA contributes directly to tumorigenesis or metastasis formation.

The over-expression and altered distribution of CEA on malignant epithelial cells may disturb the normal intercellular adhesion and lead to disruption of the tissue architecture [13]. However, the high serum levels of CEA may also play a direct role in the development of metastasis. CEA is cleared from the circulation by the liver. Kupffer cells lining the sinusoids take up CEA via specific receptors and it is transferred to the hepatocytes, where it is degraded. During this process CEA can be detected on the surface of both the Kupffer cells and the hepatocytes [16, 17]. The demonstration that CEA can function as a CAM, binding both CEA and NCA bearing cells, raises the possibility that its expression by cells lining the liver sinusoids results in the establishment of a kind of homing receptor which causes the circulating CEA positive tumor cells to stop in the liver. Recent experiments using human colorectal carcinoma cells transplanted to athymic nude mice appear to support this hypothesis. The ability of primary tumors to successfully grow in nude mice was shown to be positively correlated with the expression of high levels of CEA [18]. More directly, experimental liver metastasis and liver implantation of colorectal carcinomas could be enhanced by a prior intravenous injection of purified CEA [19]. The increased metastasis was only observed for tumors which were poorly metastatic in the nude mice. Tumors which were non-metastatic or highly metastatic were not affected, suggesting that circulating CEA did not fundamentally alter any inherent characteristic of the tumor cells. Localization of tumor cells to the liver could be directly measured and was the highest 30 min after the injection of CEA. This corresponds to the time when the maximal expression of membrane bound CEA by the Kupffer cells and hepatocytes is observed. As soluble CEA was unable to induce tumor cell aggregation *in vitro*, it appears that expression of this liver-CEA is indeed the critical event.

The extravasation of tumor cells is a critical step in the successful development of metastases. The elements within the lympho-vascular system which are responsible for the arrest of circulating tumor cells and especially those which may help to determine organ preferences in the location of metastases remain largely unknown. The expression on sinusoid lining cells of an adhesive ligand specific for tumor cells may well constitute a 'liver homing receptor' which traps the circulating tumor cells. This selective trapping may well enhance the probability that these cells successfully establish hepatic metastasis (Fig. 2D).

Changes in the expression of novel CAMs correlate with tumor progression

The search for molecules whose expression changes during tumor progression can lead to the discovery of those molecules which play a role in tumorigenesis and the development of metastases. This approach has recently led to the identification of two novel members of the Ig superfamily whose sequence homology to NCAM and related neural cell adhesion molecules indicate that they may also function as CAMs.

The development of malignant tumors is accompanied by an accumulation of functional alterations in a variety of gene products [20, 21]. While many of these changes are epigenetic, some are genetic and result from mutations, deletions or translocations. These genetic alterations have been shown to lead to the activation of genes (which are then known as oncogenes) or the inactivation of genes

(which are then known as tumor suppressor genes). While most of the oncogenes and tumor suppressor genes identified to date seem to be involved in the regulation of cell proliferation, a recently cloned tumor suppressor gene appears to be a CAM.

DCC – loss of expression of a putative CAM is associated with the development of colorectal carcinoma

Deletions in the q21 region of chromosome 18 are found in more than 70% of colorectal tumors [22]. This suggested that, in analogy to studies on retinoblastoma where deletions at 13q11 marked the location of the Rb tumor suppressor gene [23], the inactivation of a gene located at 18q21 contributes to the malignant transformation of colorectal epithelial cells. Molecular cloning of this region of chromosome 18 led to the identification of a novel gene which has been termed DCC (for deleted in colorectal carcinoma; [24]). This gene contains at least 8 exons and encodes a mRNA of approximately 12 kb which is present at low levels in most normal tissues examined, including colon mucosa. While expression of DCC mRNA was detected in tumors derived from brain and lung, only 2 of 17 colorectal carcinoma lines had measurable levels of DCC message. In colorectal tumors, the DCC gene was found to be mutated, deleted or, most commonly, interrupted by DNA insertions. The DCC gene is highly conserved between species and shows its highest level of expression in the brain in both humans and rats. Sequencing of the exons revealed that DCC encodes a molecule which is a member of the Ig supergene family. Within this family it shows the greatest sequence similarity to NCAM and related neural adhesion molecules (Fig. 1; Table 1). Since each of these molecules has been shown to mediate intercellular adhesion, it seems likely that DCC is also a CAM. Colorectal carcinomas are thought to develop in a stepwise manner, a process which is reflected in the existence of a series of precursor lesions which include hyperplasia, benign adenomas and carcinoma *in situ* [25]. Examination of these different lesions revealed that deletion of 18q21 is found in more than half of late adenomas, suggesting that inactivation of DCC is a fairly early event in the malignant transformation of intestinal epithelium. The down regulation of DCC is therefore predicted to contribute to the disruption of normal cell-cell adhesion in the intestinal epithelium (Fig. 2A). While loss of expression of a single CAM would not be expected to have the same effect on unrelated cell interaction molecules as would the expression of the h-PSA form of NCAM, DCC mediated cell adhesion may control an important cell function.

MUC18 – De novo *expression of a putative CAM is associated with the progression of malignant melanoma to metastatic disease*

The use of murine monoclonal antibodies to search for molecules which are expressed by human malignant melanoma cells but not by melanocytes in the benign proliferative precursor lesions has led to the identification of a number of molecules whose expression characterizes the various stages in the development of this tumor [26, 27]. One of the molecules which was identified by this approach is MUC18, a cell surface glycoprotein of apparent molecular weight of 113 kd [28]. MUC18 was expressed by the majority of melanomas examined but was not found on most other types of tumors. Examination of melanomas of different stages revealed that the expression of this molecule is correlated with the development of metastatic potential. In cutaneous melanoma, the probability of developing metastatic disease, as measured by the 5 year mortality rates, is closely related to the vertical thickness of the primary tumor [29, 30]. When tumors of various stages were examined for expression of MUC18, it could be seen that thin primary tumors with a very low probability of metastasis development (< 0.75 mm) were negative for this marker, while more than 70% of the metastases were positive (Fig. 3). The expression of MUC18 began to appear on tumors of approximately 1 mm in thickness and increased in frequency and in strength with increasing tumor thickness [31]. This pattern of expression which closely parallels the probability of development of metastatic disease

(Fig. 3a) is what would be expected from molecules which are involved in the development of metastatic potential.

cDNA cloning of MUC18 revealed that it is encoded by a single copy gene and that it is a member of the Ig supergene family [32]. Analysis of available sequence data banks showed that it is a novel molecule and that among the Ig supergene family members it is most closely related to the neural cell adhesion molecules (Table 1) and to the CEA gene family.

Since both these groups of molecules have been shown to function as CAMS, it seems likely that MUC18 also mediates intercellular adhesion. The expression of MUC18 by melanoma cells does not appear to be related to malignant transformation or invasion per se but to a later event in tumor progression which is associated with the establishment of metastases. Melanocytes are neural crest derivatives and migrate long distances during their differentiation. The *de novo* appearance of MUC18 on malignant melanocytes may indicate that it is an oncofetal antigen normally expressed during melanocyte development. Its reexpression on malignant cells may endow them with new adhesive properties which were needed by the developing melanocyte but which now allow new cellular interactions and which contribute to the successful establishment of metastases.

Table 1. Sequence similarity of DCC and MUC18 to neural cell adhesion molecules of the Ig supergene family[a]

	Molecule	% Identity	Overlap (AA)
Muc18	AMALGAM	26.0	169
	CONTACTIN	22.2	117
	DCC	22.5	377
	L1	22.6	288
	MAG	20.8	448
	NCAM	20.4	455
DCC[b]	AMALGAM	25,0	238
	L1	25.0	660
	MAG	28.0	198
	NCAM	24.0	585

[a] Comparisons were carried out using the FASTP algorithm [50].

[b] From reference 24.

Changes in the expression of CAMS mediating leukocyte adhesion are also associated with tumor progression

While the importance of intercellular adhesion in embryonic development has been evident for decades, it is also fundamental to the functioning of the adult organism. This has been most clearly demonstrated in the immune system, where a large number of new CAMs have been recently identified [2, 3]. The immune system consists of leukocytes, cells which travel throughout the circulatory system and migrate into tissues in search of infectious organisms. These cells are specifically attracted to areas of inflammation, and are intimately involved in information exchange with other cells, interactions which lead to the induction and expression of specific immune reactivity against the foreign invaders. These cell interactions are made possible by CAMs present on the leukocytes, endothelial cells and antigen bearing target cells which are regulated by inflammatory cytokines and by stimulation of antigen specific receptors on the T and B lymphocytes. Many of these adhesion molecules belong to the Ig supergene family and several have recently been implicated in tumor progression.

The class I and class II products of the major histocompatibility complex (MHC) function in the binding of foreign antigens and their presentation to T lymphocytes. However, they are also CAMs, specifically binding to ligands on the T cell surface. Cells transfected with cDNA encoding MHC class II molecules can bind to lymphocytes via their CD4 molecule [33], and cells expressing class I molecules bind to lymphocyte CD8 molecules [34]. The CAM function of the MHC molecules strengthens the interaction between the T cells and their targets and appears to be especially important when low affinity T cell receptors are involved. The expression of MHC molecules by tumor cells has been investigated in a variety of human tumors. Class I expression is frequently down regulated in malignant cells while class II expression is frequently induced and, in a variety of tumors, this has been shown to have a prognostic significance [31, 35, 36]. Generally the loss of MHC class I expression is associated with a poor prognosis, while the pres-

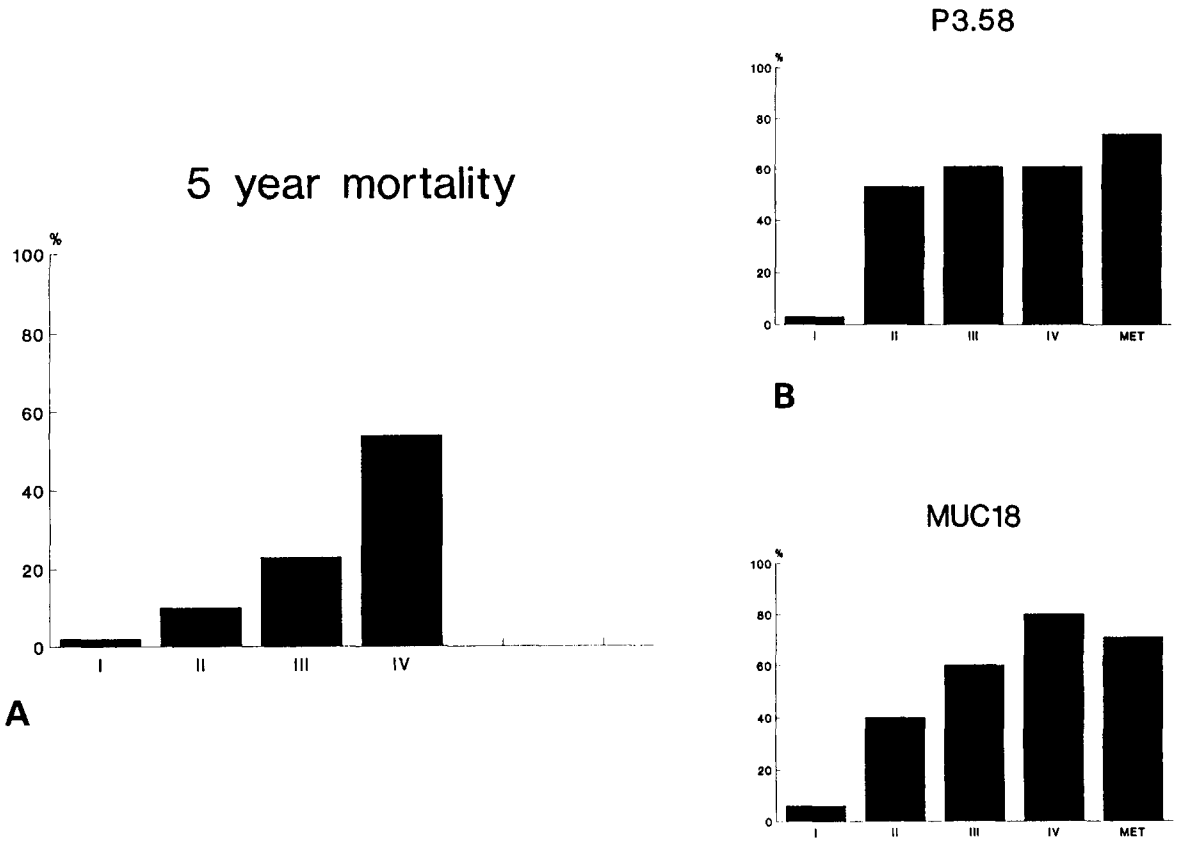

Fig. 3. Expression of P3.58 (ICAM-1) and MUC18 on cutaneous melanomas. The frequency of positive tumors is shown on the Y axis. Primary tumors are divided into 4 groups according to the vertical thickness: I = < 0.75 mm, II = 0.76–1.5 mm, III = 1.6 mm–3.0 mm, IV = > 3.0 mm, MET = metastases. Data is from references 32 and 39. The 5-year mortality data is from reference 29.

ence of class II molecules is associated with a good prognosis. These observations have been interpreted to indicate a role for specific immunological recognition in the progression of human tumors. However, the demonstration that MHC molecules can function as CAMs in the absence of T cell receptor engagement [33, 34] suggests that the expression of these molecules by tumor cells may also enhance antigen independent killing by cytotoxic macrophages or lymphocytes which express CD4 or CD8.

An important CAM, supporting interactions between T lymphocytes and other cell types, is LFA-3 (lymphocyte function associated molecule 3; [2]). LFA-3 is a cell surface glycoprotein found on virtually all cell types (including erythrocytes) and demonstrating a variable degree of N-linked glycosylation which leads to extensive size heterogeneity. Most of the variation in glycosylation reflects sialic acid content, and differences in sialic acid content have been shown to correlate with differences in the binding of LFA-3 to its ligand. LFA-3 interacts with the T lymphocyte CD2 molecule, also a member of the immunoglobulin supergene family. As antibodies to LFA-3 are able to inhibit most T lymphocyte-target cell interactions, it has been suggested that the loss of this molecule by tumor cells might aid in their escape from immune destruction. Evidence for this has in fact been obtained in B cell lymphomas [37]. Very few studies have examined the expression of LFA-3 in solid tumors [35] and therefore its role in the progression of solid tumors remains unclear.

Leukocyte adhesion is also mediated through the

interaction of the lymphokine inducible molecule, ICAM-1 (intercellular adhesion molecule-1) with the leukocyte integrins LFA-1 (lymphocyte function associated molecule-1) and MAC-1 [2]. LFA-1 is expressed by all leukocytes, while the expression of MAC-1 is primarily limited to macrophages and granulocytes. Antibody blocking studies indicate that while this interaction is particularly important in the migration of granulocytes into inflamed tissues it is also involved in effector-target cell interactions.

Although ICAM-1 can be induced by lymphokines on virtually all types of cells examined, it is not commonly observed on solid tumors examined *in situ* [38]. One of the exceptions to this is cutaneous melanoma, where the majority of tumors express ICAM-1 [38, 39]. Since ICAM-1 has been shown to strengthen interactions between immune effectors and target cells, it might be expected that its expression on tumor cells would make them a better target for cytotoxic cells and would therefore be associated with a good prognosis. However in contrast to this expectation, ICAM-1 has an expression pattern on melanomas which suggests that it may be involved in the progression of the tumor to metastatic disease [39].

One of the first monoclonal antibodies which identified ICAM-1 was P3.58, an antibody produced against melanoma cells [40]. Examination of frozen tissue sections of malignant melanoma and benign melanocytic tumors (nevi) revealed that P3.58 reacted only with melanocytes in the malignant lesions and led to the suggestion that it detected an antigen whose expression was associated with malignant transformation in the melanocyte lineage. A study of a large number of tumors of different stages revealed that the P3.58 antigen was not associated with malignant transformation but rather with tumor progression and the development of metastatic potential ([31]: Fig. 3). The antigen was no more frequently expressed on very thin malignant tumors which have a low chance of generating metastases than on the benign melanocytic tumors. However as tumors reached approximately 1 mm in thickness, the frequency of positive tumors jumped to approximately 70%. With increasing thickness, the percent of P3.58 positive cells also increased. Cloning of the P3.58 antigen cDNA from a melanoma expression library revealed the P3.58 antigen to be identical to ICAM-1 cloned from myeloid cells [41]. No evidence was obtained for qualitative differences in the mRNA or genomic organization of ICAM-1 between leukocytes and melanomas, indicating that the gene is not altered in the tumors. The correlation observed between ICAM-1 expression and poor prognosis has recently been confirmed and extended [38]. In a prospective study of stage I melanoma, the expression of ICAM-1 by the tumor was found to be a predictive parameter for the early appearance of metastasis ($P < 0.002$).

The possible role of ICAM-1 in the progression of cutaneous melanoma to metastatic disease remains unclear. *In vitro* studies indicate that as expected the presence of ICAM-1 on melanoma cells enhances their ability to serve as targets for cytotoxic T cells [42]. Perhaps cutaneous melanoma represents a type of tumor which induces predominantly a suppressive immune response. The presence of ICAM-1, strengthening the interactions between immune cells and the tumor targets, would then enhance this suppression [43].

ICAM-1 mediates adhesion with many kinds of activated leukocytes. Primary melanomas, particularly early in their development, are heavily infiltrated with mononuclear cells. When ICAM-1 begins to be expressed, the melanoma cells can now form adhesive interactions with activated lymphocytes, macrophages, and granulocytes present in this infiltrate. This heterotypic adhesion may be strong enough to effectively reduce other adhesive interactions of the melanoma cells and promote the migration of single tumor cells away from the primary tumor. In any case, it should lead to the formation of clusters of tumor cells and leukocytes (Fig. 2c). The formation of such clusters has repeatedly been shown, in experimental systems, to increase the rate of metastasis [44]. In part, this appears to be due to the fact that these clusters tend to arrest in the capillaries, particularly in regions of local inflammation (Fig. 2d; ref. [45]). Numerous studies have shown that the interaction of tumor cells with neutrophils, which can be mediated by ICAM-1, enhances endothelial binding, extravasation, and invasion into the surrounding tissue [46].

Studies using ICAM-1 deficient melanoma cells transfected with ICAM-1 cDNA indicate that the expression of this molecule can indeed lead to melanoma-leukocyte clustering (unpublished observations).

CAMs and tumor progression – A cause and effect relationship?

The function of the Ig supergene family CAMs in directing cell-cell interactions makes them attractive candidates for molecules which play decisive roles in malignant transformation and metastasis formation. That they may indeed function during this process is supported by the fact that alterations in the expression of a number of these molecules is found to correlate with malignant transformation or tumor progression. Even more provocative is the observation that a tumor suppressor gene encodes a potential CAM. However with the exception of CEA, the proposed involvement of the Ig supergene family CAMs in tumor progression lacks experimental support. This reflects, to a large degree, the lack of suitable experimental sytems. Murine models which support the growth of human tumor cells and the development of spontaneous metastases with a pattern similar to that observed in the human patients are now available for a number of different tumors [47, 48]. However, many CAM interactions may not be comparable in a heterologous system; for example, recent studies have shown that human ICAM-1 cannot interact with murine LFA-1 molecules [49]. Analysis of host cell-tumor cell adhesive interactions *in vivo* may therefore only be feasible when the homologous ligands of the tumor cell CAMs are also present and expressed in the relevant tissues. In principle this could be obtained through the use of immune deficient SCID mice reconstituted with human hematopoietic cells [48] or the use of mice transgenic for the relevant ligands.

Why alterations in CAM expression occur during tumorigenesis remains one of the most important unanswered questions. While this may, as in the case of DCC, sometimes be due to genetic changes, there is no evidence that this is a common mechanism [32, 39]. CAMs are developmentally and environmentally regulated molecules, and it seems likely that their expression pattern during tumor progression reflects a disturbance at the level of the molecular elements normally responsible for controlling their expression. For the majority of Ig supergene family CAMs these elements remain undefined. The identification of these mechanisms may shed light, not only on the normal expression of these molecules, but also on the changes which occur during the development and progression of human tumors.

Conclusions

- Many molecules which mediate intercellular adhesion belong to the immunoglobulin supergene family, suggesting that they may have evolved from a common precursor.
- Changes in CAM expression are associated with malignant transformation and tumor progression in a number of different human tumors. Such changes can involve the loss of expression, *de novo* expression or expression of a functionally altered molecule.
- New putative CAMs (DCC and MUC18) have been discovered because of differential gene expression in benign and malignant cells.
- CAMs mediating the interaction between leukocytes and other cell types also appear to be altered during tumor progression, and this may indicate a role for the immune system in this process.

Key unanswered questions

- Are the changes in CAM expression which are observed during tumor progression actually involved in this process or merely secondary phenomena?
- The expression by solid tumors of CAMs mediating leukocyte adhesion has often been found to be of prognostic significance. Does the immune system play a role in the progression of tumors?

- What are the factors which cause the expression of CAMs to be altered at particular stages in tumor progression? How many of these factors are exogenous?

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, SFB 217 (A3) and the Deutsche Krebshilfe, Mildred Scheel Stiftung, Bonn, Germany.

References

1. Edelman GM: Cell adhesion molecules in the regulation of animal form and tissue pattern. Ann Rev Cell Biol 2: 81–116, 1986
2. Springer TA: Adhesion receptors of the immune system. Nature 346: 425–434, 1990
3. Patarroyo M, Makgoba MW: Leukocyte adhesion to cells in immune and inflammatory responses. Lancet ii: 1139–1142, 1989
4. Williams AF, Barclay AN: The immunoglobulin superfamily-domains for cell surface recognition. Ann Rev Immunol 6: 381–405, 1988
5. Covault J: Molecular biology of cell adhesion in neural development. In: Glover DM, Hames BD (eds) Molecular Neurobiology. IRL Press, Oxford, pp 143–200, 1989
6. Rutishauser U, Jessel TM: Cell adhesion molecules in vertebrate neural development. Physiol Rev 68: 819–857, 1988
7. Hoffman S, Edelman GM: Kinetics of homophilic binding by embryonic and adult forms of the neural cell adhesion molecule. Proc Natl Acad Sci USA 80: 5762–5766, 1983
8. Jaffe SH, Froedlander DR, Matsuzaki F, Crossin KL, Cunningham BA, Edelman GM: Differential effects of the cytoplasmic domains of cell adhesion molecules on cell aggregation and sorting out. Proc Natl Acad Sci USA 87: 3587–3593, 1990
9. Rutishauser U, Acheson A, Hall AK, Sunshine J: NCAM as a regulator of cell-cell interactions. Science 240: 53–57, 1988
10. Patel K, Bourne S, Phimister B, Coakham H, Kemshead JT: Presence of neural cell adhesion molecule on human embryonic and brain tumors. Biochem Soc Trans 18: 408–410, 1990
11. Roth J, Zuber C, Wagner P, Taatjes DJ, Weisgerber C, Heitz PU, Goridis C, Bitter-Suermann D: Reexpression of poly(sialic acid) units of the neural cell adhesion molecule in Wilms tumor. Proc Natl Acad Sci USA 85: 2999–3003, 1988
12. Thompson J, Zimmermann W: The carcinoembryonic antigen (CEA) gene family: structure, expression and evolution. Tumor Biol 9: 63–83, 1988
13. Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K, Stanners CD: Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell 57: 327–334, 1989
14. Oikawa S, Inuzuka C, Kuroki M, Matsuoka Y, Kosati G, Nakazato H: Cell adhesion activity of nonspecific cross reactive antigen (NCA) and carcinoembryonic antigen (CEA) expressed on CHO cell surface: homophilic and heterophilic adhesion. Bioch Biophys Res Comm 164: 39–45, 1989
15. Wanebo HJ, Rao B, Pinsky CM, Hoffman RG, Stearns M, Schwartz MK, Oettgen HF: Preoperative carcinoembryonic antigen level as a prognostic indicator in colorectal cancer. N Engl J Med 299: 448–451, 1978
16. Toth CA, Rapoza A, Kowal A, Steele G, Thomas P: Receptor-mediated endocytosis by human Kupffer cells. Bioch Soc Trans 16: 1027–1028, 1988
17. Bryn RA, Medrek P, Thomas, P *et al.*: Effect of heterogeneity of carcinoembryonic antigen on liver cell membrane binding and its kinetics of removal from circulation. Cancer Res 45: 3137–3142, 1985
18. Jessup JM, Giavazzi R, Campbell D, Cleary K, Morikawa K, Fidler IJ: Growth potential of human colorectal carcinomas in nude mice: association with the preoperative serum concentration of carcinoembryonic antigen in patients. Cancer Res 48: 1689–1692, 1988
19. Hostetter RB, Augustus LB, Mankarious R, Chi K, Fan D, Toth C, Thomas P, Jessup JM: Carcinoembryonic antigen as a selective enhancer of colorectal cancer metastasis. JNCI 82: 380–385, 1990
20. Farber E, Cameron R: The sequential analysis of cancer development. Adv Cancer Res 31: 125–226, 1980
21. Weinstein IB: The origins of human cancer: molecular mechanisms of carcinogenesis and their implications for cancer prevention and treatment. Cancer Res 48: 4135–4143, 1988
22. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakumura Y, White R, Smits AMM, Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525–532, 1988
23. Lee WH, Bookstein R, Hong F *et al.*: Human retinoblastoma susceptibility gene: cloning, identification and sequence. Science 235: 1394–1399, 1987
24. Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW, Vogelstein B: Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49–56, 1990
25. Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61: 759–767, 1990
26. Clark WH, Elder DE, Guerry D IV, Epstein MN, Greene MH, Horn M van: A study of tumor progression. The precursor lesions of superficial spreading and nodular melanoma. Human Pathol 15: 1147–1165, 1984

27. Johnson JP, Stade BG, Rothbächer U, Lehmann JM, Riethmüller G: Melanoma cell antigens and their association with malignant transformation and tumor progression. In: Ferrone S (ed) Human Melanoma: Immunology, Diagnostics and Therapy. Springer Verlag, Berlin, pp 106–118, 1990
28. Lehmann JM, Holzmann B, Breitbart EW, Schmiegelow P, Riethmüller G, Johnson JP: Discrimination between benign and malignant cells of the melanocytic lineage by two novel antigens, a glycoprotein with a molecular weight of 113,000 and a protein with a molecular weight of 76,000. Cancer Res 47: 841–845, 1987
29. Sober AJ: Prognostic factors for cutaneous melanoma. In: Ruiter, Welvaart, Ferrone, Cutaneous melanoma and precursor lesions, pp 126–142 (Nijhoff, Boston), 1984
30. Breslow A: Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 172: 902–908, 1970
31. Holzmann B, Bröcker EB, Lehmann JM, Ruiter DJ, Sorg C, Riethmüller G, Johnson JP: Tumor progression in human malignant melanoma: five stages defined by their antigenic phenotypes. Int J Cancer 39: 466–471, 1987
32. Lehmann JM, Riethmüller G, Johnson JP: MUC18, a marker of tumor progression in human melanoma shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. Proc Natl Acad Sci USA 86: 9891–9895, 1989
33. Doyle C, Strominger JL: Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature 330: 256–259, 1987
34. Norment AM, Salter RD, Parham P, Engelhard VH, Littman DR: Cell-cell adhesion mediated by CD8 and MHC class I molecules. Nature 336: 79–81, 1988
35. Smith MEF, Marsh SGE, Bodmer JG, Gelsthorpek K, Bodmer WF: Loss of HLA-A, B, C allele products and lymphocyte function-associated antigen 3 in colorectal neoplasia. Pro Natl Acad Sci USA 86: 5557–5561, 1989
36. Esteban F, Concha A, Huelin C, Perez-Ayala M, Pedrinaci S, Ruiz-Cabello F, Garrido F: Histocompatibility antigens in primary and metastatic squamous cell carcinoma of the larynx. Int J Cancer 43: 436–442, 1989
37. Gregory CD, Murray RJ, Edwards CF, Rickinson AB: Downregulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus positive Burkitt's lymphoma underlies tumor cell escape from virus-specific T cell surveillance. J Exp Med 167: 1811–1824, 1988
38. Natali P, Nicotra MR, Cavaliere R, Bigotti A, Romano G, Temponi M, Ferrone S: Differential expression of intercellular adhesion molecule 1 in primary and metastatic melanoma lesions. Cancer Res 50: 1271–1278, 1990
39. Johnson JP, Stade BG, Holzmann B, Schwäble W, Riethmüller G: *De novo* expression of cell adhesion molecule ICAM-1 in melanoma and increased risk of metastasis. Proc Natl Acad Sci 86: 641–644, 1989
40. Holzmann B, Johnson JP, Kaudewitz P, Riethmüller G: *In situ* analysis of antigens on malignant and benign cells of the melanocyte lineage: Differential expression of 2 surface molecules, gp 75 and gp 89. J Exp Med 161: 366–377, 1985
41. Johnson JP, Stade BG, Hupke U, Holzmann B, Riethmüller G: The melanoma progression associated antigen P3.58 is identical to the intercellular adhesion molecule ICAM-1. Immunobiol 178: 275–284, 1988
42. Braakman, Goedegebuure PS, Vreugdenhil RJ, Segal DM, Shaw S, Bolhuis RLH: ICAM-melanoma cells are relatively resistant to CD3- mediated T cell lysis. Int J Cancer 46: 475–480, 1990
43. Guerry D, Alexander MA, Zehngebot L, Herlyn M, Mitchell KF, Zmijewski CM: HLA-DR antigens permit human melanoma cells from early but not advanced disease to stimulate autologous lymphocytes. J Clin Invest 73: 267, 1984
44. Blood CH, Zetter BR: Tumor interactions with the vasculature: angiogenesis and tumor metastasis. Biochim et Biophys. Acta 1032: 89–118, 1990
45. Starkey JR, Liggitt HD, Jones W, Hosick HL: Influence of migratory blood cells on the attachment of tumor cells to vascular endothelium. Int J Cancer 34: 535–543, 1984
46. Aeed PA, Nakajima M, Welch DR: The role of polymorphonuclear leukocytes (PMN) on the growth and metastatic potential of 13762NF mammary adenocarcinoma cells. Int J Cancer 42: 748–759, 1988
47. Cornil I, Man S, Fernandez B, Kerbel RS: Enhanced tumorigenicity, melanogenesis, and metastases of a human malignant melanoma after subdermal implantation in nude mice. JNCI 81: 938–944, 1989
48. Bankert RB, Umemoto T, Sugiyama Y, Chen FA, Repasky E, Yokota S: Human lung tumors, patients' peripheral blood lymphocytes and tumor infiltrating lymphocytes propagated in scid mice. Curr Top Microbiol Immunol 152: 201, 1989
49. Johnston SC, Dustin ML, Hibbs ML, Springer TA: On the species specificity of the interaction of LFA-1 with intercellular adhesion molecules. J Immunol 145: 1181–1187, 1990
50. Lipman DJ, Pearson WR: Rapid and sensitive protein similarity searches. Science 227: 1435–1439, 1985

Address for offprints:
J.P. Johnson,
Institute for Immunology,
Goethestrasse 31,
8000 Munich 2, Germany