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Invited Review

Adhesion molecules as targets for cancer therapy

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Summary. Adhesion molecules mediate cell-cell and cell-matrix interactions and are essential for numerous physiological and pathological processes. Recent evidence from many laboratories suggests that adhesion molecules play an important role in tumor progression and may promote tumor growth and organ-specific metastasis. Certain adhesion molecules may also function as tumor suppressors. In this review, we describe current concepts concerning the role of the adhesion molecules in the pathogenesis of cancer and the development of therapeutic approaches which make use of this information. Hence, by preventing tumor cells from interacting with each other or with their microenvironment, tumor growth and metastasis can be suppressed. The feasibility of using anti-adhesion strategies to treat cancer has been demonstrated in many animal models. Thus, monoclonal antibodies (MAbs) against adhesion molecules, synthetic peptidic and nonpeptidic analogues of the recognition sequences on their receptors, soluble adhesion molecules and antisense oligonucleotides can inhibit tumor growth and gene therapy can restore the functions of altered tumor-suppressive adhesion molecules.

Key words: Adhesion molecule, Cancer therapy, Tumor metastasis, Monoclonal antibody

Introduction

Adhesion molecules are widely expressed on the cell surface, basement membrane and extracellular matrix (ECM). They mediate cell-cell and cell-matrix interactions which are critical for a variety of physiological and pathological processes such as cell growth, differentiation and trafficking, embryogenesis, immune responses, inflammation, blood coagulation, wound repair and tumor development (Springer, 1990; Buck, 1995; Dedhar, 1995). Adhesion molecules include several distinct families such as integrins, cadherins, members of the immunoglobulin (Ig) superfamily, selectins, and some cell surface proteoglycans (Table 1). In addition to their role in adhesion, recent studies have demonstrated that these molecules may also function as signal transducers to regulate various cellular functions through G-proteins, phospholipids and protein kinases (Parsons, 1996; Ruoslahti, 1995). Alterations in the adhesive properties of tumor cells or the tumor microenvironment have been implicated in tumorigenesis and in the biological behavior of many malignancies (Albelda, 1993; Buck, 1995; Glukhovo et al., 1995). In this review, we will emphasize recent advances in our understanding of the role of adhesion molecules in tumor pathogenesis and in the development of antiadhesion approaches for improving cancer therapy.

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The role of adhesion molecules in tumor growth and metastasis

Numerous studies have indicated that adhesion molecules are involved in the growth, invasion, and metastatic properties of many types of tumors (Albelda, 1993; Juliano and Varner, 1993; Buck, 1995). An increase or decrease in the adhesion of tumor cells to neighboring tumor or host cells, ECM and endothelial cells (ECs) may occur at different stages of tumor development and progression. Different adhesion molecules may also be involved in different types of tumors. In this review, we focus only on those adhesion molecules known to be associated with tumor growth and metastasis (Table 1).

Integrins are the largest family of cell adhesion molecules that mediate cell-matrix and cell-cell adhesion. They are transmembrane heterodimers composed of an α-chain and a ß-chain. At least 15 α and 9ß subunits have been described. An α-subunit can associate with more than one ß-subunit to form different integrin molecules, and vice versa. Integrins on the cell surface serve as receptors for many proteins such as the Ig superfamily and cell-matrix proteins including fibronectin (FN), vitronectin (VN), laminin (LM) and collagen. Most integrins have multiple ligand specificities, and more than one integrin can often bind to the same ligand (Buck, 1995). A short amino acid

Table 1. Classification of cell adhesion molecules.

FAMILY	NAME	CLUSTER OF DIFFERENTIATION (CD)	LIGAND
Integrins			
I ß1 (very late activation antigens)			
	α1ß1 (VLA-1)	CD49a/CD29	Coll, LM
	α2ß1 (VLA-2)	CD49b/CD29	Coll, LM, FN
	α3ß1 (VLA-3)	CD49c/CD29	LM, Coll, FN
	α4ß1 (VLA-4)	CD49d/CD29	FN, VCAM-1, ICAM-2
	α5ß1 (VLA-5)	CD49e/CD29	FN, INV
	α6ß1 (VLA-6)	CD49f/CD29	LM, INV
	αVß1	CD51/CD29	FN
II ß2 (LeuCAM)			
	αLß2 (LFA-1)	CD11a/CD18	ICAM-1, -2, -3, E-selectin
	αMß2(Mac-1, CR3)	Cd11b/CD18	ICAM-1, FG, C3bi, Factor X, endotoxin
	αXß2 (p150/95, CR4)	CD11c/CD18	FG, C3bi
	αdß2	-/CD18	ICAM-3
III ß3 (cytoadhesins)			
	αVß3	CD51/CD61	FG, FN, vWF, VN, TSP
	αIIbß3	CD41/CD61	FG, FN, vWF, VN, TSP
IV Other integrins			
ß4	α6ß4	CD49f/CD104	LM
ß5	αVß5	CD51/-	VN
ß6	αVß6	CD51/-	VN
ß7	α4ß7	CD49d/-	FN, VCAM-1, MAdCAM-1
ß8	αVß8	CD51/-	FN
Immunoglobulin superfamily			
	ICAM-1	CD54	LFA-1, Mac-1, CD43
	ICAM-2	CD102	LFA-1
	ICAM-3	CD50	LFA-1, αdß2
	VCAM-1	CD106	α4ß1, α4ß7
	LFA-2	CD2	LFA-3, CD59
	LFA-3	CD58	LFA-2
	CD4	CD4	MHC class II
	CD8	CD8	MHC class I
	PECAM-1	CD31	PECAM-1, α4ß7, Vß3, heparin
	MAdCAM-1	-	α4ß7, L-selectin
	N-CAM	CD56	N-CAM, heparan sulphate, heparin
	C-CAM	-	C-CAM
	CEA	CD66e	CEA, integrins
	MUC18	-	?
	DCC	-	?
Cadherins			
	E-cadherin	-	E-cadherin
	N-cadherin	-	N-cadherin
	P-cadherin	-	P-cadherin
Selectins			
	P-selectin (GMP-140)	CD62P	CD15S, PSGL-1
	E-selectin (ELAM-1)	CD62E	CD15S, CD66, ESL-1
	L-selectin (Leu8, LECAM-1)	CD62L	CD15S, PNAd, MAdCAM-1, E-selectin, CD34, glycan-1, P-selectin
Cell surface proteoglucan			
	H-CAM	CD44	HA, Coll, FN

Coll: collagen; ESL: E-selectin ligand; FN: fibronectin; FG: fibrinogen; HA: hyaluronic acid; INV: invasin; LM: laminin; PNAd: peripheral lymph node vascular adhesion molecules; TSP: thrombospondin; VN: vitronectin; vWF: von Willebrand's factor.

sequence Arg-Gly-Asp (RGD) present in FN, VN and a variety of other adhesive proteins is a major recognition site of several integrins such as $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_{IIb}\beta_3$, (Akiyama et al., 1995). Other recognition sequences have been identified in FN. The cell surface integrin adhesion receptors also recognize Leu-Asp-Val (LDV) and Arg-Glu-Asp-Val (REDV) in FN. The Tyr-Ile-Gly-Ser-Ag (YIGSR) sequence in LM mediates the binding of LM to the $\alpha_6\beta_1$ integrin receptor (Humphries et al., 1987; Komoriya et al., 1991).

A number of studies have suggested that interactions of integrins with their ligands are associated with tumorigenicity and tumor progression (Akiyama et al., 1995; Buck, 1995) and altered expression of integrins on tumor cells can change their adhesive properties and biological behavior (Buck, 1995; Glukhovo et al., 1995). Signaling through integrin engagement can both protect against and promote apoptosis of tumor cells (Brooks et al., 1994; Montgomery et al., 1994; Ruoslahti, 1995). In invasive and metastatic melanoma the $\alpha_v\beta_3$, (receptor of VN, FN and LM) and $\alpha_4\beta_1$ [VCAM-1(CD106) and FN receptor] molecules are upregulated. $\alpha_v\beta_3$ has been implicated in enhancing the invasiveness of melanoma cells *in vitro* and in increasing melanoma growth *in vivo* (Felding-Habermann et al., 1992; Juliano and Varner, 1993). $\alpha_4\beta_1$ mediates the binding of melanoma cells to endothelial cells and may be involved in promoting metastasis (Albelda et al., 1990; Albelda, 1993).

A systematic study of integrin expression in normal colon, adenomas and carcinomas within the same patient has shown that as cells transit from adenomas to carcinomas there is a progressive loss in expression of both $\alpha_3\beta_1$, receptors for collagen and LM and $\alpha_5\beta_1$ receptors for FN (Pignatelli et al., 1990; Stallmach et al., 1992). It also has been suggested that $\alpha_5\beta_1$ may function as a negative growth regulator when it is not bound to its ligand, whereas when the receptor is occupied, the negative signal is relieved and/or positive growth signal is generated (Juliano and Varner, 1993). A decrease in expression of $\alpha_5\beta_1$ increases the tumorigenicity of Chinese hamster ovary (CHO) cells in nude mice (Ruoslahti, 1995). In contrast, increased expression of the $\alpha_5\beta_1$ in CHO and human colon cancer cells inhibits their tumorigenicity *in vivo* (Giancotti and Ruoslahti, 1990; Ruoslahti, 1995). However, in the absence of serum and growth factors, the binding of $\alpha_5\beta_1$ to FN might prevent apoptosis of tumor cells by enhancing the expression of bcl-2 (Zhang et al., 1995). Studies using human prostate cancer cells indicate that expression of $\alpha_6\beta_1$ and the loss of $\alpha_6\beta_4$ in tumor cells might confer an invasive phenotype (Cress et al., 1995). The α_v integrins also appear to be important in tumor growth. For example, Agrez et al. (1994) have demonstrated that $\alpha_v\beta_6$ enhances the growth of colon cancer cells *in vitro* and *in vivo*, while Friedlander et al. (1996) reported that anti-α_v and β_1 antibodies completely inhibited the migration of astrocytoma cells. Antibodies against $\alpha_v\beta_3$ blocked intercellular adhesion and organoid formation of colon carcinoma cells, resulting in rapid apoptosis of these tumor cells (Bates et al., 1994). The survival and

growth of human melanoma cells within a three-dimensional dermal collagen matrix also riquired ligation of $\alpha_v\beta_3$ to dermal collagen. Disruption of this interaction with an anti $\alpha_v\beta_3$ MAb induced cell death (Montgomery et al., 1994). Moreover, $\alpha_v\beta_3$ on tumor cells can bind to matrix metalloproteinase MMP-2 in a proteolytically active form and facilitate cell-mediated collagen degradation and thus invasion (Brooks et al., 1996). In addition, it has been demonstrated that $\alpha_v\beta_3$ is necessary for proliferation and maturation of newly forming blood vessels, an event essential for the growth and metastasis of human tumors (Drake et al., 1995). Recently, Kostenuik et al. (1996) reported that human prostatic carcinoma cells adhere to bone matrix via the $\alpha_2\beta_1$ integrin collagen receptor, and that collagen-derived peptides and antibodies against the α_2 or β_1 integrin subunits inhibit this interaction. Therefore, the integrins may be involved at several levels in both tumor development and progression.

The Ig superfamily includes molecules which function in cellular immunity (i.e., the MHC antigens, CD2, CD4, CD8 and the T cell receptor) and leucocyte trafficking [ICAM-1 (CD54), ICAM-2(CD102), PECAM-1 (CD31) and VCAM (CD106)], as well as neural cell adhesion molecule (N-CAM, CD56), epithelium-specific adhesion molecule C-CAM, carcinoembryonic antigen (CEA), «deleted in colorectal carcinoma» (DCC), vascular addressin, MAdCAM-1 and MUC18 (Johnson, 1991; Zhu et al., 1991; Kleinerman et al., 1995a,b). These molecules contain Ig homology units in their extracellular domain and they can mediate homotypic and heterotypic adhesion. Many reports have suggested that *de novo* expression or secretion of CD54 may be involved in the pathogenesis and prognosis of many types of tumors including hematopoietic and epithelial neoplasms (Johnson et al., 1989; Natali et al., 1990; Huang et al., 1995; Santarosa et al., 1995). Indeed, increased expression of CD54 on melanoma and lymphoma cells correlates with an increased risk of metastasis and a reduced number of disease-free remissions (Johnson et al., 1989; Stauder et al., 1989; Natali et al., 1990). CD106, expressed on endothelial cells, can mediate the attachment of $\alpha_4\beta_1^+$ tumor cells to endothelium and facilitate metastasis (Albelda et al., 1990). MUC18 has been identified as a progression-associated molecule in melanoma (Lehmann et al., 1989) and there is a significant correlation between MUC18 expression in melanoma cells and their ability to form metastases (Luca et al., 1993; Edward, 1995). DCC is a transmembrane protein with many similarities to the N-CAM family: 1) it is encoded by a gene that is deleted in colorectal carcinoma (Fearon et al., 1990); 2) decreased or absent DCC expression is a common alteration in several types of tumors (Fearon and Pierceall, 1995); 3) inactivation of this gene may lead to abnormalities in cell-cell interaction and the malignant phenotype. Increased CEA expression is associated with malignant transformation and high levels of CEA have been found in many human adeno-carcinomas. CEA mediates homotypic aggregation of tumor cells as well as the homing of colorectal cancer cells to liver (Johnson, 1991; Jothy et al., 1995). Kleinerman and colleagues (1995a,b) have demonstrated that C-CAM, an androgen-regulated cell adhesion molecule, acts as a tumor suppressor in prostate cancer. Transfection of an antisense C-CAM vector into a nontumorigenic prostate epithelial cell line, results in tumor formation in nude mice, while expression of C-CAM in a human prostate cancer cell line suppressed tumor growth *in vitro* and tumorigenicity *in vivo* (Hsieh et al., 1995). More recently, Zhu et al. (1992) showed that a lung-specific endothelial cell adhesion molecule (Lu-ECAM-1) which is constitutively present on lung endothelial cells, mediated metastasis of murine melanoma cells to the lung. Anti-Lu-ECAM-1 MAbs markedly reduced lung metastasis.

Cadherins are calcium-dependent cell-cell adhesion molecules. There are at least three subclasses of cadherins including epithelial cadherin (E-cadherin), neural and muscular cadherin (N-cadherin) and placental cadherin (P-cadherin). E-cadherin is expressed in almost all epithelia and plays a key role in the maintenance of epithelial structure. Inactivation of E-cadherin causes the disruption of cell-cell adhesion, and over-expression of E-cadherin by genetic manipulation induces tighter cell adhesion (Shiozaki, 1996). E-cadherin has been implicated as a suppressor of invasion since a decrease or absence of this adhesion molecule leads to an increased invasive potential of epithelial tumor cells and is associated with the progression of several kinds of epithelial neoplasma (Frixen et al., 1991; Matsuura et al., 1992; Giroldi et al., 1994; Shiozaki et al., 1996). Transfection of an E-cadherin-expressing vector into highly malignant epithelial tumor cells abrogated their invasiveness, while treatment with anti-E-cadherin antibodies reinduced invasiveness of these transfectants (Vleminckx et al., 1991). Moreover, noninvasive ras-transformed epithelial cells were rendered invasive by down-regulating E-cadhering expression following introduction of a plasmid encoding E-cadherin-specific antisense RNA (Vleminckx et al., 1991). These studies suggest that impaired functions of E-cadherin may contribute to the release of cancer cells from the primary lesion and trigger invasion and metastasis (Shiozaki et al., 1996).

Selectins are glycoproteins that have a lectin-like domain which binds to specific carbohydrate structures, such as the sialyl Lewis^x (SLe^x, CD15s) and sialyl Lewis^a (SL^a) antigens. Three types of selectins have been described, i.e., E-selectin (ELMA-1, CD62E), L-selectin (Leu-8, CD62L) and P-selectin (GMP-140, CD62P). These molecules mediate cellular interactions with the vascular endothelium and are involved in lymphocyte rolling, in recruiting leukocytes to sites of inflammation, in the activation of neutrophils and in lymphocyte homing (Crokett-Torabi and Fantone, 1995). Tumor cells show increased expression of CD15s and SLe^a (Fukuda, 1996). Highly metastatic colonic

carcinoma cells bind more avidly to CD62E on activated human endothelial cells than on low-metastatic counterparts (Fukuda, 1996). Patients with higher levels of CD15s on their tumors have a poorer prognosis than those with lower levels of CD15s (Nakamori et al., 1993). Furthermore, Saiki et al. (1996) demonstrated that anti-CD62E antibodies or synthetic CD15s inhibit tumor cell attachment to layers of activated ECs, suggesting that E-selectin on ECs may be important for the adhesive interactions of the endothelium with CD15s-expressing tumor cells in target organs to form metastasis. P-selectin is also expressed on endothelium and may facilitate the arrest of tumor cells in microvessels by mediating the binding of platelets to certain tumor cells, forming tumor cell clumps and aggregates in the circulation (Stone and Wagner, 1993).

CD44 is a broadly distributed cell surface proteoglycan which is expressed in a variety of isoforms with different molecular weights. CD44 interacts primarily with hyaluronate in the extracellular environment and pericellular layer and mediates cell-cell and cell-matrix binding. It may also bind to FN and collagen (Yamaguchi et al., 1996). It is involved in hematpoiesis, lymphocyte homing and leukocyte activation (Lazaar and Pure, 1995). CD44 has also been associated with tumor cell proliferation and metastasis (Lokeshwar et al., 1995). The upregulation of CD44 or the expression of certain isoforms of CD44 have been linked to the increased aggressiveness and metastatic potential of many human tumors (Koopman et al., 1993; Tanabe et al., 1993; Lokeshwar et al., 1995; Ermak et al., 1996; Yamaguchi et al., 1996). In rat pancreatic and mammary adenocarcinomas, expression of $CD44v_6$, a splice variant of CD44, correlates with their metastatic properties. Hence, overexpression of this variant in several non-metastasizing tumor cell lines confers full metastatic behavior to these cells (Gunthert et al., 1991; Rudy et al., 1993). Expression of a hyaluorate-binding form of CD44 (CD44H) in human tumors significantly enhances their rate of local growth and hematogenous dissemination in nude mice (Sy et al., 1991) since the capacity of CD44 to mediate tumor cell attachment to hyaluronate determines the rate of formation of the resulting tumor mass (Bartolazzi et al., 1994). It has been thought that hyaluronate might serve as a molecular bridge, promoting tumor cell interactions with host tissue ECM components which are critical for growth of both primary and metastatic tumors (Bartolazzi et al., 1994). Alternatively it may be important in the initial adhesion step leading to extravasation from the blood, as recently shown (DeGrendele et al., 1996).

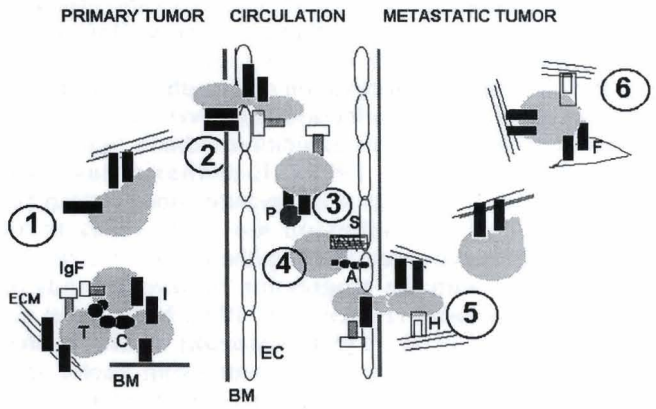

Fig. 1. A multistep process of tumor progression involving adhesion molecules. **1. Detachment and invasion.** In the primary tumor, tumor cells adhere to neighboring tumor or host cells and to extracellular matrix. The decrease of intercellular contacts and the increase in cell-matrix interactions facilitate the detachment of tumor cells from the primary tumor and morphological transitions to a more motile cell which migrates through extracellular matrix. **2. Intravasation.** The adhesion of tumor cells to extracellular matrix proteins in the subendothelium and basal surface of endothelial cells mediates the entry of tumor cells into capillary beds and lymphatic vessels. **3. Tumor embolus formation.** The tumor cells are protected against circulating immune cells by binding to platelets and to other macromolecules through integrin receptors. **4. Selection of the site of metastasis.** Tumor cells manifest a preference for binding to the endothelium in specific organs. Differential expression and affinity of adhesion receptors on luminal surfaces of endothelial cells determine the organ-specific adhesion of circulating tumor cells. **5. Extravasation.** The binding of tumor cells to endothelial cells induces retraction of endothelial cells, thereby exposing the subendothelial matrix. The interactions of tumor cells with adhesion receptors on the basal surface of endothelial cells and ECM proteins direct the extravasation of tumor cells. **6. Metastatic tumor formation.** The activation of adhesion receptors on tumor cells facilitates the following steps of the metastatic cascade: migration of tumor cells into tissue stroma, binding to surrounding host cells and ECM, induction of angiogenesis and formation of metastatic tumor. T: tumor cells; BM: basement membrane; S: stroma; EC: endothelial cells; P: platelet; F: fibroblast; C: cadherins; IgF: Ig superfamily; I: integrins; S: selectins; A: addressin; H: hyaluronate receptor; ECM: extracellular matrix.

Tumor progression is a dynamic process likely to be determined by the interactions of tumor cells with each other, with host cells and with the ECM. Adhesion receptors may also transmit growth stimulatory and/or inhibitory signals to tumor cells. Therefore, alterations in their expression or function may contribute to the uncontrolled proliferation and metastasis of malignant cells (Pignatelli and Wilding, 1996). Loss in expression or function of some adhesion molecules, such as E-cadherin and certain integrins, in tumor cells may promote the detachment of the cells form the primary tumors. Upregulation of certain adhesion molecules (i.e., CD44, integrins containing α_v or α_6) may enhance tumor invasion through adjacent stroma and intravasation through endothelial basement membrane and between endothelial cells. In the circulation, integrins and members of Ig superfamily mediate the adhesion of tumor cells to each other and activate platelets to form tumor-platelet aggregates. Fibrinogen, FN and thrombospondin in plasma may bridge tumor cells to platelets or endothelial cells through their adhesion receptors. The $\alpha_{IIb}ß_3$ receptor and P-selectin on platelets are also important in the formation of tumor emboli (Honn et al., 1995). The attachment of tumor cells to the endothelium in selective target organs is directed by integrins, selectins, organ-specific endothelial adhesion molecules and members of the Ig superfamily (Santarosa

et al., 1995; Kleinerman et al., 1995b). Finally, adhesion molecules are required in cellular extravasation, invasion of the subendothelial matrix, migration into the tissue parenchyma, neovascularization and the formation of metastasis. These processes represent potential targets for antiadhesion therapy (Fig. 1).

The development of new therapies involving anti-adhesive molecules

Knowing the importance of adhesion molecules in tumor growth, invasion, and metastasis has provided a rationale for developing new therapies. It is believed that most malignant cells use adhesion molecules to promote their growth and guide their dissemination into particular organs. Thus, by preventing tumor cells from interacting with each other or with their microenvironments, tumor growth and metastasis should be suppressed. In this regard, several approaches aimed at inhibiting the activities of adhesion molecules have been studied *in vitro* and in experimental animals and some have been evaluated in clinical trials for inflammatory diseases (Davis et al., 1995; Kavanaugh et al., 1994), transplantation (Mauff et al., 1996) and cardiovascular diseases (Jordan et al., 1996). Due to their specificity and unlimited availability, the most common approach is to use monoclonal antibodies (MAbs) which recognize adhesion molecules. Many studies have shown that neutralizing MAbs which target adhesion molecules are effective in inhibiting invasion, dissemination and/or proliferation of tumor cells in animal systems (Ruiz et al., 1993; Saiki et al., 1993; Zahalka et al., 1993; Edward, 1995; Newton et al., 1995). By directly interfering with cell-cell or cell-ECM interactions, these antibodies may disrupt invasion processes of tumor cells or induce adhesion-dependent apoptosis (Bates et al., 1994, 1995). Many adhesion molecules can also serve as signal transducing molecules and thus, antibodies directed against adhesion molecules may induce negative signals in tumor cells resulting in apoptosis or growth arrest (Lokeshwar et al., 1995; Newton et al., 1995). Other attractive approaches to inhibit tumor growth and metastasis involve the development of simple synthetic peptides (Humphries et al., 1986, 1988; Kleinerman et al., 1989; Kumagai et al., 1991), non-peptidic antagonists of adhesion molecules (Greenspoon et al., 1993; Harden et al., 1993) as well as soluble adhesion molecules including recombinant forms and proteolytic fragments (McCarthy et al., 1986, 1988; Barsky et al., 1988; Sy et al., 1992; Bartolazzi et al., 1994). These bind to recognition sites on adhesion receptors and compete with cell or matrix-associated natural adhesion molecules.

Angiogenesis is also necessary for tumor development and growth. Recently, some adhesion molecules have been found to play crucial roles in tumor neovascularization (Brooks et al., 1994). Antagonists of these adhesion molecules can induce apoptosis of proliferating angiogenic blood vessels without affecting preexisting quiescent blood vessels. They may thus disrupt ongoing angiogenesis in tumors, and consequently suppress tumor progression (Brooks et al., 1994, 1995). Cytokines can alter the expression of adhesion molecules on tumors and change their biological behavior (Garofalo et al., 1995; Herzber g et al., 1996). Hence, they may enhance tumor metastasis by upregulating the expression of certain adhesion molecules on endothelial cells or tumor cells. These effects can be blocked by specific cytokine antagonists. On the other hand, some cytokines may diminish the metastatic potential of tumors by changing the patterns of expression of adhesion molecules (Herzberg et al., 1996). Other approaches includes restoring the functions of tumor-suppressive adhesion molecules or specifically downregulating the expression of progression-associated adhesion molecules in tumor cells with pharmaceutic agents or by genetic approaches (Chiang et al., 1991; Lallier and Bronner-Fraser, 1993; Bennett et al., 1994; Jiang et al., 1995; Kleinerman et al., 1995a,b). Finally, combinations of anti-adhesion and chemotherapeutic agents are also being explored (Saiki et al., 1993).

Current status of anti-adhesion molecule therapy in cancer

Due to the importance of FN-integrin interactions in tumor cell migration, invasion and metastasis, interruption of these interactions may have significant antitumor effects. Several anti-integrin antibodies inhibit experimental metastasis in different tumor models by different mechanisms (Yamada et al., 1990; Newton et al., 1995). Thus, Akiyama et al. (1995) showed that a monoclonal anti-β_1 integrin antibody was very effective in blocking the invasion of both fibrosarcoma cells and breast cancer cells and completely inhibited invasion through reconstituted basement membrane Matrigel even when added after the cells were allowed to attach to the membrane. This suggests that the antibody might inhibit a later step in the invasion process, such as migration through the Matrigel (Yamada et al., 1990). Both anti-β_1 and anti-α_5 MAbs also inhibit experimental metastasis of breast carcinoma cells when coadministered with tumor cells to nude mice (Newton et al., 1995). Pre-treating tumor cells with Fab fragments of the antibodies was as effective as co-injection of intact antibodies with tumor cells, indicating that the antibodies might inhibit the initiation of adhesion during tumor cell arrest and cell migration. Antibody against LM-specific α_6-containing integrins inhibited experimental metastasis of B16/129 murine melanoma cells to lung (Ruiz et al., 1993; Edward, 1995). Direct examination of tissues showed that the antibody inhibited the adhesion of melanoma cells to the vascular endothelium in the lungs within five minutes of injection. IL-1 could increase lung metastasis of melanoma by inducing CD106 expression on endothelial cells. Pretreatment of tumor cells with a MAb against the CD106 ligand, $\alpha_4\beta_1$, completely abrogated IL-1-augmented lung metastasis

(Garofalo et al., 1995). Anti-$\alpha_v\beta_3$ antibodies were reported to induce apoptosis of several types of tumors by disrupting anchorage-dependent growth signals (Bates et al., 1994; Montgomery et al., 1994).

Brooks et al. (1994) have also demonstrated that $\alpha_v\beta_3$ antagonists, such as cyclic RGD peptides or anti-$\alpha_v\beta_3$ MABs inhibit tumor angiogenesis by inducing apoptosis of the proliferating angiogenic vascular cells, resulting in regression of several histologically distinct, $\alpha_v\beta_3$-human tumors transplanted into chick embryos. These $\alpha_v\beta_3$ antagonists also prevent the spontaneous pulmonary metastasis of $\alpha_v\beta_3$-human melanoma cells *in vivo* (Filardo et al., 1995). More recently, it has been shown that systemic administration of MAb against $\alpha_v\beta_3$ not only disrupts human angiogenesis but also reduces the growth and invasive properties of $\alpha_v\beta_3$-human breast carcinoma cells in SCID mice transplanted with full thickness human skin containing human breast cancer cells (Brooks et al., 1995). The $\alpha_v\beta_3$-specific murine antibody, LM609, has recently been humanized and this should facilitate its clinical application (Mousa, 1996). These findings suggest that $\alpha_v\beta_3$ antagonists might be useful for cancer treatment.

Adhesive interactions between cells and ECM can also be inhibited by synthetic peptides or soluble proteins derived from ECM components or cell membrane such as FN (Humphries, 1986; Saiki et al., 1989), LM (Iwamoto et al., 1987) and CD15s (Saiki et al., 1996). They have been used to inhibit metastasis of experimental tumors by interfering with tumor-host interactions and metastasis functions such as attachment and mobility (Johnson, 1991). The RGD sequence appears critical for cell-FN interaction (Felding-Habermann et al., 1992). Humphries et al. (1986) have shown that a synthetic peptide, GRDS, containing the RGD sequence inhibited lung metastasis of melanoma in mice and that one injection of the peptide dramatically increased survival. Various analogues of the RGD peptide such as cyclic RGD peptides or polymers containing repeating RGD sequences were much more effective than monomeric RGD peptides for inhibiting experimental lung or liver metastasis of various murine and human tumors (Saiki et al., 1989; Brooks et al., 1994; Greenspoon et al., 1994; Saiki et al., 1996) because they have a higher affinity and longer half-life *in vivo* (Edward, 1995). Small, synthetic nonpeptide analogues of RGD and LDV (Greenspoon et al., 1993, 1994) have also been developed. These nonpeptidic RGD mimetics prevent adhesion of tumor cells to FN and VN *in vitro* and inhibit experimental and spontaneous metastases of melanomas in animals (Harden et al., 1993). The ligation of tumor cells to LM in the basement membrane is also important for invasion and metastasis since it allows cellular attachment and consequently activates invasiveness (Terranova et al., 1984; Komoriya et al., 1991; Yamamura et al., 1993). Anti-LM antibodies and a proteolytic fragment of LM-containing the LM receptor-binding region inhibited pulmonary metastasis (Terranova et al., 1982; Barsky et al., 1986). The YIGSR sequence in the ß1 chain of LM has been identified as a major binding site for LM receptors on tumor cells. YIGSR inhibits the invasiveness of tumor cells *in vitro* and reduces tumor growth and metastasis *in vivo* (Komoriya et al., 1991; Yamamura et al., 1993). This pentapeptide may not only block tumor cells binding to basement membranes by competing with LM for the LM receptor on tumor cells, but also suppress tumor-induced angiogenesis. Many synthetic peptides containing the YIGSR sequence are effective in inhibiting the growth and dissemination of different types of tumors (Yamamura et al., 1993). A $\alpha_5\beta_1$-specific peptide has been isolated and can induce apoptosis of tumor cells under serum-free condition (Koivunen et al., 1994; Ruoslahti, 1995). Its efficacy in experimental metastasis model has been studied and by using positron-emission tomography (PET), Saiki et al. (1996) recently demonstrated that synthetic CD15s inhibits tumor-cell arrest in the lungs following i.v. injection of B16-BL6 melanoma cells while the FN-derived RGDS peptide analogues [Ar(DRGDS)$_3$] inhibit the invasion of tumor cells into basement membranes. Therefore, different adhesion molecule antagonists can target cells at different stages of the metastasis processes.

Morla et al. (1994) have found that soluble FN can be converted into fibrils (superfibronectin) following treatment with a small recombinant fragments derived from the III_1-C domain of FN. Superfibronectin is ten-fold more adhesive to cells than native FN and effectively inhibits cell migration *in vitro* and tumor growth *in vivo* (Matsumoto et al., 1991; Saiki et al., 1991, 1995; Yoneda et al., 1994; Ruoslahti, 1995) reported that a recombinant fusion polypeptide CH271 containing both cell- and heparin-binding domains of FN is very effective in inhibiting liver and lung metastasis of three different types of tumors. Hence, it reduced the arrest and retention of tumor cells in target organs, blocked their adhesion to subendothelial matrix and inhibited invasion of the basement membrane. Murata et al. (1989) demonstrated that 6-O-carboxyl-methyl-chitin (SCM-chitin), which structurally mimics heparan and heparin sulfate but does not have heparin-like anticoagulant properties, inhibited lung colonization of murine melanoma cells. The antimetastatic activity of SCM-chitin might be due to the suppression of tumor cell invasion and tumor-induced angiogenesis by specific binding to LN and FN and/or by inhibiting the enzymatic activities of cell-derived heparanase and type-IV collagenase (Saiki et al., 1990; Murata et al., 1991). A new synthetic compound SCM-chitin-RGDS, in which the cell-adhesive RGDS peptide was conjugated to SCM-chitin has great therapeutic potential in cancer metastasis (Komazawa et al., 1993). This conjugate effectively inhibited liver metastasis of lymphomas and colon carcinomas in mice, and increased survival rates of tumor-bearing animals. Combined treatment with CH271 and the anti-cancer drugs, doxorubicin or mitomycin-C, resulted in enhanced inhibitory effects on

tumor metastasis and invasion, and significantly prolonged the survival of mice with lymphomas (Saiki et al., 1993). This study suggested that anti-adhesion therapy in combination with chemotherapy might result in additive antitumor activity.

Because altered expression of variant forms of CD44 is associated with proliferation and metastasis of tumor cells, blocking the ability of CD44 to interact with its ligands has been investigated as a means of suppressing the growth of tumors *in vitro* and *in vivo*. Coinjection of an anti-CD44v_6 with metastatic tumor cells led to retardation or even complete blockade of lymph node and lung metastases in mice (Seiter et al., 1993). A neutralizing anti-CD44 antibody, IM7.8.1, inhibited proliferation of human prostate cancer cells and significantly decreased their invasive activity (Lokeshwar et al., 1995). Zahalka et al. (1993, 1995) also demonstrated that the IM7.8.1 prevented lymph node invasion by subcutaneous (s.c.) murine lymphoma LB cells but not spleen infiltration, which was blocked by anti-LFA-1 MAbs. Administration of the anti-human CD44, which completely inhibits the binding of the human melanoma cell line SMMU-2 to hyaluronic acid *in vitro*, suppressed the growth and metastatic potential of SMMU-2 tumor cells *in vivo* (Guo et al., 1994). Hence, early i.v. administration of this antibody significantly inhibited the growth of s.c. melanomas. Furthermore, when antibody treatment was initiated one week after tumor inoculation, it did not suppress local tumor development but did inhibit the formation of metastatic tumors leading to prolongation of survival. Sy et al. (1992) have shown that a soluble recombinant CD44-Ig fusion protein, which binds to hyaluronic acid in ECM, effectively suppressed hematogenous dissemination of $CD44H^+$ human B-lymphoma cells in nude mice. Bartolazzi et al. (1994) also showed that the soluble fusion protein blocked local development of murine melanomas without affecting the growth of the tumor cells *in vitro*. These studies indicated that interference with CD44-hyaluronic acid interaction can suppress tumor growth and metastasis.

The interaction of CD54 with its ligand (LFA-1 ($\alpha_L\beta_1$, CD11a/CD18) might be involved in the pathogenesis of lymphoma and multiple myeloma (Van Riet and van Camp, 1993). Anti-CD18 and anti-CD54 MAbs inhibit invasion and metastasis of murine and human lymphomas (Harning et al., 1993; Rocha et al., 1996). One anti-CD54 antibody had strong antitumor activity in SCID mice with disseminated human myeloma although it was not cytotoxic to tumor cells *in vitro*. This antibody also suppressed the growth of advanced tumors, suggesting that the antibody may interfere with invasion and/or the homing of tumor cells to anatomical sites crucial for their growth *in vivo* (Huang et al., 1995).

Cell-cell interaction mediated by carbohydrate components of major tumor-associated carbohydrate antigens has been suggested to play a role in tumor progression (Hakomori, 1991). In this regard, glycosphingolipid-containing liposomes, oligosaccharide derivatives of glycosphingolipids or MAbs against carbohydrates on tumor cells inhibit tumor metastasis of mouse melanomas, most likely by interfering with the early stages of interaction between tumor cell surface glycosphingolipids or carbohydrates and endothelial cell glycosphingolipids or carbohydrates (Hakomori, 1991; Honn et al., 1992). Modified citrus pectin, a soluble component of a plant fiber derived from citrus fruit, interferes with cell-cell interactions mediated by cell surface carbohydrate-binding galectin-3 molecules. An *in vitro* study showed that the modified citrus pectin inhibited both the adhesion of prostate cancer cells to rat endothelial cells and colony formation in semisolid media. Oral administration of the modified citrus pectin markedly reduced spontaneous metastasis of prostate cancer in rats. The effect of modified citrus pectin might be manifested in the early stages of metastasis, possibly by inhibiting the formation of tumor cell emboli as well as by inhibiting the interactions between tumor cells and the endothelium of a target organ, thus, acting as anti-adhesion agent (Pienta et al., 1995).

Several approaches aimed at restoring or enhancing the expression and functions of tumor-suppressive adhesion molecules or downregulating progression-associated adhesion molecules in tumor cells have been investigated (Kleinerman et al., 1995b; Jiang et al., 1995; Rummel et al., 1996). Using a recombinant adenoviral delivery system, Kleinerman and coworkers (1995a,b) demonstrated that expression of tumor suppressor C-CAM slowed the growth of human prostate tumors in nude mice. Vleminckx et al. (1991) showed that introducing the E-cadherin gene into highly invasive epithelial tumor cells significantly suppressed their invasive behavior *in vivo*. Jiang et al. (1995) have also reported that gamma-linolenic acid, a n-6 polyunsaturated fatty acid, upregulated E-cadherin expression in a range of human cancer cells, increased cell aggregation, and inhibited their motility and invasion. More recently, Rummel et al. (1996) have demonstrated that exposure to PMA reduced MUC18 expression in tumor cells. Antisense oligonucleotides or expression of antisense RNA in tumor cells by gene therapy can also specifically inhibit the expression of metastasis-associated proteins in tumor or host cells (Vleminckx et al., 1991; Bennett et al., 1994). In addition, several cytokines such as tumor necrosis factor (TNF) and IL-4 can induce changes in integrin expression and adhesive properties of tumor cells and decrease the metastatic potential of colon carcinoma cells (Herzberg et al., 1996). These studies strongly suggest that it may be possible to devise strategies to regulate the expression of certain adhesion molecules in tumor cells and thereby influence tumor growth and/or metastatic behavior.

Conclusions

The mechanisms controlling tumor growth and metastasis are extremely complex and involve numerous biochemical processes. These include adhesion

molecule-mediate signaling and cell-cell and cell-matrix interactions which are essential biological processes in tumor development and dissemination. A number of preclinical studies have demonstrated the feasibility of using agents aimed at altering adhesion processes in controlling tumor growth and metastasis. In particular, anti-adhesion therapy holds promise as an adjuvant therapy, specifically in metastasis. It may also be used to enhance the cytotoxic effects of conventional chemotherapeutic agents and reverse drug resistance (Bates et al., 1995; Kerbel, 1995).

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