MINIREVIEW

Role of Integrins in Cancer: Survey of Expression Patterns (44435)

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Abstract. **Tumor cells are characterized by uncontrolled growth, invasion to surrounding tissues, and metastatic spread to distant sites. Mortality from cancer is often due to metastasis since surgical removal of tumors can enhance and prolong survival. The integrins constitute a family of transmembrane receptor proteins composed of heterodimeric complexes of noncovalently linked α and β chains. Integrins function in cell-to-cell and cell-to-extracellular matrix (ECM) adhesive interactions and transduce signals from the ECM to the cell interior and vice versa. Hence, the integrins mediate the ECM influence on cell growth and differentiation. Since these properties implicate integrin involvement in cell migration, invasion, intra- and extra-vasation, and platelet interaction, a role for integrins in tumor growth and metastasis is obvious. These findings are underpinned by observations that the integrins are linked to the actin cytoskeleton involving talin, vinculin, and α-actinin as intermediaries. Such cytoskeletal changes can be manifested by rounded cell morphology, which is often coincident with tumor transformation *via* decreased or increased integrin expression patterns. For the various types of cancers, different changes in integrin expression are further associated with tumor growth and metastasis. Tumor progression leading to metastasis appears to involve equipping cancer cells with the appropriate adhesive (integrin) phenotype for interaction with the ECM. Therapies directed at influencing integrin cell expression and function are presently being explored for inhibition of tumor growth, metastasis, and angiogenesis. Such therapeutic strategies include anti-integrin monoclonal antibodies, peptidic inhibitors (cyclic and linear), calcium-binding protein antagonists, proline analogs, apoptosis promotors, and antisense oligonucleotides. Moreover, platelet aggregation induced by tumor cells, which facilitates metastatic spread, can be inhibited by the disintegrins, a family of viper venom–like peptides. Therefore, adhesion molecules from the integrin family and components of angiogenesis might be useful as tumor progression markers for prognostic and for diagnostic purposes. Development of integrin cell expression profiles for individual tumors may have further potential in identifying a cell surface signature for a specific tumor type and/or stage. Thus, recent advances in elucidating the structure, function, ECM binding, and signaling pathways of the integrins have led to new and exciting modalities for cancer therapeutics and diagnoses.** [P.S.E.B.M. 1999, Vol 222]

The integrin superfamily consists of a major class of transmembrane glycoproteins that mediate cell-matrix and cell-cell adhesion (1). Extracellular matrix (ECM) molecules serve as ligands for the integrins and are crucial for the orderly development of tissues during morphogenesis, maintenance of adult tissue, wound healing, and oncogenesis (2). Cell adhesion interactions with the

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ECM are mediated through the heterodimeric α- and β-chains of the integrins (3). Detailed studies of integrin expression in benign breast lesions (fibroadenoma or papilloma) and mammary adenocarcinomas show patterns of integrin type and distribution that are altered from normal breast tissue. Since altered expression of the various integrins occurs during tumor growth and progression, the integrins and their associated proteins could be potential targets for cancer diagnosis and therapy. Ironically, it is this altered integrin expression that may further contribute to the invasive and metatastic potential of tumor cells.

The objectives of this review are twofold: first, to organize, collate, and discuss recent advances concerning the role of integrins in cancer, and second, to survey integrin expression patterns during tumor transformation, growth and progression, invasion/metastases, angiogenesis, and apoptosis of various malignancies with special reference to breast cancer. Since this review was not intended to be encyclopedic, the reader is directed to the many reviews cited as the first several papers in the reference section. Comprehensive details on the structure, function, and physicochemical properties of integrins cannot be considered here. However, the variable names and classification schemes used in this rapidly advancing field justify a review that attempts to link structural descriptions with physiological data.

Integrins and Their Ligands

The integrins are composed of a large family of heterodimeric integral cell surface receptors that mediate cell-to-extracellular matrix (ECM) and cell-to-cell interactions (1–3). Derangement of integrin expression may be responsible for a number of aberrant cellular activities during tumor onset, progression, and metastatic dissemination (4–6). ECM molecules play an important adjunct role in ontogenetic development, maintenance of adult cell physiology and tissue repair, hyperplastic growth, and tumor development (7–9). The integrins, composed of α- and β-chain heterocomplexes, serve as integral cell membrane receptors that form focal adhesion contacts with various ECM-ligands (i.e., fibronectin, laminin, vitronectin, the collagens, thrombospondin, entactin, fibrinogen, intercellular adhesion molecule (ICAM), and the vascular cell adhesion molecule (VCAM) (2, 8) (Tables I and II). Recent investigations have further linked integrin interactions with cytoplasmic cytoskeletal filament-associated proteins such as actin, vinculin, talin, α-actinin, paxillin, and divalent cation-dependent proteins such as calreticulin (2, 4). (Table I and Fig. 1).

The integrins play a major role in cell adhesion phenomena (8, 9). Each integrin subfamily is characterized by a limited number of β-chains associated with a larger number of α-chains. To date, 8 different β-chains and 14 different α-chains have been described, accounting for at least 20 combinations of the heterodimeric receptors (9). Both the α- and β-subunits are integral membrane glycoproteins containing long extracellular domains that constitute the ligand binding regions (Fig. 1). The α-chains exhibit four repeat amino acid segments believed to bind calcium (Ca^{++}) and possibly other divalent cations such as Mg^{++} and Mn^{++} (7, 10). The β-subunits display at least four cysteine-rich repeats, in linear juxtaposition, that stabilize the large extracellular amino terminal loop. Both chains appear to contribute to the formation of an interface for the ligand binding pocket (see Fig. 1). Ligand binding specificity depends in large part on the specific α-and β-subunit chains present in the heterodimer.

It has been proposed that the N-terminal half of the integrin α-chain is folded into a β-sheet propeller motif that contains seven weak amino acid (FG-GAP) sequence repeats (11). The β-sheets (secondary structure) are thought to be arranged in a toroidal geometric configuration around a central axis with Mg^{++} ions bound to the upper faces of the propeller (blades) and Ca^{++} ions bound to the lower faces. Subsequent studies have led to the proposal that ligand binding occurs on the upper surfaces of the propeller blades within the FG-GAP repeats as previously demonstrated by cross-linking to ligands and site-directed mutagenesis experiments (12, 13).

In contrast to their extracellular domains, the intracellular domains of both the α- and β-subunits are relatively short-chain segments (except β4) following their transmembrane insertion. The short β-cytoplasmic tails contain regions capable of binding to cytoskeletal-associated proteins that link the integrins to the actin cytoskeletal system (Table I and Fig. 1). In comparison to the β-chains, little is known concerning α-chain binding to cytoplasmic-associated proteins other than a calreticulin association that regulates calcium transmembrane channel influx (14). These cytoplasmic peptide tails could serve as prime targets for the development of therapeutic strategies aimed at uncoupling or disrupting signal transduction from the cell membrane to the nucleus and vice versa (15, 16).

Ligand binding of integrins is thought to be controlled by a mechanism requiring either a) receptor clustering alone; b) ligand occupancy plus receptor clustering; or c) clustering, ligand occupancy, and tyrosine kinase activation (17). The process of binding ligands to the integrins involves outside-in signaling initiated by receptor clustering accompanied by conformational changes in the α/β chains culminating in an affinity modulation for the ligand (18). In the course of this process, adhesion plaques form at the cytoplasmic face of the cell membrane that serve as focal points for recruitment of proteins (talin, veniculin, paxillin, etc.) to provide cascade interfaces for actin, G-proteins, tyrosine kinases, and transcription factors (see below and Fig. 1).

The Integrin-ECM Relationship

Studies of ECM interaction with cells *via* their integrin receptors have shown that the integrins function as bidirectional transducers of extra- and intracellular signals (19, 20). The regulation of cell proliferation, differentiation, survival,

Table I. Members of the Integrin Supergene Family Categorized by Receptor Name, Designation, Structural Components, and Cell Distribution

Integrin subunit	Original receptor name	CD designation	Subunit chain (Kd)* α,β	Human chromosome number	Cell/tissue distribution
**$\alpha_1 \beta_1$	VLA-1	CD49a; CD29	200; 140	5; 10	Smooth muscle, T cell, endothelium, hepatocyte
$\alpha_2 \beta_1$	VLA-2 (Platelet 6P Ia)	CD49b; CD29	160; 140	5; 10	Epithelium, endothelium, leukocytes, platelets
$\alpha_3 \beta_1$	VLA-3	CD49c; CD29	150; 140	17; 10	Epithelium, endothelium
$\alpha_4 \beta_1$	VLA-4	CD49d; CD29	140; 140	2; 10	Leukocytes, melanomas
$\alpha_5 \beta_1$	VLA-5 (Platelet GPIc)	CD49e; CD29	155; 140	12; 10	Endothelium, platelets, hepatocytes, lymphocytes
$\alpha_6 \beta_1$	VLA-6	CD49f; CD29	140; 140	2; 10	Most cells, platelets, epithelium, endothelium
$\alpha_7 \beta_1$	VLA-7	NR	140; 140	NR, 10	Muscle, melanoma
$\alpha_8 \beta_1$	VLA-8	NR	160; 140	NR, 10	Epithelium, brain, endothelium, myeloid
$\alpha_V \beta_1$	VNR	CD51; CD29	150; 140	2; 10	Fibroblasts, tumor cells, osteoblasts
$\alpha_L \beta_2$	LFA-1	CD11a; CD18	180; 95	16; 21	Leukocytes, myeloid cells
$\alpha_m \beta_2$	MAC-1	CD11b; CD18	170; 95	16; 21	Neutrophils, lymphocytes, monocytes
$\alpha_x \beta_2$	p150, 95	CD11c; CD18	150; 95	16; 21	Granulocytes, monocytes
$\alpha_{11b} \beta_{3a}$	Major platelet receptor	CD41; CD61	150; 105	17; 17	Megakaryocytes, platelets, melanoma
$\alpha_V \beta_{3a}$	Platelet IIIA, VN receptor	CD51; CD61	150; 105	2; 17	Osteoclasts, tumors, endothelium, fibroblasts
$\alpha_6 \beta_4$	$VLA\text{-}6_{alt}$	CD49f; CD104	140; 2400	2; 17	Neurons, fibroblasts, epithelium
$\alpha_V \beta_5$	VNR_{alt}	CD51; CD19	105; 105	2; NR	Pancreas, fibroblasts, carcinoma cells
$\alpha_V \beta_6$	VNR-β	CD51; CD61	150; 105	2; NR	Epithelium, carcinoma cells

* = Apparent molecular weight of nonreduced forms.
NR = not reported.
** = all α-chains are associated with calreticulin.
Note. Some of the original (earlier) receptor names are not now commonly used.
Data were extracted and compiled from Refs. 1, 3, 4, 7, 10, and 21.

and immediate gene expression is influenced by integrin mediation of cell interaction with the ECM. The disruption of epithelial and endothelial cell interactions with the ECM induces programmed cell death, whereas fibroblast integrin adhesion can affect cell cycle activities by influencing cyclin-A and D expressions (21, 22). In addition to signal transduction to the actin cytoskeleton, the cytoplasmic domains of the integrins interact in cascade fashion with protein kinases, calcium-binding proteins, focal adhesion kinases, Na^+/H^+ antiporters, tyrosine and MAP kinases, and transcription nuclear factors such as NFκB and API (23, 24). Recent reports that some of the signaling activated by integrins and growth factor receptors shares similar pathways suggest the possibility of cross-talk between ECM-induced and growth factor–induced (hormones, cytokines, etc.) signal transduction (15, 25). It has been observed that many members of the integrin subfamilies bind to more than one ECM ligand and demonstrate specificity in a cell-dependent fashion (26, see also Table II). The reason for the redundancy in ligand specificity is unclear; however, cell cooperative interactions and transmission of different information from the environment remain viable rationales. Correspondingly, the inhibitory effect on cell adhesion by individual anti-integrin antibodies is rarely reported as 100% (27). This again is likely due to the presence of multiple integrins on the cell that recognize the same ligands, thus bestowing overlapping specificities. Such backup systems are common in nature to provide failsafe systems for cell growth, homeostasis, and development.

The Biodistribution of Integrins

The phylogenetic expression of cellular integrins and their tissue distribution appear to be universal from fungi to mammals (Table I). In fact, the sequences and genes of the Drosophila integrins are about as closely related to chordates as those comprising the most divergent vertebrate subunits (1, 8). The cell and tissue distribution of the integrins is indeed widespread. Integrins have been found on virtually every cell and tissue studied (28). During development, integrins are ubiquitously expressed. They are involved in

Table II. Members of the Integrin Supergene Family Categorized According to Their Extra-Cellular Matrix Ligands, Amino Acid Recognition Sites, and Function

Integrin subunits	Extra-cellular matrix Protein/Cell Ligand	Recognition site (AA)*	RGD sensitive (+) (−)	Presence‡ Absence I-Domain +	Functional activities
$\alpha_1\beta_1$	LAM, Col	YIGSR, RHDS	−	+	Reduced expression in breast carcinoma
$\alpha_2\beta_1$	Col, PLT, LAM, ENC, cell-to-cell	DGEA, RHDS	−	+	Inhibits malignancy of mammary carcinoma
$\alpha_3\beta_1$	FBN, Col, INV, EPL, $\alpha_2\beta_1$, $\alpha_3\beta_1$, LAM, KAL, ENT	RGD	+	−	Expressed in most tumor cells
$\alpha_4\beta_1$	FBN, VCAM-1 PP-HEV, CSF	EILDV, IDAPS, REDV	−	−	Inhibits metastases in melanoma and β-lymphoma
$\alpha_5\beta_1$	FBN, INV, PLT	LDV, RGD, PHSRN	+	−	Overexpression suppresses growth and tumorigenesis
$\alpha_6\beta_1$	LAM, INV, PLT, EPL	VIGSR?	−	−	Elevated in breast, liver, and NSCL carcinoma
$\alpha_7\beta_1$	LAM	VGVAPG, YIGSR	−	−	Increased expression in melanomas
$\alpha_8\beta_1$	BAL, TEN	IDG, LDV, IDA	−	−	Basement membrane-associated
$\alpha_9\beta_1$	MER, TEN	IDG, LDV, IDA	−	−	Expressed in colon carcinoma
$\alpha_V\beta_1$	FBN, VTN	RGD	NR	−	Increased in anaplastic tumors
$\alpha_1\beta_2$	$ICAM_{1,2,3}$	KELLLPGNNRKV	−	+	Leukocyte to endothelial cell adhesion
$\alpha_m\beta_2$	C3bi, FBN, FAX, ICAM	KQAGDV	−	+	Adhesion, phagocytosis, complement binding
$\alpha_x\beta_2$	C3bi, FBN	GPRP	−	+	Leukocyte adhesion, complement binding
$\alpha_{11b}\beta_{IIIa}$	FBN, FBG, FIB, VWF, VTN, TSP	KQAGDV, RGD, HHLGGAKQAGDV, RYD	+	+	Required for activated platelet aggregation
$\alpha_V\beta_3$	BSP, FIB, VTN, FBN, FBG, PLC, αCol, PBP, VWF, TSP, OSP, Col	RGD, RYD	+	−	Essential for angiogensis for melanoma and angiogenic cells
$\alpha_6\beta_4$	LAM, KAL, MER	VGVAPG? YIGSR?	?	−	Correlates with malignancy in keratinocytes
$\alpha_V\beta_5$	VTN, FBN, PBP, TAT	RGD	+	−	Suppresses anchorage independence
$\alpha_V\beta_6$	FBN	RGD	+	−	Virus-associated fusion
$\alpha_4\beta_7$	FBN, VCAM, MADCAM	EILDV	NR	NR	Endothelial and mucosal lining-associated actions
$\alpha_V\beta_8$	NR	NR	NR	NR	Reproductive/development activities
$\alpha_?\beta_9$	NR	NR	NR	NR	NR
$\alpha_pS2\beta_p$	FBN, BMP	β-subunit	NR	NR	ECM interaction during Drosophila development

Note. NR = not-reported.
Amino acid single letter code; Abbreviations: BAL = basal lamina; BMP = basement membrane protein; BSP = bone sialoprotein; C3Bi = Complement Component 3B inactivated; COL = collagen; CSF = connecting strand, fibronectin; ENC = endothelial cell; ENT = entactin (nidogen); EPL = epiligrin; FAX, clotting blood factor X; FBN = fibronectin; ICAM = intercellular cell adhesion molecule; INV = invasin, protein product of INV gene; KAL = kallikrein; LAM = laminin; MADCAM = mucosal adherens in cell adhesion molecule; MER = merosin (laminin-2); OSP = osteopontin; PBP = penton base protein of human adenovirus; PLC = perlecan; PLT = platelet; PPHev = Peyer's Patch high endothelial venules; TAT = HIV tat protein; TEN = tenascin (cytotactin); TSP = thrombospondin; VCAM = vascular cell adhesion molecule; VTN = vitronectin; VWF = Von Willibrand factor; NSCL = non-small cell lung (carcinoma).
Data were extracted and compiled from Refs. 1–5, 8, 20, 140, and 141.
‡ I = Domain is a collagen binding subdomain distinct from the cation-repeats on the α-chain subunits.

regulating morphogenetic cell movements and migration; they are especially numerous during gastrulation, neurulation, and histogenesis (2). Integrin expression levels tend to decrease gradually during differentiation as adult structures emerge. The integrins further serve as receptors in inflammation, wound healing, and thrombotic events such as platelet aggregation (4, 6). In this regard, integrin antibodies and RGD peptides have been used to prevent or treat thrombus formation and related hemostatic events (29, 30). Finally, integrins are able to mediate adhesive events during various cancer stages such as malignant transformation, tumor growth and progression, invasion and metastasis, and apoptosis, as discussed below.

Integrin Signal Transduction

Signal transduction in the course of integrin functioning is known to be bidirectional; that is, two-way signaling occurs from outside-to-inside and from inside-to-outside (22, 31, 32). The integrins must be activated to undergo adhesion and binding to the ECM. Activation of integrins (33) occurs by local stimuli such as soluble mediators (hormones, cytokines, growth factors, etc.) or by solid interfaces (ECM or other cells). Thus, cell activation may involve adhesion by clusters of various stimulated integrins culminating in signals triggered by local events in the cellular environment (i.e., thrombogenic agonists, antigen stimulation/processing, and T-cell activation (26). Equally important, integrins must then be inactivated to avoid cell adhesion and ECM binding at inopportune times and locations. Inappropriate adhesion can culminate in unwanted thrombosis and inflammation whereas adhered cells need to detach to undergo mitosis or migration (34, 35). It is obvious why cells require finely tuned attachment-detachment signals mediated by integrin interaction during rapid periods of

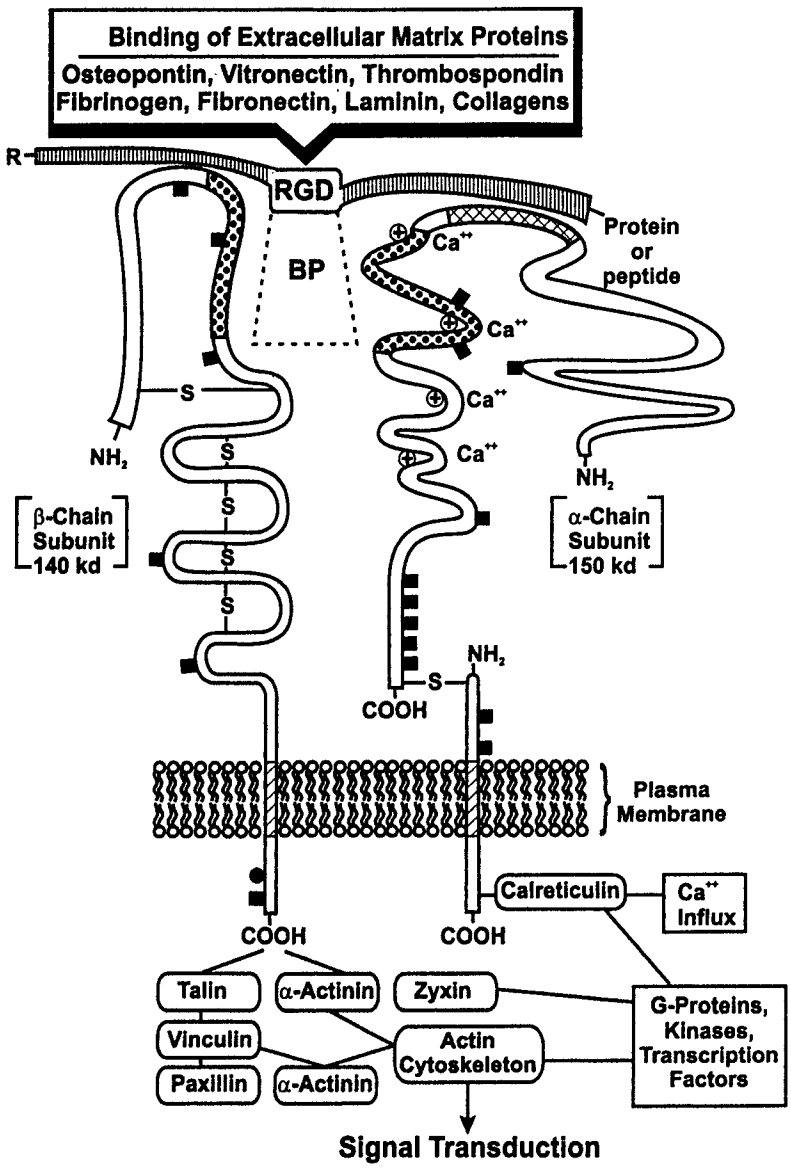

Figure 1. A diagramatic representation of a universal integrin receptor heterocomplex is depicted. The α- and β-chains are displayed as noncovalently linked parallel subunits with insertions through the cell membrane. The long extracellular domains of both chains are contrasted with their short intracytoplasmic subunit chains. The binding pocket (BP) is depicted as the stippled portion of the opposing sides of both the α- and β-chains. The various extracellular matrix protein ligands are contained within the rectangular box at the top of the diagram. An RGD-containing peptide (adhesion inhibitor) is interposed between the ligand and the integrin binding site. Nonactivated integrins are thought to contain divalent cation salt bridges; the cations are then extruded upon binding of the integrin to the ECM-ligand. Linkage of signal transduction pathways *via* the β- and/or α-chains are pictured in schematic fashion at the bottom of the diagram. Symbols: NH_2, amino terminal end of the peptide; COOH, carboxy terminal end of the peptide. ⊠, binding pocket (interface) composed of β- and α-chain lengths; ▩I-, domain of the α-chain that binds collagen; S-S, disulfide bridges; Ca^{++}, divalent cations such as calcium, magnesium, manganese; ■, glycosylation sites, ●, phosphorylation site.

flux such as embryogenesis and histogenesis (2). One may deduce that normal adult cells have homeostatic control of such signaling events whereas malignant cells may have lost these regulatory mechanisms of growth/no growth maintenance. Most probably, chemical factors that promote reversible forms of integrin inactivation, within controllable limits, will provide a potential source of antiproliferative agents for cancer therapy. Fetal proteins, such as α-fetoprotein, that are associated with morphogenetic movements and migration could be a valuable source of such agents (36).

Cell Attachment and Spreading

Most cells (except blood cells) require attachment and subsequent spreading on the ECM substrate for proper growth, function, and survival (27). In tissue formation, cells are attached to each other and to the meshwork of the ECM, mediated by the family of integrins. Normal epithelial cells often undergo inappropriate apoptosis when deprived of the ECM (28). Cell growth and survival on substratum (matrix) attachment has been termed anchorage dependence (35, 37). In cell culture, this linkage occurs at specialized membrane structures referred to as focal adhesions consisting of clusters of integrins that are firmly bound to the ECM interface (31, 37). More loosely bound focal contacts, characteristic of epithelial tissues, probably allow for some flexibility and cell movement. These integrin clusters serve as attachment points for intracellular actin stress fibers on the cytoplasmic surface of the plasma cell membrane, thus influencing cell shape. It is also at the focal point (see above) that integrins can trigger signaling pathways that cross-talk with growth factors, cytokines, and kinase pathways (38), the latter of which include the focal adhesion kinase (FAK) (39, 40). Such cascade interactions are reportedly linked to G-proteins and the tyrosine kinases

of the *src* family. It has also been reported that tissue-derived cells that spread and then flatten appear to thrive, whereas cells that retain a rounded form fail to thrive (41). Depending on the integrin heterodimer involved, differing antiapoptotic effects of cell spreading have been delineated and described (35, 39, 41). These investigations revealed that following the ECM determination of cell morphology, cell shape, was a major factor in determining subsequent cell growth and survival. It becomes evident that therapeutic intervention at the integrin–ECM interface might provide a means of altering the cell signals responsible for the balance between cell death and cell survival.

Cation-Dependent Processes

Calcium influx and mobilization appear to be constituent parts of the intracellular signaling pathways associated with integrin ligation and occupation (7, 23). The position of the ECM-ligand binding site on the integrins has been deduced from chemical cross-linking data and has been localized to the four divalent cation-binding repeat regions of the integrin subunits (7). These Ca^{++} binding regions on the α-chain combine with amino acids 100–200 on the β-chain to contribute to the interface of the ligand binding pocket (BP) (see Fig. 1). The binding of the integrins to their ligands (ECMs) is thus a cation-dependent process, which generally occurs with low binding affinities (10^{-6} *M*). The presence of the divalent cations maintains the integrity and form of the BP, but the cations are extruded in the course of ligand binding and occupancy. Integrins recognize specific amino acid sequences in their ligands, such as RGD and related sequences (Table II) found in fibronectin, fibrinogen, thrombospondin, vitronectin, laminin, and the various collagen types (9, 42, 43). Other amino acid sequences such as DGEA, EILDV, GPRP, and REDV also serve as recognition sites for these and other ECMs, including ICAM and C3B (32). Calcium, and to a lesser extent magnesium (Mg^{++}), provide the cation requirements for ligand binding of the ECMs to integrins (13, 43, 44). Thus, Ca^{++} and Mg^{++} are potential physiological regulators of integrin-mediated cell adhesion *via* ECM-ligation. The two cations can exert different and/or opposite effects on the binding function of the various integrins, inhibiting binding in one instance and enhancing it in another. It is of special interest that manganese (Mn^{++}) at low concentrations also fulfills the divalent requirement for cell spreading on fibronectin (42).

T lymphocytes bind to ICAM-1 through LFA-1 complexes. Mn^{++} can alter the conformation of the LFA-1 to favor ligand binding (44) whereas Ca^{++} ions serve to maintain LFA-1 in an inactive state. Calcium is also required for the establishment of multilayered basement membranes that contain laminin and entactin (45, 46). Both Mg^{++} and Ca^{++} form salt bridges that stabilize the α-β-integrin binding complex. These structures influence both the ECM binding activity and proteolytic susceptibility of the integrin complex (23, 43, 44). Although these cation binding regions of the integrin are very similar to the Ca^{++} binding loop in the EF band motif of calmodulin, these sequences more closely resemble the bacterial galactose-binding protein in lacking one of the conserved coordinating side chains (42,47). Since a glutamate (Glu) is missing in the Ca^{++} binding loop of the eukaryotic homologs, integrin binding to the ECM might alter the receptor conformation to accommodate the missing Glu. In the presence of high Mn^{++} or Mg^{++}, this conformation could be supplied by the RGD sequence, which resembles the lysine-isoleucine-glycine (KIG) of the galactose binding protein.

Calcium-Related Proteins; Calreticulin

Calcium ions constitute a second messenger system that encompasses one or more of the following processes: a) regulation of metabolic pathways; b) synthesis and release of hormones and neurotransmitters; c) muscle and non-muscle cell motility; d) lipid and carbohydrate metabolism; e) programmed cell death; and f) mitosis (23). A stringent requirement for cytosolic Ca^{++} at submicromolar levels is maintained by Ca^{++} buffering proteins in an intracellular system of storage and transport pathways. Such buffering proteins may include calmodulin, calsequestrin, calponin, calbindin, and calreticulin (48). Although the major Ca^{++} binding/storage protein in the sarcoplasmic reticulum (ER) is calsequestrin, the major nonmuscle ER Ca^{++} binding protein is calreticulin (CRT), which is especially abundant in smooth muscle and liver (45, 48, 49). The CRT molecule provides both high affinity/low capacity and low affinity/high capacity Ca^{++}-binding sites and, thus, is well suited for Ca^{++} sequestration and deposition within the cell (49).

There is recent evidence that CRT, a 46-kDa polypeptide, is a highly conserved, ubiquitously expressed Ca^{++} binding protein of the cytosol, which may serve multiple functions (50, 51). It constitutes a portion of the systemic lupus antigen nucleoprotein complex that acts as a human auto-antigen (52). Additional isoforms exist at various cell locations including the cell surface membrane, the nucleus, and the ER. An additional form circulates as a plasma anticoagulant protein (53, 54). Although most CRT isoforms contain a terminal KDEL for ER retention, CRT appears capable of shuttling between the cytosol, the ER, and the nucleus (55–57). One form of CRT is found complexed with the cytoplasmic domains of all integrin α-subunits bound through the integrin sequence motif KXGFFKR (58, 59). A similar amino acid sequence (KXFFKVR, where X is G, A, or V) is also present in the DNA-binding domain (zinc fingers) of all known members of the steroid receptor superfamily (60–62). Amino acids in this region of the nuclear receptors make direct contact with nucleotides of the hormone response element since the receptors themselves are transcription factors. CRT has been demonstrated to inhibit the transcriptional activities of the retinoic acid (63), androgen (61), vitamin D_3 (64), and glucocorticoid receptors *in vitro* and *in vivo* (62) and the peroxisome proliferator-activated receptor/retinoid X receptor heterodimers *in vitro*

(65). Thus, CRT can act as a modulator of the regulation of steroid-inducible gene transcription and expression. It would follow logically that CRT may then serve as a signal transduction modifier between the cell membrane, the cytoplasm, and the nucleus by virtue of its binding to the α-integrin subunit (66). Interruption or uncoupling of this transduction signal could influence cell growth (mitosis) and differentiation, and possibly, tumor transformation and progression.

Previous reports have indicated further that CRT is an essential modulator of both integrin-mediated calcium adhesive functions and integrin-initiated signaling (65, 66). Signals can be transduced to transcription factors by two main groups of receptors: 1) integral plasma membrane receptors; and 2) the intracellular nuclear steroid/thyroid hormone receptor superfamily. Although the classical activation pathway of the nuclear receptors is *via* direct transmembrane diffusion of the hormone into the cytoplasm, an alternate pathway of nuclear receptor activation has been identified and described (67). This alternate pathway involves a nonligand activation of the steroid receptors by signals transduced by growth factor receptors on the cell surface (67, 68). A level of further control can be interspersed in this pathway by direct protein-to-protein interactions of the receptors with other cytoplasmic transcription factors such as AP-1 (*fos/jun*) and NfκB (69, 70). Thus, CRT bound to the α-subunit of the integrins has been proposed to serve as a second messenger in the alternate pathway based on the above postulates. If that is the case, CRT and proteins of its associated pathways might be used as potential targets for cancer therapy/treatment.

Disintegrins and Tumor-Induced Platelet Aggregation

Recently, a wide array of true viper and pit viper venoms has been demonstrated to contain peptides, termed disintegrins, that block integrin function and are potent inhibitors of platelet aggregation (71, 72). Such polypeptides are readily distinguished from the cobra-like venoms, which act as neurotoxins. Most disintegrins contain the RGD cell attachment recognition sequence, are rich in cysteine, and function to block platelet aggregation. Inhibition results from blocking the binding of the GPIIa/IIIb platelet-integrin complex to ECM proteins such as fibrinogen and von Willebrand's factor (73–75). Since most disintegrins possess an RGD sequence, they are capable of further inhibiting the adhesive functions of other RGD-dependent integrins such as $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (vitronectin receptors) and $\alpha_5\beta_1$, a fibronectin receptor (76, 77). However, by comparison with the integrins, other amino acid adhesion sequences, such as Lys-Gly-Asp, may be operational in the disintegrins. The disintegrins may represent models for designing novel and potent compounds with therapeutic value in the inhibition of platelet aggregation and the blockage of the tumor-induced platelet aggregation stage of metastasis (see below), due to their broad spectrum of reactivity with many integrins.

Tumor cell–induced platelet aggregation (TCIPA), as a required component of metastasis, was first described by Gasic in the early 1970s (78). Tumor cells in the vasculature are frequently observed in complexes with platelets and this association, together with the hypercoagulable state of malignant disease, appears to be essential for successful metastasis (79, 80). The ability of tumor cells to induce platelet aggregation is widespread among cancers including breast carcinoma, colon adenocarcinoma, lung carcinoma, melanomas, and others (81, 82). Platelet participation in the metastatic process is thought to result from a) direct binding of platelets to tumor cells, and b) the release of soluble inducer agents from the tumor cells. These agents would include the classical platelet aggregation activators such as ADP, cathepsin B, thrombin-like proteinases, collagen, and tissue factor-generated thrombin (83). Thus, platelets may act to facilitate all the intermediate steps of transvascular metastasis including tumor cell retention and arrest, subendothelial interaction, and extravasation from the microvasculature. Blockage at these steps might retard or reduce tumor cell metastasis.

The disintegrins, purified components from viper snake venoms, contain the RGD and related adhesion sequences and bind with high affinity to the surface of platelets, affecting aggregation inhibition. Recently, two disintegrin antiplatelet peptides, naturally occurring trigramin and rhodostomin, were shown to be 6,000–18,000 times more potent than synthetic RGD-peptides in the inhibition of TCIPA (79, 82). However, the two disintegrins employ different adhesion inhibition pathways to achieve the TCIPA blockade. Trigramin is a specific antagonist of platelet-to-platelet membrane GPIIb/3a integrin interaction with breast cancer cells, whereas rhodostomin inhibits platelet aggregation by antagonism of the GPIIb/3a-fibrinogen interaction with colon adenocarcinoma cells (79). With breast, prostate, and colon cancer cells, TCIPA, induced by tissue factor activation of thrombin, could be inhibited by both trigramin and rhodostomin (79, 80, 82). Thus, the disintegrins represent a class of chemicals whose therapeutic potential for metastasis has not yet been fully realized.

Tumor Transformation

Malignant transformation is characterized by disruption of cytoskeletal organization, decreased adhesion, and altered adhesion-dependent responses. Studies of integrin expression in transformed cells suggest that various integrin subunits may contribute either positively or negatively to the transformed cell phenotype (84). Changes in the expression of fibronectin- binding integrins have been observed in some types of transformed fibroblasts, whereas other changes in integrin expression have been observed among a myriad of malignant cells (85). In general, a transformed cell phenotype may contain several alterations in cell adhesion receptors (86, 87) (Table III). For example, high levels of $\alpha_5\beta_1$-integrin seem to correlate with low levels of transformation for certain tumors. However, increased expres-

Table III. Various Integrin Heterocomplexes Detected by Immunohistochemical Techniques on Selected Human Transformed, Primary, and Metastatic Tumor Cells

		Integrin heterocomplexes—alpha/beta chains										
Tumor tissue	Tumor state	$\alpha_1\beta_1$	$\alpha_2\beta_1$	$\alpha_3\beta_1$	$\alpha_4\beta_1$	$\alpha_5\beta_1$	$\alpha_6\beta_1$	$\alpha_V\beta_1$	$\alpha_{11b}\beta_{111a}$	$\alpha_V\beta_3$	$\alpha_6\beta_4$	$\alpha_V\beta_5$
Breast	Trans.		±	±	−	−	±				±	
	Prim.	−	±	±	−		±	−		−	±	
	Metas	+	±	±	−	−	+	+			±	
Colon	Trans.			±		−						
	Prim.	−	−	±		−	±			−		
	Metas		±		−	+					+	
Kidney	Trans.						−	+		+		+
	Prim.			+			±					
	Metas		±	±		+		±				±
Lung	Trans.	−	+	+		−	+	+		−		+
(NSCLC)	Prim.		−	±			−	±		±		
	Metas			+					+			
Melanoma	Trans.		+						+			
	Prim.	−	±	+	+	±	−			+	±	
	Metas	+	+	±	±	+	±		+	+	+	
Ovary	Trans.					+		−				
	Prim.			+		−						
	Metas			+		−		−				
Skin	Trans.					+					+	+
(squamous)	Prim.		−	−		−	−			+		
	Metas		+	+	−		+	−		−	+	

Note. Blanks indicate paucity of data in the literature.
$^+$ = Upregulation of integrin expression
$^-$ = Downregulation of integrin expression
* = Disorganized/redistribution of integrin expression and/or reduced expression
NSCLC = Nonsmall cell lung carcinoma
Trans = transformed cells
Prim = primary tumor cells
Metas = metastatic tumor cells
Data were extracted and compiled from Refs. 4, 6, 8, 19, 22, 27, 79–92, 95, and 96.

sion of $\alpha_v\beta_3$ appear to be positively correlated with increased malignancy in melanomas (88). A consistent finding is the lack of spatial organization of integrin expression in epithelial tumors. In carcinomas, the spatial arrangement of integrins becomes quite disordered, with a diffuse and less abundant cellular distribution. These changes in integrin cell-surface distribution can affect ligand binding affinity, and correlate with a concomitant disorganization of the structure of the basement membrane itself. Reduced levels of α_5, α_3, and α_2 integrin expression have been reported in carcinomas, whereas increased levels of $\alpha_6\beta_4$ appear in head, neck, and skin tumors.

In sarcoma virus transformation of several rodent cell lines, the fibronectin receptor $\alpha_5\beta_1$ disappears from the cell surface; $\alpha_3\beta_1$ levels remain constant (4). After viral transformation of human lung WI-38 fibroblasts, the number of β_1-integrin subunits on the cell surface was unchanged, but their distribution was in disarray. Further transformation-related changes involved increased phosphorylation, lowered binding affinities for ligands, and increased glycosylation of N-linked oligosaccharides of the integrin. As stated above, $\alpha_5\beta_1$ integrins support a normal cell phenotype, and overexpression of $\alpha_5\beta_1$ has been shown to normalize a transformed phenotype (89). In comparison, viral-transformed human osteosarcoma cells require the $\alpha_2\beta_1$ collagen/laminin receptor as do rhabdomoysarcoma, nonsmall cell lung carcinoma, and melanoma. In these studies, matrix (collagen) reorganization seems to be at least one reason why the $\alpha_2\beta_1$ is important for tumor formation by transformed cells. Thus, it seems that several combinations of changes in integrin expression may precede tumor formation in a given tissue. Overall, transformation represents a precancerous state requiring preventive strategies rather than anticancer therapies. One such strategy might be the parallel determination of specific cell integrin expression profiles in normal and tumor cells from the same tissue. (See breast example, Table IV).

Integrin Expression During Primary Tumor Growth and Progression

In cancer growth, the modification of integrin structure is often associated with a change in integrin expression (Table III). Both quantitative and qualitative alterations in integrin cell surface patterns have been observed *in vitro* and *in vivo*. Some integrins are either overexpressed or no longer expressed whereas others become phosphorylated, affecting their cytoskeletal and extracellular ligand binding properties (5, 90). Thus, formulation of a cell signature of integrin expression from biopsies may have potential as a diagnostic aid for the detection of both transformation and

Table IV. Comparison of Integrin Expression Patterns in Specific Cell Types Found in Normal Versus Neoplastic Breast Tissue by Immunohistochemical Staining*

Cell type	Myoepithelial cells		Luminal/ductual cells		Stromal cells		Muscle cells		Endothelial cells	
Integrin subunit	Normal	Neoplastic	Normal	Neoplastic	Normal	Neoplastic	Normal	Neoplastic	Normal	Neoplastic
α_1	+2/+3	–	+/–	+1	+1/+2	+/–	+1/+2	+/+2	+1/+2	+1/+2
α_2	+2/+3	–	+1	+/–	–	–	–	NR	+/–	–
α_3	+3	+1	+1	+	–	–	+2	+1	+/–	+/–
α_4	+/–	–	–	–	–	–	–	NR	+/–	NR
α_5	+/–	–	–	–	+/–	+1/+2	+3	+2	+/–	+/–
α_6	+/3	+/–	+/–	+1	–	–	–	–	+1/+2	+1/+2
α_v	+1/+2	+1	+/–	+1/+2	+1/+2	+1/+2	+/–	–	+1/+2	+1/+2
β_1	+2/+3	+1	+1/+2	+1	+2	+1/+2	+2	+1	+2	+/–
β_3	+/–	–	–	–	–	–	–	–	+/–	–
β_4	+2	–	+/–	–	–	–	–	–	+/–	+1/+2

Note. Immunohistochemical Staining:
+1, +2, +3 = increasing grades of fluorescence staining intensities
+/– = faint fluorescence staining
– = lack of fluorescence staining
* Data were compiled from Refs. 92, 93, 95, 130, and 140.

tumor progression. For example, Chinese hamster ovary cells that overexpress $\alpha_5\beta_1$ demonstrate reduced malignancy (8), whereas melanoma progression has been correlated with $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_2\beta_3$ up-regulation (90). Further studies showed that progression of melanomas is associated with changes in $\alpha_6\beta_1$ expression (91). Changes in the expression of $\alpha_6\beta_4$ integrins are also observed in breast cancer, with reduced levels at the primary site and constant levels at metastatic sites (92, 93). In pancreatic cancer cell lines, the integrin subunit chains α_2, α_3, α_6, β_1, β_4, and β_5, were associated with adenocarcinomas and ampullary tumors, whereas highly differentiated cell lines showed variable loss of one or more of the integrin chains (94, 95). The promiscuous $\alpha_3\beta_1$ receptor in human solid tumors has further been shown to maintain a high frequency of expression in both the primary and metastatic tumor state (96). Finally, in human malignant mammary tumor progression, $\alpha_2\beta_1$ and $\alpha_3\beta_1$ were present in non-neoplastic and fibroadenomas but were low or absent in invasive mammary carcinomas (27, 97). Thus, the loss or altered patterns of the ECM-binding integrins appears to be one of the abnormalities underpinning tumor progression (Table III).

Although the expression of integrin receptors is altered in malignant compared to normal cells, most tumors maintain normal expression of at least some of their integrins. The changes in integrin expression and the cell surface distribution are specific to the tumor cell type (Tables III and IV). In one group, carcinomas of breast, prostate, and colon exhibit a loss of $\alpha_3\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, and $\alpha_6\beta_4$ integrin expression; however, the highly malignant sarcomas (osteosarcoma, rhabdomyosarcoma) overexpress the $\alpha_2\beta_1$ collagen receptor believed to play a role in metastasis (98, 99). In a third group, the melanomas demonstrate overexpression of the $\alpha_2\beta_3$ integrins, which are known to promote cell proliferation while inhibiting apoptosis (93).

In general, the loss or gain of expression of particular integrins appears to be indirectly implicated in malignant transformation and directly involved with tumor progression and metastasis. Therefore, the concept of therapeutic targeting of integrins specific to certain tumors *via* antibodies, peptide antagonists, and/or disintegrins may provide viable options for nontoxic therapeutic treatment modalities (see below, angiogenesis). Such therapeutic modalities might also bypass the development of drug resistance, which has emerged as a confounding factor in cancer chemotherapy.

Tumor Cell Invasion and Metastasis

Metastasis is a process in which cancer cells detach from the primary tumor site, enter the circulation, and extravasate at distant sites. Tumor cell entrance into the vascular system involves the loss of cell adhesion and the release of proteolytic enzymes to digest a tunnel through a number of tissue membrane barriers. The metastatic process involves making and breaking contacts with different ECM components at these sites, and may require changes in the integrins expressed by the tumor cells (Table III). Invasive tumor cells express genetic alterations that permit development of cell surface integrin-controlled signal transduction pathways that respond to paracrine and autocrine stimulation in an abnormal manner. Moreover, the most frequent cause of death in breast cancer patients can be attributed to metastasis of tumor cells and their growth at distal regions rather than the *in situ* effects of the malignancy at the primary site (38).

It is clear that tumor cells can migrate effectively on ECM (i.e., fibronectin) substrates, and that multiple integrins functioning in concert contribute to this process (100, 101). Clearly, cell adhesion *via* receptor clustering is required so that cells can pull themselves along a migration path. Integrin clustering greatly influences ECM binding affinity. Chemical agents that modulate cell adhesion could

alter cell migration, and thereby, cell invasiveness. Thus, the overall process of invasion involves the adhesion of tumor cells to basement membrane matrices, partial proteolytic digestion of basement membrane layers, followed by cell penetration (migration) through the disrupted membranes. Interestingly, $\alpha_v\beta_3$ integrin colocalizes with the ECM metalloproteinase-MMP2 on the surface of invasive melanoma cells thereby facilitating tumor cell invasion by degradation of the ECM (102).

Currently, anti-integrin antibodies, disintegrins, and synthetic peptides have been reported to be effective antimetastatic agents. The antibodies and peptides function by preventing clustering of the receptors, occupying the receptors at the sites to which ligands attach, and inducing conformational subunit chain alterations (see Integrins and Their Ligands). Such agents have a potential for clinical treatment (20). In addition, blocking of receptors by monoclonal antibodies or synthetic peptides may also influence wound healing, wound contraction, and angiogenesis (30, 103). The RGD-directed integrins, for example $\alpha_v\beta_3$, have been demonstrated to be essential components of newly developing capillaries, both in granulation tissue (inflammation) and the developing stroma of tumors. Blocking angiogenesis is thus a prime objective for the interference and regression of tumor growth.

Blocking the binding of tumor cells to platelets is also regarded as a potential method to inhibit metastasis (as described in Disintegrins and Tumor-Induced Platelet Aggregation). Tumor cell attachment to platelets is an early stage in increased expressions of vascular invasion and transudation (6, 32). Among the integrins, $\alpha_4\beta_1$ initiates the growth and spread of melanoma cells (27, 32). $\alpha_5\beta_1$ and $\alpha_v\beta_3$ are expressed in advanced melanoma and metastases suggesting that integrins may have prognostic value (Table III). In human melanoma patients, the expression of increased $\alpha_4\beta_1$ together with decreased $\alpha_6\beta_1$ significantly correlate with the occurrence of metastases (26, 93). Thus, the correlation of the expression and derepression of integrins coupled with the use of anti-integrin antibodies (i.e., anti-$\alpha_6\beta_1$ integrins for melanoma), disintegrins, and peptides such as RGD and YIGSR suggest that treatment regimes for specific cancers may be feasible.

Tumor Angiogenesis

Angiogenesis, defined as the initiation and control of capillary growth, provides an exciting potential for future targets of anticancer therapy. During tumor growth, new blood vessels are recruited first at the outer tissue rim and later within the interstitial mass of the growing tumor. The increased mass of the developing tumor requires continual neovascularization since cell proliferation requires continuous supplies of both oxygen and nutrients. The limitations of oxygen and nutrient diffusion require that tumor cells induce new capillary ingrowth to form solid masses exceeding 1.0 mm^3 in size (103). Anti-angiogenesis agents seem to be capable of suppressing tumor growth specifically without causing systemic toxicity. These agents also decrease the likelihood of developing drug resistance in the tumor. Toxic side effects should be minimal in nontumor tissues since normal capillary endothelial cells grow very slowly (express low levels of integrins) whereas tumor vessels multiply at rapid rates and display increased expression of integrins (30).

The control of capillary cell growth, vascular differentiation, or involution can be affected *in vitro* by varying the ECM coating densities for vascular endothelial cells in culture (32). Also, altering the ability of the substratum to support cell tension appears to play a role. Data suggest that ECM molecules may control capillary morphogenesis by binding specific endothelial cell surface integrins and resisting mechanical loads applied to these ECM-receptors (103). In this context, cell shape is a major factor of mechanical loading since extended cells proliferate more rapidly as ECM coating densities are increased (32). Further, ECM molecules (i.e., fibronectin) regulate capillary cell growth by altering the setpoint of the cell surface integrin Na^+/H + antiporter systems (100, 101). Activation of antiporter exchange in control of intracellular pH is a property shared by many integrin family members, including α_5, α_v,β_1,β_2, and β_3 subunits (24).

Previous studies have shown that angiogenesis inhibitors, including collagen cross-linking/deposition blockers, proline analogs, retinoids, disintegrins, and steroid/heparin combinations show promise *in vivo* as antiangiogenic cancer therapies (103). Furthermore, inhibition of basement membrane biosyntheses was shown to prevent tumor angiogenesis. Finally, the role of integrins in tumor angiogenesis can be aptly demonstrated by $\alpha_v\beta_3$ in melanomas; differential integrin expression was found on newly formed vessels but not on pre-existing vessels (32).

Vascular cell integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have now been implicated in neovascularization and tumor-induced angiogenesis. In particular, $\alpha_v\beta_3$ contributes to the survival, proliferation, and metastasis of melanomas and ovarian tumors (104, 105). Since angiogenesis is a critical process for the growth and metastasis of most solid tumors, it should be feasible to devise therapeutic modalities to disrupt and uncouple signal transduction specifically and selectively in vascular tumor cells undergoing angiogenesis within tumors. Antagonists to the vascular cell integrins (monoclonal antibodies, peptides inhibitors, antisense β_3 and β_5 oligonucleotides) cause regression of pre-established human tumor xenografts in animals and may ultimately prove effective in human patients (106, 107). Thus, the inhibition of angiogenesis by attacking the tumor's blood supply and preventing new vessel outgrowth is one of the most promising areas of anticancer drug development that is now approaching clinical trials (108–110).

Apoptosis in Tumor Cells

The term anoikis was coined to denote the specific apoptosis that occurs in cells that undergo matrix detach-

ment (110). This anchorage-dependent programmed cell death was observed in epithelial and endothelial cells that were physically dissociated from their extracellular matrices. It was further shown that integrin-signaling *via* protein kinase pathways controlled both the positive and negative aspects of anoikis and that the process was reversible *in vitro* by rapidly replating the dissociated cells (111). Anoikis can be distinguished from necrosis by cell/nuclear morphology, internucleosomal DNA cleavage, nuclear lamina cleavage, and loss of *Bcl*-2. Anoikis occurs both *in vitro* and *in vivo,* preventing cells from colonizing promiscuously when detached (112). It is an important process in embryonic cells undergoing cavitation (113) and in cancer cells of low tumorigenic potential (114). Only specific α- and β-integrins appear capable of suppressing anoikis in a single cell type, demonstrating that the various integrins differ in their ability to downregulate cell death (115). It is for this reason that tumor cells might often express aberrant, substitute, or altered integrin patterns to evade and escape cell death of the anoikis type.

As discussed in the Tumor Angiogenesis section, antagonists to the integrin $\alpha_v\beta_3$ disrupt neovascularization in melanomas; this occurs by promotion of the unscheduled apoptosis of newly sprouted blood vessels (116). In human cells undergoing angiogenesis, tumor regression can be promoted by intravascular injection of cyclic peptides or monoclonal antibodies directed against the integrin receptors. Interruption of ligand binding of α_v β_3 in melanoma cells induces apoptosis in the proliferative angiogenic vascular cells, leaving pre-existing quiescent blood vessels unaffected (117). Finally, $\alpha_5\beta_1$ integrin was shown to prevent apoptosis of cells attached to fibronectin by activating the *Bcl*-2 pathway that protects against apoptosis (118).

Recent reports have further shown that components of the ECM can function as cell survival factors through the suppression of apoptosis (119). For example, ECM proteins such as fibronectin suppress apoptosis in normal human melanocytes through integrin-dependent, anchorage-dependent regulation (120). Apoptosis could be reversed in these cells by anti-$\alpha_5\beta_1$ monoclonal antibodies and/or RGD peptides; furthermore, apoptosis was only detectable in $\alpha_5\beta_1$-positive human tumor cell lines (121). Such studies have suggested that fibronectin induced programmed cell death *via* its interaction with $\alpha_5\beta_1$ integrin. However, in rectal carcinomas, the laminin-binding $\alpha_6\beta_4$ integrins contribute to the function of epithelial cells and their oncogene-transformed derivatives (122). These studies demonstrated that activation of the $\alpha_6\beta_4$ integrin induced both the p21 cyclin-dependent kinases and apoptosis in its signal transduction pathway (123). Furthermore, the rectal carcinoma cells that lacked expression of the $\alpha_6\beta_4$ but included $\alpha_6\beta_1$ integrins bound laminin less avidly. In studies of canine kidney tumor cells, collagen-sepharose affinity chromatography and immunoblotting demonstrated both the presence and collagen- binding (Mg^{++}-dependent) activity of the $\alpha_2\beta_1$ integrins. Loss of this integrin class resulted in increased apoptosis, cyst formation, and other morphogenetic changes (124).

In some neuroblastomas, abrogation of cell adhesion through downregulation of integrin receptors plays a crucial role in the induction of apoptosis (124–126). In this regard, it was reported that cell growth and survival are mediated by β_1 integrins in normal breast epithelium but not breast carcinomas (125). Using anti-β_1 and anti-α_2 antibodies, cellular binding to collagen type-I was clearly demonstrated in the normal epithelium, but was impaired or lost in breast carcinomas. Thus, the loss of proper integrin-mediated cell-ECM interaction may be crucial to breast tumor formation and cell survival. In subsequent studies employing colon cancer cells, certain patterns of integrin expression on normal and tumor cells regulated both cell proliferation and programmed cell death (124–127).

Summary Update of Integrins in Breast Cancer

An important part of present day research is a focus on integrin expression patterns and their involvement in adhesion processes and signal transduction in human breast cancer. The $\alpha_2\beta_1$ (collagen/laminin receptor) is highly expressed on the epithelium of ductules of normal breast tissue and benign fibroadenomas (102, 126, 128–132). In contrast, markedly decreased $\alpha_2\beta_1$ expression together with loss of estrogen receptor expression were found on poorly differentiated breast adenocarcinoma cells. Similar but less extensive decreases were also observed with $\alpha_5\beta_1$ (fibronectin receptor) and $\alpha_v\beta_3$ (vitronectin receptor) integrin expression in these mammary tumors (127, 128). In comparison, highly differentiated breast adenocarcinoma cells exhibited intermediate expression levels of these integrins. Table IV further displays integrin expression patterns on various cell types in normal versus neoplastic breast tissue. Furthermore, significant downregulation of α_1, α_3, α_6, β_1, and β_4 subunits were observed in all neoplastic breast epithelia. These integrins have been shown to regulate anchorage-independent growth of mammary tumor cells in culture (133, 134).

A reduction in breast cancer cell contacts is also crucial for the initiation of metastatic growth, again implicating the $\alpha_2\beta_1$ integrin whose expression may be downregulated by oncogenes (135). The regulation of $\alpha_2\beta_1$ expression by oncogenes is associated with the disruption of tissue architecture that precedes invasive breast cancer. In comparison, increased or maintained expression of the $\alpha_6\beta_4$ integrin is predictive of a poor prognosis, especially in laminin-positive breast tumors (136, 137). In tissue-culture studies, substantial $\alpha_v\beta_3$ expression in breast tumors was positively correlated with the cell's ability to adhere and migrate, thus increasing its metastatic potential. Furthermore, the $\alpha_v\beta_5$ and $\alpha_v\beta_1$ integrins, which mediate adhesion to vitronectin, were observed to be widely distributed among a variety of breast cancer cell lines. The survival of metastatic human breast carcinoma was found to be promoted by the expression of $\alpha_6\beta_1$ integrin and its ligand, epiligrin, which also

regulates metastatic potential (138). Finally, a reduction in the expression of breast tumor integrins has been correlated with positive lymph node status (139). These integrins include $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_1$, and $\alpha_v\beta_5$. When challenged for contact to their respective ligands, all the integrins showed significantly less adhesion potential. Such studies demonstrate a link between altered integrin expression and function in primary breast cancer cells predisposed to metastasize.

Recent studies have now shown that breast tumorigenesis depends not only on genetic constitution but also on cellular milieu factors such as cell-cell and cell-ECM contacts (140). Investigators have demonstrated that monoclonal antibodies to β_1-integrins can block intracellular signaling, causing breast cancer cells to revert to nontumorigenic cells. The antibody-treated cells 1) stopped dividing; 2) formed cell-to-cell contacts; 3) produced an organized cytoskeleton; and 4) became polarized by secreting a basal-surface basement membrane and displaying apical surface integrin subtypes. These same cells could then be converted back to the malignant type by exposure to anti-β_4 or anti-α_6 monoclonal antibodies using a similar protocol. Thus, it is increasingly evident that breast cancer progression results not only from genetic clonal expansion (genetic constitution), but also from interactions between the cell and its microenvironment such as the ECM (141).

Concluding Remarks

It now seems feasible that interference with integrin signaling could provide a basis for the development of treatments for cancer growth, progression, metastases, and angiogenesis. Anti-integrin antibodies and RGD and related peptides have shown promise in both tumor therapy and diagnosis. Interruption of the adhesive interaction of tumor cells may serve to arrest disease progression (142). The observations that different integrins present on various tumor types are differentially expressed during tumor transformation, progression, and metastasis, suggesting that integrins may also be prognostic markers. Agents that block or interfere with the initial attachment of integrins to ECM components, signal events leading to proliferation, protease induction, cell migration/invasion, or angiogenesis could thus constitute a formidable armamentarium of detection markers and nontoxic anticancer agents. Such antiadhesive agents may find application in the treatment of the four major classes of human diseases: neoplasia, inflammation, trauma, and infection.

1. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell **69:**11–25, 1992.
2. Albelda SM, Buck CA. Integrins and other cell adhesion molecules. FASEB J **4:**2868– 2880, 1990.
3. Ginsberg MH, Loftus JC, Plow EF. Cytoadhesions, integrins, and platelets. Thromb Hemostasis **59:**1–6, 1988.
4. Heino J. Integrin-type extracellular matrix receptors in cancer and inflammation. Ann Med **25:**335–342, 1993.
5. Fawcett J, Harris AL. Cell adhesion molecule and cancer. Curr Opin Oncol **4:**142–148, 1992.
6. Rudolph R, Cheresh D. Cell adhesion mechanisms and their potential impact on wound healing and tumor control. Clin Plast Surg **17:**457–462, 1990.
7. Ginsberg MH, Loftus JC, D'Souza S, Plow EF. Ligand binding to integrins: Common and ligand-specific recognition mechanisms. Cell Differ Dev **32:**203–214, 1990.
8. Cheresh DA. Strucural and biologic properties of integrin-mediated cell adhesion. Clin Lab Med **12:**217–236, 1992.
9. Bosman FT. Integrins: Cell adhesives and modulators of cell function. Histochem J **25:**469–477, 1993.
10. Loftus JC, Smith JW, Ginsberg MH. Integrin-mediated cell adhesion: The extracellular face. J Biol Chem **269:**25235–25238, 1994.
11. Springer TA. Folding of the N-terminal, ligand-binding region of integrin α-subunits into a β-propeller domain. Proc Natl Acad Sci U S A **94:**65–72, 1997.
12. Loftus JC, Halloran CE, Ginsberg MH, Feigen LP, Zablock JA, Smith JW. The amino terminal one-third of the α_{IIb} defines the ligand recognition specificity of integrin $\alpha_{IIb}\beta_3$. J Biol Chem **271:**2033–2039, 1996.
13. Staatz WD, Peters KJ, Santoro SA. Divalent cation-dependent structure in the platelet membrane glyoprotein Ia-IIa VLA-2 complex. Biochem Biophys Res Commun **168:**107–113, 1990.
14. Michalak M, Milner RE, Burns K, Opas M. Calreticulin: A review. Biochem J **285:**681–692, 1992.
15. Diaz-Gonzalez F, Forsyth J, Steiner B, Ginsberg MH. Trans-dominant inhibition of integrin function. Mol Biol Cell **7:**1939–1951, 1996.
16. Du X, Gu M, Weisel JW, Nagaswami C, Bennett JS. Long-range propagation of conformational changes in integrin $\alpha_{11b}\beta3$. J Biol Chem **268:**23087–23092, 1993.
17. Akiyama SK. Integrins in cell adhesion and signaling. Hum Cell **9:**181–186, 1996.
18. Hato T, Pampori N, Shattil SJ. Complementary roles for receptor clustering and conformational change in the adhesive and signaling functions of integrin $\alpha_{IIb}\beta_3$. J Cell Biol **141:**1685–1695, 1998.
19. Felding-Habermann B, Cheresh DA. Vitronectin and its receptors. Curr Opin Cell Biol **5:**864–868, 1993.
20. Akiyama SK, Olden K, Yamada KW. Fibronectin and integrins in invasion and metastases. Cancer Metastasis Rev **14:**173–189, 1995.
21. Ruoslahti E. Fibronectin and its $\alpha_5\beta_1$ integrin receptor in malignancy. Invasion Metastasis **14:**87–97, 1994–95.
22. Schwartz MA, Ingber DE. Integrating with integrins. Mol Biol Cell **5:**389–393, 1994.
23. Kirchhofer D, Grzesiak J, Pierschbacher MD. Calcium as a potential physiological regulator of integrin-mediated cell adhesion. J Biol Chem **266:**4471–4477, 1991.
24. Schwartz ML, Lechene C, Ingber DE. Insoluble fibronectin activates the Na/H antiporter by clustering and immobolizing integrin $\alpha_5\beta_1$, independent of cell shape. Proc Natl Acad Sci U S A **88:**7849–7853, 1991.
25. Plopper GE, McNamee HP, Dike LE, Bojanowski K, Ingber DE. Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. Mol Biol Cell **6:**1349–1365, 1995.
26. Juliano RL, Varner JA. Adhesion molecules in cancer: The role of integrins. Curr Opin Cell Biol **5:**812–818, 1993.
27. Gui GP, Puddlefoot JR, Vinson GP, Wells CA, Carpenter R. *In vitro* regulation of human breast cancer cell adhesion and invasion *via* integrin receptors to the extracellular matrix. Br J Surg **82:**1192–1196, 1995.
28. Virtanen I, Korhonen M, Kariniemi AL, Gould VE, Laitinen L, Ylanne J. Integrins in human cells and tumors. Cell Differ Dev **32:**215–228, 1990.
29. Swaim MW, Chiang HS, Huang TF. Characterization of platelet aggregation induced by PC-3 human prostate adeno-carcinoma cells and inhibited by venom peptides, trigramin, and rhodostomin. Eur J Cancer **32A:**715–721, 1996.
30. Liapis H, Adler LM, Wick MR, Rader JS. Expression of $\alpha_v\beta_3$ integrin is less frequent in ovarian epithelial cells of low malignant potential in contrast to ovarian carcinomas. Hum Pathol **28:**443–449, 1997.

31. Dedhor S. Integrin mediated signal transduction in oncogenesis: An overview. Cancer Metastasis Rev **14:**165–172, 1995.
32. Danen EH, van Muijen GN, Ruiter DJ. Role of integrins as signal transducing cell adhesion molecules in human cutaneous melanoma. In: Hart I, Hogg N, Eds. Cell Adhesion and Cancer. Cancer Surveys: Advances and Prospects in Clinical, Epidemiological, and Laboratory Oncology. Plainview, NY: Cold Spring Harbor Laboratory Press, Vol **24:**pp43–65, 1995.
33. Juliano R. Signal transduction by integrins and its role in the regulation of tumors. Cancer Metastasis Rev **13:**25–30, 1994.
34. Akiyama SK, Yamada KM. Introduction: Adhesion molecules in cancer. Sem Cancer Biol **4:**215–218, 1993.
35. Juliano RL. The role of B1 integrins in tumors. Sem Cancer Biol **4:**227–283, 1993.
36. Mizejewski GJ. α-Fetoprotein as a biologic response modifier: Relevance to domain and subdomain structure. Proc Soc Exp Biol Med **315:**333–365, 1997.
37. Ruoslahti E. Stretching is good for a cell. Science **276:**1345–1346, 1997.
38. Lin Z, Brattain MG, Appert H. Differential display of reticulocalbin in the highly invasive cell line MDA-MB-435, versus the poorly invasive cell line, MCF-7. Biochem Biophys Res Commun **231:**283–289, 1997.
39. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. Ann Rev Cell Dev Biol **12:**463–518, 1996.
40. Edwards JG, Hameed H, Campbell G. Induction of fibroblast spreading by Mn^{2+}: A possible role for unusual binding sites for divalent cations in receptors for proteins containing Arg-Gly-Asp. J Cell Sci **89:**507–513, 1988.
41. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science **276:**1425–1428, 1997.
42. Staatz WD, Rajpara SM, Wayner EA, Carter WG, Santoro SA. The membrane glycoprotein Ia-IIa (VLA-2) complex mediates the Mg^{++}-dependent adhesion of platelets to collagen. J Cell Biol **108:**1917–1924, 1989.
43. Paulsson M. The role of Ca^{2+} binding in the self-aggregation of laminin nidogen complexes. J Biol Chem **263:**5425–5430, 1988.
44. Dransfield I, Cabanas C, Craig A, Hogg N. Divalent cation regulation of the function of the leukocyte integrin LFA-1. J Cell Biol **116:**219–226, 1992.
45. Ramsden JJ. Calcium-dependence of laminin binding to phospholipid membranes. Biopolymers **33:**475–477, 1993.
46. Franzen B, Linder S, Alaiya AA, Eriksson E, Uruy K, Hirano T, Okuzawa K, Auer G. Analysis of polypeptide expression in benign and malignant human breast lesions: Downregulation of cytokeratins. Br J Cancer **73:**1632–1638, 1996.
47. Fliegel L, Burns K, Opas M, Michalak M. The high affinity calcium binding protein of sarcoplasmic reticulum: Tissue distribution and homology with calregulin. Biochim Biophys Acta **982:**1–8, 1989.
48. Heilmann C, Spamer C, Leberer E, Gerok W, Michalak M. Human liver calreticulin: Characterization and Zn^{++} dependent interaction with phenyl-sepharose. Biochem Biophys Res Commun **193:**611–616, 1993.
49. D'Souza SE, Ginsberg MH, Burke TA, Plow EF. The ligand binding site of the platelet integrin receptor $GP_{IIb}\beta_3$ is proximal to the second calcium binding domain of its α-subunit. J Biol Chem **265:**3440–3446, 1990.
50. Milner RE, Baksh S, Shemanko C, Carpenter MR, Smille L, Vance JE, Opas M, Michalak M. Calreticulin, and not calsequestrin, is the major calcium binding protein of smooth muscle sarcoplasmic reticulum and liver endoplasmic reticulum. J Biol Chem **266:**7155–7165, 1991.
51. Opas M, Dziak E, Fliegel L, Michalak M. Regulation of expression and intracellular distribution of calreticulin, a major calcium binding protein of nonmuscle cells. J Cell Physiol **149:**160–171, 1991.
52. Sueyoshi T, McMullin BA, Marnell LL, Du Clos TW, Kisiel W. A new procedure for the separation of protein z, prothrombin fragment 1.2, and calreticulin from human plasma. Thrombosis Res **63:**569–575, 1991.
53. Dedhar S. Novel functions for calreticulin: Interaction with integrins and modulation of gene expression? Trends Biochem Sci **19:**269–271, 1994.
54. Burns K, Atkinson EA, Bleackley RC, Michalak M. Calreticulin: From Ca^{2+} binding to control of gene expression. Trends Cell Biol **4:**152–154, 1994.
55. Nash PD, Opas M, Michalak M. Calreticulin: Not just another calcium-binding protein. Mol Cell Biochem **135:**71–78, 1994.
56. Rojiani MV, Finlay BB, Gray V, Dedhar S. *In vitro* interaction of a polypeptide homologous to human Ro/SS-A antigen (calreticulin) with a highly conserved amino acid sequence in the cytoplasmic domain of integrin α-subunits. Biochemistry **30:**9859–9866, 1991.
57. Leung-Hagesteijn CY, Milankov K, Michalak M, Wilkins J, Dedhar S. Cell attachment to extracellular matrix substrates is inhibited upon downregulation of expression of calreticulin, and intracellular integrin α-subunit-binding protein. J Cell Sci **107:**589–800, 1994.
58. Fuller PJ. The steroid receptor superfamily. Mechanisms of diversity. FASEB J **5:**3092–3099, 1991.
59. Dedhar S, Ronnie PS, Shago M, Leung-Hagesteijn CY, Yang H, Filmus J, Hawley H, Bruchovsky N, Cheng H, Matusik RJ, Giguere V. Inhibition of nuclear hormone receptor activity by calreticulin. Nature **367:**480–484, 1994.
60. Burns K, Duggan B, Atkinson EA, Famulski KS, Nemer M, Bleackley RC, Michalak M. Modulation of gene expression by calreticulin binding to the glucorticoid receptor. Nature **367:**476–480, 1994.
61. Desai D, Michalak M, Singh NK, Niles RM. Inhibition of retinoic acid receptor function and retinoic acid–regulated gene expression in mouse melanoma cells by calreticulin: A potential pathway for cyclic AMP regulation of retinoic action. J Biol Chem **271:**15153–15159, 1996.
62. Wheeler DG, Horsford J, Michalak M, White JH, Hendy GN. Calreticulin inhibits vitamin D_3 signal transduction. Nucleic Acids Res **23:**3270–3273, 1995.
63. Michalak M, Burns K, Andrin C, Mesaeli N, Jass GH, Busaan JL, Opas M. Endoplasmic reticulum form of calreticulin modulates glucocorticoid-sensitive gene expression. J Biol Chem **271:**29436–29445, 1996.
64. Winrow CJ, Miyata KS, Marcus SL, Burns K, Michalak M, Capone JP, Rachubinske RA. Calreticulin modulates the *in vitro* DNA binding but not the *in vivo* transcriptional activation by peroxisome proliferator-activated receptor/retinoid X-receptor heterodimers. Mol Cell Endocrinol **III:**175–179, 1995.
65. Coppolino MG, Woodside MJ, Demaurex N, Grinstein S, St.-Armand R, Dedhar S. Calreticulin is essential for integrin-mediated calcium signalling and cell adhesion. Nature **386:**843–847, 1997.
66. Coppolino M, Leung-Hagesteijn CY, Dedhar S, Wilkin J. Inducible interaction of integrin $\alpha_2\beta_1$ with calreticulin: Dependence on the activation state of the integrin. J Biol Chem **270:**23132–23138, 1995.
67. Power RF, Mani SK, Codina J, Conneely OM, O'Malley B. Dopaminergic and ligand-independent activation of steroid hormone receptors. Science **254:**1636–1639, 1991.
68. Ignar-Trowbridge DM, Nelson KG, Bidwell MC, Curtis SW, Washburn TF, McLachan JA, Korach KS. Coupling of dual signaling pathways: Epidermal growth factor action involves the estrogen receptor. Proc Natl Acad Sci U S A **89:**4658–4662, 1992.
69. Philips A, Chalbos D, Rochefort H. Estradiol increases and anti-estrogens antagonize the growth-factor induced activator protein-1 activity in MCF-7 breast cancer cells without affecting c-*fos* and c-*jun* synthesis. J Biol Chem **268:**14103–14108, 1993.
70. Shyamala G, Guiot MC. Activation of KB-specific proteins by estradiol. Proc Natl Acad Sci U S A **89:**10628–10632, 1992.
71. Scarborough RM, Rose JW, Hsu MA, Phillips DR, Fried VA, Campbell AM, Nannizzi L, Charo IF. Barbourin: A $GP_{IIb-IIIa}$-specific integrin antagonist from the venom of *Sistrurus m. barbouri.* J Biol Chem **15:**9359–9362, 1991.
72. Chiang HS, Swaim MW, Huang TF. Characterization of platelet aggregation induced by human colon adenocarcinoma cells and its inhibition by snake venom peptides, trigramin, and rhodostomin. Br J Haematol **87:**325–331, 1994.
73. Chiang HS, Swaim MW, Huang TF. Characterization of platelet aggregation induced by human breast carcinoma and its inhibition by snake venom peptides, trigramin, and rhodostomin. Breast Cancer Res Treat **33:**225–235, 1995.
74. Coller BS, Anderson KM, Weisman HF. The anti-$GP_{IIb-IIIa}$ agent: Fundamental and clinical aspects. Haemostasis **26**(Suppl 4)**:**285–293, 1996.
75. Nurden AT. New thoughts on strategies for modulating platelet func-

tion through the inhibition of surface receptors. Haemostasis **26**(Suppl 4):78–84, 1996.
76. Sheu JR, Lin CH, Peng HC, Huang TF. Triflavin, an Arg-Gly-Asp-containing peptide, inhibits the adhesion of tumor cells to matrix proteins *via* binding to multiple integrin receptors expressed on human hepatoma cells. Proc Soc Exp Biol Med **213**:71–80, 1996.
77. Nierodzik ML, Klepfish A, Karpatkin S. Role of platelet integrin $GP_{IIb\text{-}IIIa}$, a fibronectin, von Willebrand factor, and thrombin in platelet–tumor interaction *in vitro* and metastasis *in vivo*. Sem Hematol **31**:278–288, 1994.
78. Gasic GJ, Gasic TB, Galanti N, Johnson T, Murphy S. Platelet-tumor-cell interactions in mice: The role of platelets in the spread of malignant disease. Int J Cancer **11**:704–718, 1973.
79. Gould RJ, Polokoff MA, Friedman PA, Huang TF, Holt JC, Cook JJ, Niewarowski S. Disintegrin: A family of integrin inhibitory proteins from viper venoms. Proc Soc Exp Biol Med **195**:168–172, 1990.
80. Huang TF, Wang WJ, Teng CM, Ouyang C. Mechanism of action of the anti-platelet peptide, arietin, from Bitis arietans venom. Biochim Biophys Acta **1074**:144–150, 1991.
81. Sheu JR, Yen MH, Peng HC, Chang MC, Huang TF. Trifavin, an Arg-Gly-Asp containing pepide, prevents platelet plug formation in *in vivo* experiments. Eur J Pharm **294**:231–238, 1995.
82. Sheu JR, Ko WC, Hung WC, Peng HC, Huang TF. Interaction of thrombin-activated platelets with extra-cellular matrices: Comparison of the activity of Arg-Gly-Asp-containing venom peptides and monoclonal antibodies against $GP_{IIb\text{-}IIIa}$ complex. J Pharm Pharmacol **49**:78–84, 1997.
83. Zhou Q, Smith JB, Grossman MH. Molecular cloning and expression of catrocalla statin, a snake venom protein from *Crotalus atrox* which inhibits platelet adhesion to collagen. Biochem J **307**:411–417, 1995.
84. Ruoslahti E. Control of cell motility and tumor invasion by extracellular matrix interactions. Br J Cancer **66**:239–242, 1992.
85. Plantefaber LC, Hynes RO. Changes in integrin receptors on oncogenically transformed cells. Cell **56**:281–290, 1989.
86. Aklyama SK, Larjava H, Yamada KM. Differences in the biosyntheses and localization of the fibronectin receptor in normal and transformed cultured human cells. Cancer Res **50**:1601–1607, 1990.
87. Giancotti FG, Ruoslahti E. Elevated levels of the $\alpha_5\beta_1$ fibronectin receptor suppress the transformed phenotype of chinese hamster ovary cells. Cell **60**:849–859, 1990.
88. Chan BM, Matsuma N, Tabada Y, Zetter BR, Hemier M. *In vitro* and *in vivo* consequences of the VLA-2 expression on rhabdomyosarcoma cells. Science **251**:1600–1602, 1991.
89. Chen FA, Repasby EA, Bankert RB. Human lung tumor-associated antigen identified as an extracellular matrix adhesion molecule. J Exp Med **173**:1111–1119, 1991.
90. Klein CE, Steinmayer T, Kaufman D, Weber L, Broker EB. Identification of a melanoma progression antigen as integrin VLA-2. J Invest Dermatol **96**:281–284, 1991.
91. Natali PG, Nicotra MR, Cavaliere R, Giannarelli D, Bigotti A. Tumor progression in human malignant melanoma is associated with changes in $\alpha_6\beta_1$ laminin receptor. Int J Cancer **49**:168–172, 1991.
92. Natali PG, Nicotra MR, Botti C, Mottolese M, Bigotti A, Segatto O. Changes in expression of $\alpha_6\beta_4$ integrin heterodimer in primary and metastatic breast cancer. Br J Cancer **66**:218–322, 1992.
93. Pignatelli M, Hanby AM, Stamp GW. Low expression of $\beta_1\alpha_2$, and α_3 subunits of VLA integrins in malignant mammary tumors. J Pathol **165**:25–32, 1991.
94. Bartolazzi A, Cerboni C, Nicotra MR, Mottolese M, Bigotti A, Natali PG. Transformation and tumor progression are frequently associated with expression of the $\alpha_3\beta_1$ heterodimer in solid tumors. Int J Cancer **58**:488–491, 1994.
95. Koukoulis GK, Virtanen I, Korhonen M, Laitinen L, Quaranta V, Gould VE. Immunohistochemical localization of integrins in the normal, hyperplastic, and neoplastic breast: Correlations with their functions as receptors and cell adhesion molecules. Am J Pathol **139**:787–789, 1991.
96. Albelda SM. Biology of disease: Role of integrins and other cell adhesion molecules in tumor progression and metastasis. Lab Invest **68**:4–17, 1993.
97. Hall PA, Coates P, Lemoine NR, Horton MA. Characterization of integrin chains in normal and neoplastic human pancreas. J Pathol **165**:33–41, 1991.
98. Grinstein S, Rotin D, Mason MJ. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. Biochim Biophys Acta **988**:73–97, 1989.
99. Ingebar DE, Prusty D, Frangioni JV, Cragoe EJ Jr., Lechene C, Swarta MA. Control of intracellular pH and growth by fibronectin in capillary endothelial cells. J Cell Biol **110**:1803–1811, 1990.
100. Ingebar DE. Extracellular matrix as a solid state regulator in angiogenesis: Identifications of new targets for anti-cancer therapy. Sem Cancer Biol **3**:57–63, 1992.
101. Varner JA. The role of vascular cell integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in angiogenesis. EXS **79**:361–390, 1997.
102. Koukoulis GK, Howeedy AA, Korhonen M, Virtanen I, Gould VE. Distribution of tenascin, cellular fibronectins, and integrins in the normal, hyperplastic, and neoplastic breast. J Submicrosc Cytol Pathol **25**:283–295, 1993.
103. Varner JA, Cheresh DA. Integrins and cancer. Curr Opin Cell Biol **8**:724–730, 1996.
104. Varner JA, Cheresh DA. Tumor angiogenesis and the role of vascular cell integrin $\alpha_v\beta_3$. Imp Adv Oncol **1**:69–87, 1996.
105. Max R, Gerritsen RR, Nooijen PT, Goodman SL, Sutter A, Keilholz U, Ruiter DJ, DeWaal RM. Immunohistochemical analysis of integrin $\alpha_v\beta_3$ expression on tumor-associated vessels of human carcinomas. Int J Cancer **71**:320–324, 1997.
106. Gladson CL. Expression of integrin $\alpha_6\beta_3$ in small blood vessels of glioblastoma tumors. J Neuropathol Exp Neurol **55**:1143–1149, 1996.
107. Voest EE. Inhibitors of angiogenesis in a clinical perspective. Anticancer Drugs **7**:723–727, 1996.
108. Brooks PC, Stromblad S, Klemke D, Sarkar FH, Cheresh DA. Anti-integrin $\alpha_v\beta_3$ blocks human breast cancer growth and angiogenesis. J Clin Invest **96**:1815–1822, 1995.
109. Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature **390**:404–407, 1997.
110. Frisch SM, Ruoslahti E. Integrins and anoikis. Curr Opin Cell Biol **9**:701–706, 1997.
111. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol **124**:619–626, 1994.
112. Meredith JE, Swartz M. Integrins, adhesion, and apoptosis. Trends Cell Biol **7**:146–150, 1997.
113. Coucouvanis E, Martin G. Signals for death and survival: A two-step mechanism for cavitation in the vertebrate embryo. Cell **83**:279–287, 1995.
114. Montgomery A, Reisfeld R, Cheresh D. Integrin $\alpha_v\beta_3$ rescues melanoma cells from apoptosis in three-dimensional dermal collagen. Proc Natl Acad Sci U S A **91**:8856–8860, 1994.
115. Boudreau N, Sympson CJ, Werb Z, Bissell MJ. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science **267**:891–893, 1995.
116. Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA. Integrin $\alpha_v\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell **79**:1157–1164, 1994.
117. Scoot G, Cassidy L, Busacco A. Fibronectin suppresses apoptosis in normal human melanocytes through an integrin-dependent mechanism. J Invest Dermatol **108**:147–153, 1997.
118. Zhang Z, Vuori K, Reed JC, Ruoslahti E. $\alpha_5\beta_1$ integrin supports survival of cells on fibronectin and upregulates *bcl*-2 expression. Proc Natl Acad Sci U S A **92**:6161–6165, 1995.
119. Sugahara H. Induction of apoptosis by fibronectin *via* its interaction with VLA-5. Nippon Rinsho-Jap J Clin Med **54**:1809–1814, 1996.
120. Terui Y, Furukawa Y, Sakai T, Kikuchi J, Sugahara H, Kanakura Y, Kitagwa S, Miura Y. Upregulation of VLA-5 expression during monocytic differentiation and its role in negative control of the survival of peripheral blood monocytes. J Immunol **156**:1981–1988, 1996.
121. Agrez MV, Bates RC. Colorectal cancer and the integrin family of cell adhesion receptors: Current status and future directions. Eur J Cancer **30A**:2166–2170, 1994.
122. Clarke AS, Lotz MM, Chao C, Mercurio AM. Activation of the p21 pathway of growth arrest and apoptosis by the β_4 integrin cytoplasmic domain. J Biol Chem **270**:22673–22676, 1995.
123. Saelman EU, Keely PJ, Santoro SA. Loss of MDCK cell $\alpha_2\beta_1$ integrin expression results in reduced cyst formation, failure of hepato-

cyte growth factor–induced branching morphogenesis, and increased apoptosis. J Cell Sci **108**:3531–3540, 1995.
124. Rozzo C, Chiesa V, Caridi G, Pagnan G, Ponzoni M. Induction of apoptosis in human neuroblastoma cells by abrogation of integrin-mediated cell adhesion. Int J Cancer **70**:688–698, 1997.
125. Giancotti FG. Integrin signaling: Specificity and control of cell survival and cell cycle progression. Curr Opin Cell Biol **9**:691–700, 1997.
126. Howlett AR, Bailey N, Damsby C, Petersen OW, Bissell MJ. Cellular growth and survival are mediated by β_1 integrins in normal human breast epithelium but not in breast carcinoma. J Cell Sci **108**:1945–1957, 1995.
127. Hata H, Matsuzaki H, Takeya M, Yoshida M, Sonoki T, Nagasaki A, Kuribayashi N, Kawano F, Takatsuki K. Expression of Fas/Apo-1 (CD95) and apoptosis in tumor cells from patients with plasma cell disorders. Blood **86**:1939–1945, 1995.
128. Green LJ, Mould AP, Humphries MJ. The integrin β-subunit. Int J Biochem Cell Biol **30**:179–184, 1998.
129. Zutter MM, Mazoujian G, Santoro SA. Decreased expression of integrin adhesive protein receptors in adenocarcinoma of the breast. Am J Pathol **137**:863–870, 1990.
130. Zutter MM, Krigman HR, Santoro SS. Altered integrin expression in adenocarcinoma of the breast: Analysis by *in situ* hybridization. Am J Pathol **142**:1439–1448, 1993.
131. Damjanovich L, Fulop B, Adany R, Nemes Z. Integrin expression on normal and neoplastic human breast epithelium. Acta Chir Hung **36**:69–71, 1997.
132. Meyer T, Marshall JF, Hart IR. Expression of α_V integrins and vitronectin receptor identify in breast cancer cells. Br J Cancer **77**:530–536, 1998.
133. Saulnier R, Chan B, Uniyal S, Chan T, Patrzykat A, Elliott BE. The role of integrins and extracellular matrix in anchorage-dependent growth of a mammary carcinoma cell line. Cell Mol Biol **43**:455–468, 1997.
134. Gui GP, Puddlefoot JR, Vinson GP, Wells CA, Carpenter R. Altered cell-matrix: A prerequisite for breast cancer metastases? Br J Cancer **75**:623–633, 1997.
135. Alford D, Pitha-Rowe P, Taylor-Papdimitriou J. Adhesion molecules in breast cancer: Role of α_2, β_1 integrin. Biochem Soc Symp **63**:245–259, 1998.
136. Tagliabue E, Ghirelli C, Squicciarini P, Aiello P, Colnaghi MI, Menard S. Prognostic value of α_6, β_4 integrin expression in breast carcinomas is affected by laminin production from tumor cells. Clin Cancer Res **4**:407–410, 1998.
137. Jones JL, Royall JE, Critchley DR, Walker RA. Modulation of myoepithelial-associated α_6, β_4 integrin in a breast cancer cell line alters invasive potential. Exp Cell Res **235**:325–333, 1997.
138. Wewer UM, Shaw LM, Albrechtsen R, Mercurio AM. The integrin $\alpha_6\beta_1$ promotes the survival of metastatic human breast carcinoma cells in mice. Am J Pathol **151**:1191–1198, 1997.
139. Gui GP, Wells CA, Browne PD, Yeomans P, Jordan S, Puddefoot JR, Vinson GP, Carpenter R. Integrin expression in primary breast cancer and its relation to axillary nodal status. Surgery **117**:102–108, 1995.
140. Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsby C, Bissell MJ. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and *in vivo* by integrin blocking antibodies. J Cell Biol **137**:231–245, 1997.
141. Schehr RS. Rethinking the development of breast cancer. Nat Biotech **15**:517–518, 1997.
142. Ben-Ze'ev A. Cytoskeletal and adhesion proteins as tumor suppressors. Curr Opin Cell Biol **9**:99–108, 1997.