Adhesion, Invasion, Integrins, and Beyond

5

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KEY POINTS

- A precise and concerted interplay between both the cellular components, i.e., the cells and their mutations, and the acellular components, i.e., the tumor microenvironment, drives tumor growth and spread beyond physiological boundaries as well as promotes cellular resistance to conventional radiotherapy and chemotherapy.
- One of the prominent microenvironmental modulators of the sensitivity of tumor tissue and tumor-associated normal tissue to therapy is the interaction of cells with the extracellular matrix. Besides serving as structural support for the cells in a tissue, the extracellular matrix participates in the regulation of essential cell functions such as survival, proliferation, differentiation, adhesion, and migration.
- Adhesion and invasion are controlled by integrin receptors and are frequently dysregulated in cancer, with disastrous consequences such as local destruction of normal tissue, metastases, and ineffective local tumor control by anticancer therapeutics. Particularly compromised local tumor control evolves from the combination of genetic alterations and changes in the tumor microenvironment.
- Integrins and their associated signaling molecules are attractive target molecules to be inhibited by pharmacological small molecules aiming at optimization of conventional radio- and chemotherapy.
- Unraveling the intra- and extracellular networks a tumor cell exploits for its growth and spreading capability in more depth may foster the diagnosis of early-stage cancer, the development of novel drugs, and eventually improved patient survival.

Abstract

The importance of the tumor microenvironment for tumor development and progression becomes increasingly evident. A precise and concerted interplay between both the cellular components, i.e., the cells and their mutations, and the acellular components, i.e., the tumor microenvironment, drives tumor growth and spread beyond physiological boundaries as well as promotes cellular resistance to conventional radiotherapy and chemotherapy. One of the prominent microenvironmental modulators of the sensitivity of tumor tissue and tumor-associated normal tissue to therapy is the interaction of cells with the extracellular matrix. Besides serving as structural support for the cells in a tissue, the extracellular matrix participates in the regulation of essential cell functions such as survival, proliferation, differentiation, adhesion, and migration. In this chapter, the overarching function of the tumor-related extracellular matrix is depicted and summarized with regard to the molecular, pathophysiological, and radiobiological aspects associated with tumor biology, radiation, and chemoresistance in the context of cell adhesion molecule families, their interactions with other types of cell surface receptors, and the downstream network of signal transducers.

5.1 Introduction

A large body of evidence illustrates the complexity of tumor biology on both a cellular and an acellular level. Examples for cellular factors are the variety of genetic gain-of-function or loss-of-function mutations in key molecules involved in proliferation, multidrug resistance, and apoptosis, which are concertedly responsible for the commonly recognized "hallmarks of cancer" (Croce 2008; Halazonetis et al. 2008; Varmus et al. 2005).

Beyond these cellular factors, the acellular elements complementing the tumor as complex, autonomous tissues are increasingly identified as critical and potent carcinogenic promoters and modulators of tumor cell sensitivity to conventional radiation and chemotherapies. The panel of acellular elements comprises, among others, soluble growth factors, oxygen, metabolites, and the proteins of the extracellular matrix (ECM) (Cordes and Park 2007; Durand 1994; Kim et al. 2006; Petersen et al. 2003; Tannock 1996; Vaupel and Mayer 2005; Wedgwood and Younes 2006). The distinguishable parameter between a normal cell and a malignant cell is the acquisition of an autonomous positive feedback loop from those extracellular signals by the malignant cell. Most importantly, this process must not necessarily be associated with independence from extracellular signals as misleadingly reported. Uncoordinated reactions and the intracellular channeling and execution of these signals into action on extracellular growth signals integrate into perpetual mitosis of both the tumor cells themselves and of the normal cell types, which are overflowed with tumor cell-derived soluble growth factors. Similar to growth factors, tumor cells synthesize and secret proteins of the extracellular matrix and tissue-remodeling matrix-metalloproteinases to construct their own unique and progrowth microenvironment (Chang and Werb 2001; Chung et al. 1992; Lopez-Otin and Matrisian 2007; Lynch and Matrisian 2002). Within this environment, specific niches provide a microenvironment that confers resistance to therapy with ionizing radiation and/or chemotherapeutics (Dalton 2003; Diaz-Montero and McIntyre 2003; Hehlgans et al. 2007b; Hodkinsons et al. 2007; Li and Dalton 2006; Paszek and Weaver 2004; Weaver et al. 2002; Zahir and Weaver 2004). Moreover, remodeling of the extracellular matrix generates migratory avenues for local tumor cell invasion and metastasis. Particularly the latter as limiting factor for patient's survival challenges systemic anticancer strategies and fosters the development of more targeted approaches against the primary tumor (Board and Valle 2007; Cochran et al. 2008; Ljungberg 2007; Morabito et al. 2007).

In addition to the intrinsic insensitivity of the majority of tumor cells to antigrowth signals, it can be hypothesized that some tumor cells grow in the before mentioned microenvironmental insensitivity-conferring niche or represent one of the tumor-initiating cells characterized by a great self-renewal potency and a slow doubling time.

Owing to an overarching function of the microenvironmental component called the extracellular matrix, this chapter depicts and summarizes the molecular, pathophysiological, and radiobiological aspects associated with tumor biology, radiation, and chemoresistance in the context of the cell adhesion molecule families, their interactions with other types of cell surface receptors and the downstream network of signal transducers.

5.2 Extracellular Matrix, Cell–Matrix Interactions, and Cell–Cell Interactions

The extracellular matrix represents the structural support for the cells in a tissue and further serves in regulating essential cell functions via cell surface receptors and as depot for growth factors. In connective tissues, the extracellular matrix either is between cells as interstitial matrix or organized as basement membrane providing anchorage for epithelial or endothelial cells (Alberts et al. 2002; Lodish et al. 2004). Either type of cell-matrix interaction can be found in all tissues and is mediated by different cell adhesion molecules (CAMs) mostly as transmembrane proteins (Hynes 2004). In general, the features mechanical stability and integrity of signal transduction in a tissue are also conducted by interactions between neighboring cells. These kinds of interactions are similarly accomplished by transmembrane proteins. Some of them are CAMs; others form, for example, intercellular tubes called gap junctions, which allow cell–cell communication via passage of molecules with a molecular weight below 1,000 kDa (Rousset 1996). To date, it is clear that all of these processes contribute to the proper regulation of normal tissue function and homeostasis as well as of malignant tissue in a differential and complex manner determined by the cumulated diversity of cellular alterations during the development of an individual tumor.

5.2.1 Extracellular Matrix

The presence of the extracellular matrix is essential for a large number of processes such as survival, proliferation, differentiation, adhesion, migration, and tissue integrity (Alavi and Stupack 2007; Blaschke et al. 1994; LaBarge et al. 2007; Petersen et al. 1998). The composition of the ECM based on the distribution of different molecules, as detailed below, depends on the tissue with its unique functions and its role in separating one tissue/organ from another.

An aspect of utmost importance is the direct impact of the ECM on the cell's dynamic in terms of cell shape, tissue tension, and motility resulting from membrane-spanning outside-in and inside-out signal transduction (Giancotti and Ruoslahti 1999; Hynes 2002). Signals are transduced by both CAMs and growth factor receptors, and their intracellularly converging cascades. Regarding growth factors, the ECM sequesters a wide range of cellular growth factors (Vlodavsky et al. 1990, 1991). On changes of the microenvironmental conditions, a set of proteases is able to release these deposited molecules to expeditiously act locally via their cognate transmembrane growth factor receptors. In this case, no de novo synthesis is required.

The molecular components assembling the ECM are produced and secreted by resident cells like fibroblasts (Hay 1989; Sugrue and Hay 1981). Subsequent to secretion, these components aggregate with the existent network of hydrophilic glycosaminoglycans and fibrillar and elastic proteins.

5.2.1.1 Proteoglycan Components

Glycosaminoglycans (GAGs) are carbohydrate polymers, which are usually attached to ECM proteins to form proteoglycans (Alberts et al. 2002; Bolender et al. 1981). Being molecules with a net negative charge, proteoglycans are hydrophilic, thus, attracting water molecules. This hydration characterizes the gel-like consistency of the ECM essential for the hydration of cells and as basis for the fibrillar and elastic proteins. Proteoglycans found in the ECM are outlined in the following:

1. *Keratan sulfate proteoglycans*. Keratan sulfate has variable sulfate content but does not contain uronic acid. Present in, e.g., cartilage, bone.
2. *Heparan sulfate proteoglycans*. Heparan sulfate, as linear polysaccharide, is ubiquitously expressed in human tissues. Its proteoglycanic form binds to various protein ligands/receptors to participate in the regulation of biological functions like embryonic development and metastasis.
3. *Chondroitin sulfate proteoglycans*. Chondroitin sulfate contributes to the elastic capacity and strength of tendons, ligaments, and cartilage.

5.2.1.2 Hyaluronic Acid

Hyaluronic acid is a polysaccharide consisting of alternative residues of D-glucuronic acid and *N*-acetylglucosamine. Hyaluronic acid, as major compound of the ECM and thus of the hydrophilic gel, realizes a high degree of absorbability to protect specific tissues against compression. Furthermore, hyaluronic acid contributes to the regulation of embryonic development, healing processes, inflammation, and tumor development

(Adamia et al. 2005; Nagano and Saya 2004). Its specific cognate transmembrane receptor is CD44.

5.2.1.3 Fibronectin

Fibronectin, a high-molecular-weight glycoprotein, contains ~5% carbohydrate that facilitates adhesion to the CAM family of integrins as well as other ECM proteins such as collagen and heparan sulfate (Czirok et al. 2006; Gospodarowicz et al. 1979; Ruoslahti 1999). On binding of fibronectin, the cellular cytoskeleton is reorganized allowing, e.g., cell movement along ECM structures. This process is particularly important for wound healing and blood clotting. In cancer, fibronectin has been suggested to support tumor development and to mediate resistance to chemo- and radiotherapy as a consequence of an increased expression (Cordes and Park 2007; Damiano 1999, 2002; Hehlgans et al. 2007b).

5.2.1.4 Collagen

The most abundant matrix proteins in the ECM are collagens (Alberts et al. 2002; Ramachandran and Kartha 1954). These fibrillar proteins are mainly responsible for the structural support essential for many cell functions of the resident cells. Upon synthesis of procollagen, packaging of procollagen in the Golgi apparatus, and exocytosis, procollagen is cleaved at specific peptide sites by procollagen peptidases. The evolved tropocollagen units are organized into fibrils, which subsequently assembled in collagen fibers. The fibers then attach to cell membranes through diverse types of proteins such as fibronectin and integrins. To date, 28 types of collagens have been reported. The majority—over 90%—are the collagens I, II, III, and IV. Subgroups of collagens are fibrillar (types I, II, III, V, XI), fibril-associated collagens with interrupted triple helices (FACITs) (types IX, XII, XIV), short chains (type VIII, X), basement membrane (type IV), and others (type VI, VII, XIII). Several diseases have been ascribed to genetic defects in collagen-encoding genes. A few examples are osteogenesis imperfecta (collagen I) and scurvy, which results from defective collagen due to the lack of Vitamin C. In this case, Vitamin C is an essential enzyme for a posttranslational modification process of the collagen molecule.

5.2.1.5 Laminin

Laminins represent the major noncollagenous scaffolding molecules particularly of basal laminae (Johnson 1980). The members of this glycoprotein family are secreted and then integrated into existent ECM. In contrast to the collagenic fiber formation, laminins are organized as web-like networks, which effectively maintain a great capacity of tension force. Each laminin molecule is a heterotrimer assembled from alpha, beta, and gamma chains. Identified are the following chains: alpha chains (LAMA1, LAMA2, LAMA3, LAMA4, LAMA5), beta chains (LAMB1, LAMB2, LAMB3, LAMB4), and gamma chains (LAMC1, LAMC2, LAMC3). In summary, 15 different laminin heterotrimers are known. Laminins bind, for example, collagens and entactins.

5.2.1.6 Elastin

Elastin is synthesized by fibroblasts and smooth muscle cells. Elasticity in the tissue is given by elastin (Ardelt 1964). Tissues dependent on a great degree of elasticity are, e.g., blood vessels, lung, elastic ligaments, bladder, and skin.

The understanding of ECM structure, composition, and function is critical in comprehending the altered responsiveness of cancer cells upon irradiation and chemotherapy as well as the complex dynamics of local tumor cell invasion and distant metastasis. In the following, the variety of molecules involved in cell–matrix interactions and cell–cell contact are described in a more thorough manner.

5.2.2 Cell–Matrix Interactions

Interactions between cells and the ECM are facilitated through specifically organized areas of the cell membrane. Two well-known types of these interactions are the focal adhesions and the hemidesmosomes (Fig. 5.1; Table 5.1) (Broussard et al. 2008; Burridge et al. 1990; Martin et al. 2002).

The common feature of these adhesion-mediating sites is the presence of transmembrane integrin receptors forming an ECM-cytoskeleton nexus (see Sect. 5.3.1). In addition, many adapter proteins and signaling molecules congregate to build up a multiprotein complex at the cytoplasmic face of the cell membrane,

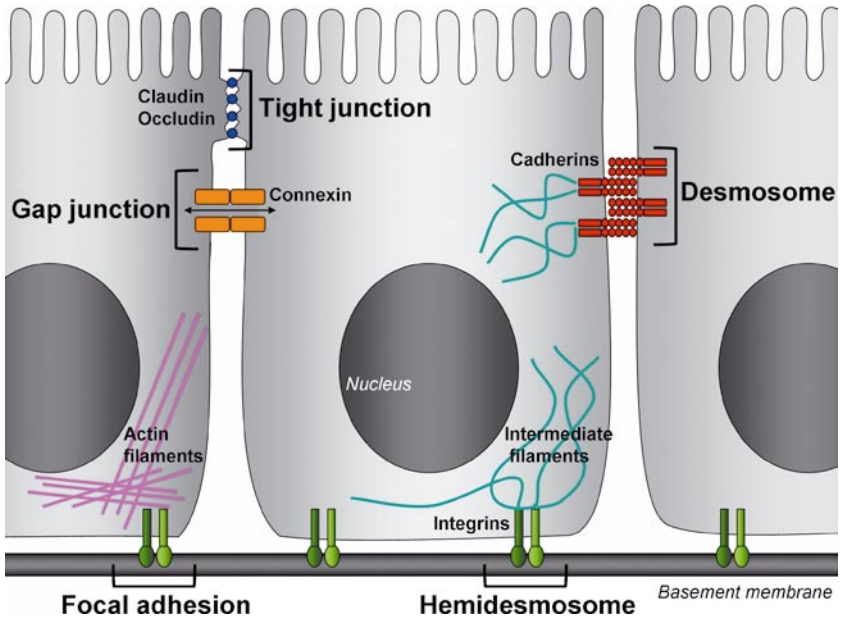

Fig. 5.1. Schematic delineation of cell–ECM and cell–cell interactions

Table 5.1. Different types of cell–ECM interactions

	Function	CAM	Occurrence
Focal adhesions	Anchorage	Integrins	E.g., cell migration, muscle–tendon connection
Hemidesmosomes	Anchorage	$\alpha_6\beta_4$ integrin	E.g., epithelium

termed a focal adhesion (Lo and Chen 1994). Courses of assembling and disassembling take place in remodeling tissues, during cell migration and self-renewal in turnover tissues. More stable focal adhesions are formed, e.g., in muscle cells anchored to their tendons. Hemidesmosomes, molecularly organized similarly to focal adhesions, play an important role in epithelial tissue mediating anchorage of epithelial cells to the basement membrane. The terminus hemidesmosome evolved from the terminus desmosome (i.e., macula adherents [Latin for "adhering spot"]; see Sect. 5.2.3), which represents a spot-like cell structure specialized for cell–cell contact on the lateral side of an epithelial cell. As hemidesmosomes are also integrin dependent, the $\alpha_6\beta_4$ integrin, as common example, facilitates a cytoplasmic connection between the anchor protein plectin to keratin intermediate filaments. This composition helps to compensate tensile or shearing forces and contributes therefore to tissue integrity.

Concerning focal adhesions in epithelial cells, these sites of adhesion connect the ECM to actin filaments via transmembrane integrin receptors (Hynes 2002).

To note, intermediate filaments are cytoskeletal structures that are formed by different members of a family of related, highly conserved proteins (Godsel et al. 2008). Most types of intermediate filaments are located in the cytoplasm. The nuclearly localized intermediate filaments are called lamins. Categorization of intermediate filaments into six groups has been done on the basis of similarities in amino acid sequence and protein structure. Types I and II intermediate filaments are acidic and basic keratins, namely epithelial keratins and trichocytic keratins (e.g., in hair and horns). Type III intermediate filaments are, e.g., vimentin (widely expressed in fibroblasts), Type IV intermediate filaments are, e.g., neurofilaments, type V intermediate filaments are nuclear lamins, type VI intermediate filaments are nestin. A well-known disease resulting from

gene mutations in keratin 5 or keratin 14 intermediate filament genes is, e.g., epidermolysis bullosa simplex (Fine and Griffith 1985; Ishida-Yamamoto et al. 1991).

Actin is one of the most highly conserved proteins and the monomeric subunit of microfilaments (Alberts et al. 2002; Perry and Cotterill 1965). Microfilaments belong to one of the three major components of the cytoskeleton. Actin is also a component of thin filaments managing contractility in muscle cells. Overall, actin serves in a variety of critical cellular mechanisms such as cell division, motility and shape, vesicle movement, signal transduction, and assembly and maintenance of cell junctions.

5.2.3 Cell–Cell Interactions

Desmosomes (macula adherents) are the subcellular correlates clenching neighboring cells together in simple/monolayer and stratified/multilayer epithelia and in muscle cells (Fig. 5.1; Table 5.2) (Green et al. 2007). These button-like attachment sites are located at the lateral side of a cell and contain transmembrane adhesion molecules of the cadherin family as well as different anchor proteins linking to the intracellular keratin cytoskeletal filaments. The extracellular portion of a cadherin, including five domains with calcium-binding motifs, binds to an identical cadherin on an adjacent cell for mediating cell–cell contact (Pettitt 2005). According to their structural composition, desmosomes fulfill different functions.

Tight junctions (zonula occludens), as second type of cell–cell contacts, serve as diffusion barrier to prevent the leakage of molecules and fluids through the intercellular space (Fig. 5.1, Table 5.2) (Niessen 2007; Niessen and Gottardi 2008). The barrier function is conferred by a branching network of independently sealing strands to result in a linkage of the cytoskeletons of neighboring cells. Multiple proteins, claudins and occludins representing the majority of components, cross-link the opposing cell membrane strands. Hence, the efficacy of tight junctions in preventing diffusion exponentially increases with the number of strands. In general, tight junctions achieve (1) attachment of cell to adjacent cells, (2) blocking of molecule diffusion between cells, and (3) preserving cellular polarity by preventing the motion of integral membrane proteins between the apical and basolateral surfaces of the cell. This includes the preservation and control of effective active transcellular transport or passive diffusion through the cell. They prevent the passage of molecules and ions through the space between cells. Tissues critically dependent on proper tight junction function are the epithelial tissue of the intestine, the epithelial tissue of the urinary tract and the endothelium of the brain, i.e., blood–brain barrier.

As third type of cell–cell contacts, gap junctions are composed of connexin monomers (Fig. 5.1; Table 5.2) (Mese et al. 2007; Wang and Mehta 1995). Six monomers form a connexon hexamer, which serves as a hemichannel. When two hemichannels of adjacent cells associate, they establish a gap junction. This intercellular communication tunnel allows different molecules and ions, mostly small intracellular signaling molecules (intracellular mediators), with a molecular weight below 1,000 kDa to pass freely between cells (Rousset 1996). This type of cell–cell interaction is localized, for example, in the heart muscle, where it enables coordinated contraction.

Table 5.2. Different types of cell–cell interactions

	Function	CAM	Occurrence
Desmosomes	Anchorage	Cadherins	E.g., epithelium, heart muscle
Tight junctions	Occlusion	Claudins, occludins	E.g., intestine, urinary tract
Gap junctions	Communication	Connexin	E.g., heart, nervous system, bones

5.3 Cell Adhesion Molecules

5.3.1 Integrins

As outlined under Sect. 5.2.2, the CAMs of the integrin family present the main cell surface receptors for binding of cells to ECM proteins like fibronectin, collagen, or laminin (Table 5.3) (Hynes 2002; Martin et al. 2002; Schmidt et al. 1993; Schwartz 2001). Moreover, integrins also serve as adhesion molecules for cell–cell interactions, especially on blood cells. Integrins are composed of two different transmembrane glycoproteins, known as α and β subunits, which bind noncovalently to form an αβ heterodimer (Fig. 5.2). To date, 24 different integrin receptors have been identified (Hynes 2002). The ligand binding specificity of the heterodimers is influenced by the subunit combination and by cell-type specific factors. The binding of integrins to ECM proteins is accomplished by short amino acid sequences located at the large extracellular domain. Motifs for such integrin-binding sequences are RGD (arginine–glycine–aspartate), found in fibronectin or laminin or DGEA (aspartate–glycine–glutamic acid–alanine), and GFOGER (glycine–phenylalanine–glycine–glutamic acid–arginine) found in collagen (Calderwood et al. 1995, 1997; Evans and Calderwood 2007; Liu et al. 2000; Ruoslahti 2003). Inside the cells, adapter proteins like talin, α-actinin, and vinculin bridge the gap between the cytoplasmic integrin domain and the cytoskeleton. This multiprotein complex forms the structural basis for an association with a large set of signal transduction molecules like focal adhesion kinase (FAK) and the Rous sarcoma oncogene (Src), eventually assembling a focal adhesion (Brakebusch and Fassler 2003). The signaling works in opposite directions. While ligand binding to the integrin extracellular domain leads to activation of numerous intracellular signaling pathways (outside-in signaling), certain intracellular processes stimulated by, e.g., docking of growth factors to their cognate transmembrane receptor alter the binding affinity and avidity of integrins (inside-out signaling) (Hynes 2002). These mechanisms are poorly understood, but may be due to conformational changes of the receptor. Because integrin function is independent from de novo synthesis and/or degradation, the integrin-related adhesion response in both directions can proceed within seconds. For example, this allows platelets to circulate unimpeded in the blood until damage of the vascular wall activates the integrins in the

Table 5.3. Families of cell adhesion molecules assigned to morphological, functional, and molecular characteristics

Characteristics	Integrins	Cadherins	Ig CAMs	Selectins
Ligands	ECM proteins, Ig CAMs	Cadherins	Ig CAMs, integrins, ECM proteins	Carbohydrates
Binding	Heterophilic	Homophilic	Homophilic, heterophilic	Heterophilic
Adhesion sites	Focal adhesions, hemidesmosomes, nonjunctional adhesion	Desmosomes, adherens junctions	Nonjunctional adhesion	Nonjunctional adhesion
Main function	Cell–matrix interactions	Cell–cell interactions	Cell–cell interactions	Cell–cell interactions
Tumor overexpression	E.g., HNSCC, breast cancer		E.g., neuroblastomas, SCLC	
Tumor downregulation		E.g., breast cancer, gastric cancer	E.g., gastrointestinal tumors	

HNSCC head and neck small cell cancer, *SCLC* small cell lung cancer

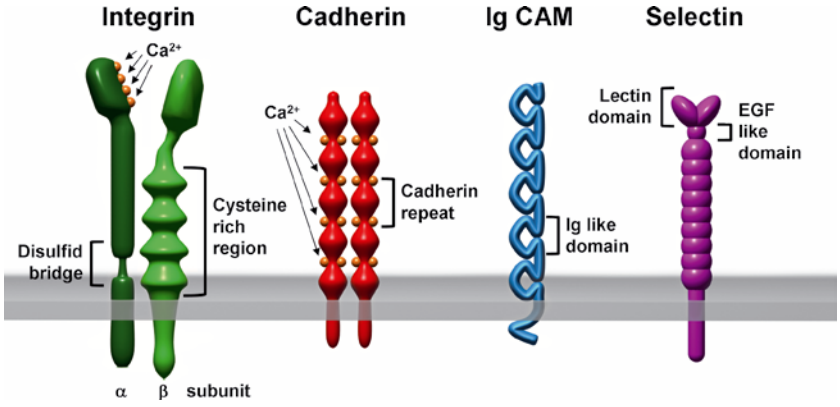

Fig. 5.2. The four families of cell adhesion molecules. Depiction of their heterodimeric, homodimeric or single chain structure, important functional domains, and Ca^{2+}-dependent sites in each one of the receptor types

platelet membrane enhancing the affinity for fibrinogen (Moroi and Jung 1998). The fibrinogen connects the platelets to a clot and prevents bleeding. This switch in binding activity is also important for lymphogenic or hematogenic metastasis of cancer cells when entering and leaving the vessel. Regarding integrin function, interactions between these CAM and transmembrane growth factor receptors build the basis for optimized and most efficient intracellular signaling and regulation of all types of cellular mechanisms (Porter and Hogg 1998). Whether this mutual and cooperative interrelation is caused by transactivation mediated by a panel of membrane-associated cytoplasmic signaling molecules or through direct interactions remains to be solved.

In cancer, integrins fulfill the same functions as they do in normal tissue (Chung et al. 2008; Danen 2005; Mochizuki and Okada 2007; Ramsay et al. 2007). Although widely examined, the most common feature in various human tumor entities including breast cancer or squamous cell carcinomas is an abnormal integrin expression relative to the corresponding normal tissue. However, the expression can differ within one single tumor and between tumors of the same entity.

Despite this diversity and unclear pathophysiological consequences of an altered integrin expression, recent findings suggest an association of β_1 integrin expression and overall survival of patients with invasive-ductal breast carcinomas (Yao et al. 2007). Accordingly, in vitro experiments showed a reversion of the transformed phenotype to a morphological and functional normal phenotype in breast cancer cells concomitant to a reduced tumor formation capability in vivo upon β_1 integrin inhibition (Park et al. 2006). These observations indicate a strong contribution of integrins to oncogenic transformation.

Besides the expression of integrins, malignant cells acquire the ability to grow anchorage independent as a result of gain-of-function mutations in oncogenes localized within integrin-associated signaling pathways. In contrast, normal cells usually undergoing apoptosis upon detachment from ECM, a mechanism called anoikis. In vitro studies have shown that $\alpha_v\beta_6$ but not $\alpha_v\beta_5$ expression leads to reduced anoikis in squamous cell carcinoma cells (Janes and Watt 2004). This could be an explanation, why upregulation of $\alpha_v\beta_6$ seems to be a prognostic factor in human squamous cell carcinomas.

Another impact of integrins in cancer comes from gene mutation analyses. The poorly differentiated cell line SCC4, which originates from a human carcinoma of the tongue, is heterozygous for the point mutation T188I (T, threonine; I, isoleucine) in the β_1 integrin subunit (Evans et al. 2004). This modification leads to constitutively active ligand binding independent from the type of associated β subunit. After transfection with wild-type β_1 integrin, the SCC4 cells begin to differentiate, indicating that this mutation may contribute to the neoplastic phenotype. Although these findings are remarkable because they show that alterations of integrin activation can influence the malignancy of cancer cells without changing the level of integrin expression, mutations in the β_1 integrin gene are rare. Screening of 124 human oral squamous cell carcinomas revealed six nucleotide changes, all of which could be also found in normal tissue of the patients (Evans et al. 2004). Only one mutation resulted in an altered amino acid sequence of β_1 integrin. Analysis of the predicted structure suggests that this sequence variation does not interfere with the function of the heterodimeric receptor. Whether mutations of β_1 integrin or other integrin subunits play a general role in tumor development with respect to other cancer entities remains to be examined in further studies.

There are many ways how integrins can modulate the malignant characteristics of tumors including a modu-

lation of the behavior of the primary tumor in terms of invasion as well as a modulation of the tumor's metastatic abilities. In addition to cadherins (see Sect. 5.3.2), the invasiveness of tumors depends on integrins (Hood and Cheresh 2002; Ramsay et al. 2007). Cell migration representing one part of the complex process of cell invasion is a highly dynamic sequence of focal adhesion assembly and focal adhesion disassembly. Starting with smaller focal adhesions at the leading edge of a migrating cell, called focal complexes, the assembly of a focal complex into a larger, stable focal adhesion progresses through the recruitment of additional proteins (Small and Resch 2005). These stable focal adhesions remain stationary, providing the cells an anchor from which to move in any direction. During the course of migration, the focal adhesions move from the front to the rear of the cells in a caterpillar, traction-like manner. Focal adhesions reaching the rear edge are disassembled. Referring to the frequent increased migratory potential of tumor cells, upregulation, e.g., of the basement membrane integrin receptor $\alpha_6\beta_4$ correlates with poor prognosis in a variety of different cancers not only due to promoted tumor progression, but also particularly due to enhanced invasiveness (Lipscomb and Mercurio 2005). In glioblastoma cell lines, for example, inhibition of either β_1 integrin or β_3 integrin with specific inhibitory monoclonal antibodies strongly impairs cell invasion into basement membrane (Cordes et al. 2003). This effect yielded from an integrin-dependent alteration of the proteolytic activity of matrix metalloproteinases (MMPs), which represent key enzymes to degrade the ECM and enable cells to invade. Correspondingly, it has been shown that $\alpha_v\beta_3$ integrin modulates MMP activity directly in endothelium and melanoma cells in vivo (Brooks et al. 1996).

5.3.2 Cadherins

Cadherins are the main receptors for calcium-dependent cell–cell adhesion in most solid tissues (Alberts et al. 2002). Besides being responsible for the mechanical stability, they coordinate the integration of cells in functional structures like the epithelium and control cell movement in tissue development and organization, especially during embryogenesis (Table 5.3). Classical cadherins are transmembrane glycoproteins, which bind almost exclusively to the same type of receptor expressed on the other cell in a homophilic manner (Fig. 5.2). The intracellular domain is connected to the cytoskeleton via a group of anchor proteins known as catenins (Pettitt 2005; Reynolds 2007). This linkage is essential for strong adhesive activity. Disassembly of this functional complex and therefore disruption of cell–cell contact can be caused by tyrosine phosphorylation of either cadherin or catenin by a variety of receptor tyrosine kinases (RTK) like epidermal growth factor receptor (EGFR) or insulin-like growth factor-1 receptor (IGF-1R). Contrariwise, cadherins are able to modulate RTK signaling, consequently interfering with many critical cellular processes (Pettitt 2005). Epithelial cadherin (E-cadherin)-mediated adhesion, for example, has been reported to reduce ligand-dependent EGFR activation, which results in decreased DNA synthesis and inhibition of cell growth (Qian et al. 2004).

Considering these effects, it is not astonishing that cadherins are discussed to play a major role in tumorigenesis. E-cadherin especially is deemed a tumor suppressor (Cowin et al. 2005; Reynolds 2007). Many types of epithelial cancers show an inverse correlation of E-cadherin expression and patient outcome. According to the results of several studies, it has been postulated that the downregulation of E-cadherin is necessary for tumor cell invasion and formation of distant metastasis, and that reconstitution of the functional cadherin/catenin complex might lead to reduced malignancy (Reynolds 2007). The loss of cadherin-mediated cell–cell adhesion in cancer cells can be due to transcriptional mechanisms or increased proteolytic degradation by matrix metalloproteinases. For example, overexpression of dysadherin, a cancer-associated membrane protein, inactivates E-cadherin in a posttranscriptional manner, which induces experimental metastasis (Ino et al. 2002). Mutations of the E-cadherin gene resulting in expression of a nonfunctional receptor have been found in lobular breast cancers, diffuse gastric cancers, and gynecological cancers (Chan 2006; Cowin et al. 2005). Such mutations arise either de novo or can be inherited, which is the case with patients suffering from familiar diffuse gastric cancer.

5.3.3 Immunoglobulin Superfamily

The immunoglobulin-like (Ig-like) CAMs are widely expressed in different cell types including neurons, leukocytes, endothelial, and epithelial cells (Table 5.3) (Alberts et al. 2002). Although mediating mainly cell–cell adhesion, Ig-like CAMs are also capable of binding to ECM proteins (Acheson et al. 1991). In contrast to integrins or cadherins, their binding affinity is much weaker and not dependent on the presence of divalent cations like Ca^{2+} or Mg^{2+}. Therefore, Ig-like CAMs are regarded to be responsible for the fine-adjustment

mechanisms of adhesive processes and tissue organization. All members of this family have in common that the extracellular part contains one or more Ig-like domains, which are typical for antibodies (Fig. 5.2). Either the receptor can be tied to the membrane by a glycosylphosphatidylinositol anchor, or it can interact with intracellular signaling molecules via a transmembrane/cytoplasmic tail (Hemperly et al. 1990; Pollerberg et al. 1987).

The neural CAM (NCAM) is among the best-studied members of this group critical for brain development and memory formation (Mileusnic et al. 1999). It is expressed not only in neural cells, but also in a variety of other tissues like epithelium, colon, and pancreas. Interestingly, in numerous tumors, an altered NCAM expression pattern correlates with poor prognosis. Studies with transgenic mice have shown that loss of NCAM function increases lymphatic metastasis of pancreatic cancer by induction of vascular endothelial growth factor (VEGF) and tumor lymphangiogenesis (Crnic et al. 2004). Accordingly, downregulation of NCAM was found to be associated with enhanced malignancy and reduced survival of patients with colorectal, gastric, or pancreatic carcinomas (Fogar et al. 1997; Roesler et al. 1997; Tascilar et al. 2007). But there exist also contrary observations. NCAM overexpression in neuroblastomas and small cell lung cancer, for example, correlates with advanced stage and fatal course of disease and is used as prognostic marker (Gluer et al. 1998; Miyahara et al. 2001). Overall, the biological significance of NCAM for tumor development and progression is unclear to date and depends strongly on the tumor entity.

5.3.4 Selectins

Selectins are cell–cell adhesion molecules, which play a critical role in leukocyte diapedesis through the vascular wall due to inflammation or tissue injury (Table 5.3) (Alberts et al. 2002). The three closely related family members mainly expressed by leukocytes (L-selectin), platelets (P-selectin), and endothelial cells (E- and P-selectin) contain a characteristic extracellular lectin-domain that binds to carbohydrate ligands (Fig. 5.2). In contrast to other CAMs like cadherins or integrins, selectin function is confined to the vascular system.

Several studies have indicated that selectins also recognize cancer cells and therefore facilitate hematogenic metastasis. Overexpression of E-selectin in the liver of transgenic mice leads to redirection of melanoma cells in this organ (Biancone et al. 1996). Specific targeting E-selectin with antibodies has been shown to significantly decrease the number of experimental metastasis in vivo (Brodt et al. 1997). Not only E-selectin, but also other members of the selectin family are suggested to promote metastasis. P-selectin-deficient as well as L-selectin-deficient mice show a reduction of tumor metastasis in different mouse models. Another therapeutic approach uses the anticoagulant heparin for potentially blocking selectin-mediated adhesion of tumor cells to endothelium (Borsig et al. 2002). Taking into account the prominent impact of normal cells on tumor progression and tumor microenvironment, L-selectins on leukocytes have been hypothesized to contribute to cancer development and progression (Coussens and Werb 2002).

5.4 Integrin Signaling Molecules

Integrins, together with a range of structural molecules and signaling molecules, provide a connection between the outside and the inside of the cell (Brakebusch and Fassler 2003; Chung and Kim 2008; Hehlgans et al. 2007b; Hynes 2002; Schwartz 2001). This specific cell membrane area is called focal adhesion. It is characterized by specific types of macromolecular protein assemblies transmitting mechanical force and regulatory signals over the cell membrane. Effective regulation of an adequate cell behavior results from the interactions of cells with their surrounding ECM. Furthermore, integrin- and receptor tyrosine kinase-mediated signaling are connected to control the cellular fate, e.g., survival, cell cycle progression, proliferation, adhesion, migration, differentiation, and apoptosis (Fig. 5.3) (Schwartz 2001; Watt 2002).

One of the molecules, which plays a major role in the above-mentioned processes and also holds a central position in the growth factor receptor-integrin network, is the putative serine–threonine kinase integrin-linked kinase (ILK). ILK is bound to the cytoplasmic tail of β-integrin subunits through its C-terminal kinase domain (Figs. 5.3, 5.4a) (Hannigan et al. 1996). Downstream, ILK has been reported to phosphorylate the prosurvival protein kinase Akt on serine 473 and glycogen synthase kinase-3β (GSK3β) on serine 9 in a phosphatidylinositol-3 kinase (PI3K)-dependent manner (Delcommenne et al. 1998; Lynch et al. 1999). More recent findings suggest ILK to be a pseudokinase (Boudeau et al. 2006) and the RICTOR-mammalian target of rapamycin (mTOR) complex to be responsible for phosphorylation of Akt on serine 473 (Sarbassov et al. 2005). Pseudokinases are proteins that lack at least one of the highly conserved catalytic residues/motifs

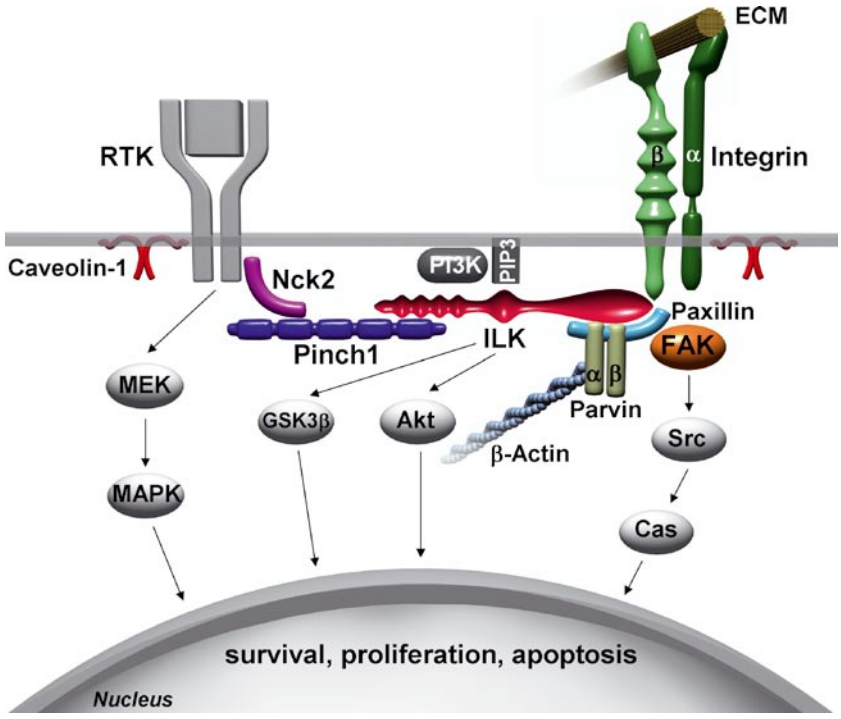

Fig. 5.3. Scheme of transmembrane integrins and selected integrin signaling mediators. Cooperative and mutual signal transduction between integrins and receptor tyrosine kinases optimally control critical cell functions like survival, proliferation, and apoptosis. *Akt* v-akt murine thymoma viral oncogene homolog 1, *ECM* extracellular matrix, *FAK* focal adhesion kinase, *GSK3*β glycogen synthase kinase-3β, *ILK* integrin-linked kinase, *Nck* non-catalytic (region of) tyrosine kinase adaptor protein, *Cas* (p130Cas), Crk-associated substrate, *Pinch1* particularly interesting new cysteine-histidine rich protein, *PI3K* phosphatidylinositol-3-kinase, *RTK* receptor tyrosine kinase, *MEK* mitogen-activated protein kinase kinase, *MAPK* mitogen-activated protein kinase, *PIP3* phosphatidylinositol (3,4,5)-triphosphate, *Src* Rous sarcoma oncogene

in the kinase-like domain. This event suggests these proteins to be inactive in terms of regular protein kinases. Phosphatidylinositol 3,4,5-triphosphate (PIP3) being a phospholipid component in the cytosolic side of cell membranes, seems to activate ILK through interaction with the central pleckstrin homology ([PH] PH domains facilitate protein recruitment to membranes, cellular compartments or enable protein-protein interactions) domain of ILK (Delcommenne et al. 1998). The N-terminal ankyrin repeat domain contains four ankyrin (ANK) repeats and is responsible for binding to the particularly interesting new cysteine-histidine-rich protein 1 (Pinch1). Ankyrins are important for attachment processes between integral membrane proteins and the cytoskeleton.

Pinch1 and its homologue Pinch2 are so-called LIM-only proteins, each consisting of five LIM domains (Braun et al. 2003; Dougherty et al. 2005; Stanchi et al. 2005). The name LIM derives from the initials of the three first described proteins containing LIM domains: *L*IN-11, *I*SL1, and *M*EC-3. LIM domains mediate protein–protein interactions and are composed of two cysteine-rich zinc finger structures. The first N-terminal LIM domain 1 of Pinch1 is essential for binding to the N-terminal ankyrin repeat domain of ILK (Figs. 5.3, 5.4b) (Tu et al. 1999; Velyvis et al. 2001). Moreover, Pinch1 serves as an important structural component in the RTK-integrin connective network by forming a ternary complex with ILK and Nck2, a Src homology (SH)2/SH3 adaptor protein (Tu et al. 1998; Vaynberg et al. 2005). Responsible for this interaction is the fourth LIM domain of Pinch1 and the third SH3 domain of Nck2. Nck2 itself binds to growth factor receptors like epidermal growth factor receptor or platelet-derived growth factor receptor β (PDGFRβ) with its C-terminal SH2 domain (Fig. 5.3, 5.4c) (Tu et al. 1998).

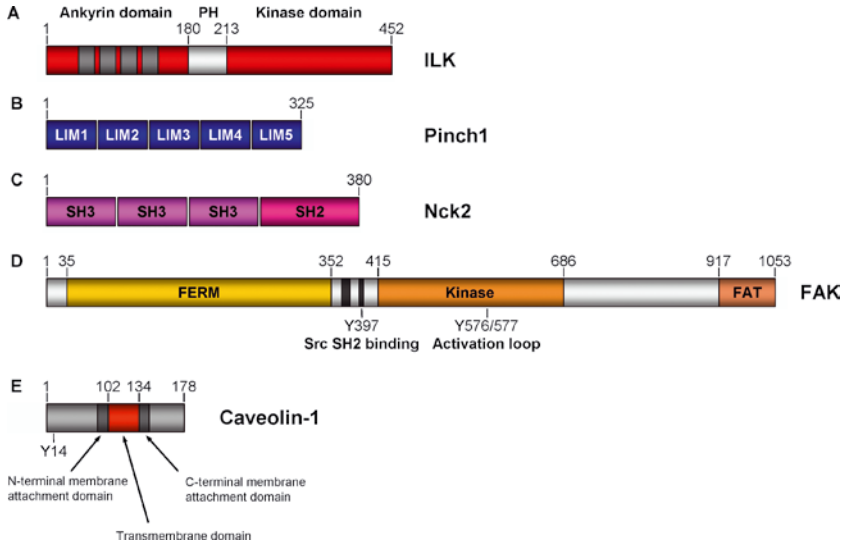

Fig. 5.4a–e. Essential transducers and modulators of integrin signals. Important functional domains and phosphorylation sites

A second important mediator of integrin signals is the 125-kDa protein focal adhesion kinase (Fig. 5.3) (Parsons 2003; Tachibana et al. 1995). FAK is a nonreceptor tyrosine kinase, which transmits signals from both integrins and RTKs to regulate cell shape, growth, survival, motility, adhesion, and migration. FAK activation, for example by adhesion, leads to autophosphorylation on tyrosine 397, which is then followed by recruitment of a signaling complex consisting of phosphorylated p130 Crk-associated substrate (p130Cas) on tyrosine 410, Src and phosphorylated paxillin on tyrosine 31 and 118 (Fig. 5.4d) (Calalb et al. 1995; Mitra et al. 2005; Parsons 2003). Once phosphorylated, FAK signals to mitogen-activated protein kinase (MAPK) and calpain-2 or recruits c-*Jun* N-terminal kinase (JNK) to focal adhesion sites to influence cell proliferation, migration, and apoptosis. FAK consists of 1,053 amino acids and contains an amino terminal region, which displays sequence homology to band 4.1 and ezrin/radixin/moesin (ERM) membrane-cytoskeletal linker proteins (Figs. 5.3, 5.4d) (Girault et al. 1999). This approximately 300–amino acid region, called FERM, is found in a number of membrane-targeted proteins (Chishti et al. 1998). FERM domains mediate interactions with cytoplasmic regions of transmembrane receptors and with phosphoinositides to efficiently localize FERM domain-containing proteins to membranes (Barret et al. 2000; Bompard et al. 2003; Hirao et al. 1996). The central FAK kinase domain spans approximately amino acids 415 to 618 and contains tyrosine 576/577 phosphorylation sites within the activation loop of FAK (Nowakowski et al. 2002). Within the linker region between the FERM and kinase segment lies the tyrosine 397 phosphorylation site, which is not strictly an autophosphorylation site but is also activated by Src SH2 binding (Mitra et al. 2005; Siesser and Hanks 2006). This phosphorylation further stimulates FAK activity through phosphorylation of other phosphorylation sites, including tyrosine 576/577 residues (Caron-Lormier and Berry 2005). A second element in this linker region is the Src SH3-binding motif (Ceccarelli et al. 2006). The C-terminal focal adhesion targeting (FAT) domain is responsible for binding to paxillin and talin, an integrin-associated protein, and for localization of the protein to focal adhesions (Hayashi et al. 2002; Schlaepfer et al. 2004).

Another protein that has been lately discovered to be associated with integrin signaling is the integral membrane protein Caveolin-1 (Fig. 5.3, 5.4e). Caveolin proteins are major components of caveolae, invaginations of the cell membrane, which participate in important physiological functions of the cell including endocytosis, membrane trafficking, lipid homeostasis, and a number of signaling events (Anderson 1998; Fielding and Fielding 2003; Salanueva et al. 2007). So far, three Caveolin proteins, named Caveolin-1, -2, and -3, have been described. Caveolin-1 and -2 are mostly co-expressed with high expression levels in differentiated cells like endothelial, epithelial and smooth muscle cells, fibroblasts, adipocytes, and pneumocytes. Caveolin-2 is primarily expressed in muscle tissue-types (Song et al. 1996; Tang et al. 1996). All Caveolin isoforms contain a

central transmembrane domain and cytosolic carboxy- and amino-terminal domains. The C-terminal membrane attachment domain contains three palmitoylation sites for anchoring of the protein to the membrane. The N-terminal membrane-proximal oligomerization domain mediates also interaction with other proteins for regulation of their activity (Couet et al. 1997; Li et al. 1996). Pathophysiological functions have been described for Caveolin-1, which is also involved in tumorigenesis, tumor suppression, differentiation, and oncogenic transformation (Carver and Schnitzer 2003; Galbiati et al. 1998; Williams and Lisanti 2005). Tyrosine 14–phosphorylated Caveolin-1 seems to accumulate at focal adhesion sites where it triggers extracellular signals (Lee et al. 2000; Mettouchi et al. 2001). Direct inhibition of Src and EGFR as well as direct activation of the insulin receptor by Caveolin-1 has been reported (Okamoto et al. 1998; Yamamoto et al. 1998). Caveolin-1 also interacts with β1 integrins and promotes Fyn-dependent Shc phosphorylation and MAPK activation (Wary et al. 1998; Wei et al. 1999).

5.5 Matrix Metalloproteinases

Matrix metalloproteinases are required for degradation of the extracellular matrix and therefore have important functions in tissue remodeling (Alberts et al. 2002). Tissue remodeling takes place not only under several physiological conditions like embryogenesis, angiogenesis, and wound healing, but also during pathological processes, namely tumor invasion, metastasis, and arthritis (Ra and Parks 2007).

On the cellular basis, MMPs are involved in all events requiring a change in ECM composition forming an optimized microenvironment for a cell to adhere, migrate, proliferate, apoptose, or differentiate. To date, 28 different MMPs have been identified, at first on the basis of genomic screening, from which 24 MMP proteins can be found tissue specifically in humans (Greenlee et al. 2007). In contrast to other endopeptidases, MMPs require a zinc ion as cofactor for their catalytic activity. They can be functionally classified in dependence on their substrate specificity in collagenases, gelatinases, stromelysins, and membrane-type MMPs (MT-MMPs) (Table 5.4).

Additionally, there are a number of MMPs, which do not fit exactly in this classification but are ordered with regard to structural similarities, evolutionary classification, or differential expression (Chang and Werb 2001; Ra and Parks 2007).

For tight regulation of function, MMPs are initially synthesized as inactive zymogens (i.e., a proenzyme or an inactive enzyme precursor) (Chang and Werb 2001). Responsible for this inactive state is a highly conserved prodomain consisting of the amino acids PRCGxPD (proline–arginine–cysteine–glycine–x–proline–aspartate), which inhibits enzymatic function of the protein by covering the catalytic site through direct interaction of the cysteine residue with the zinc ion in the active site. This event prevents substrate binding and cleavage resulting in the active form of a MMP. As well known mediator of MMP cleavage, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) have been assigned critical roles in cancer progression and metastasis development (Blasi and Carmeliet 2002; Kucharewicz et al. 2003; Sternlicht and Werb 2001). Other proteases involved in activation of MMPs are chymotrypsin, trypsin, and MMPs itself. Apart from the membrane bound MT-MMPs, which contain a transmembrane domain and are intracellularly activated once inserted into the cell membrane, MMPs are secreted into the extracellular space as inactive proenzyme (Hernandez-Barrantes et al. 2002; Nagase 1997). The second conserved domain is the catalytic domain with the structural metal binding 106 to 119 residues. The zinc-binding active site within this domain consists of 52 to 58 amino ac-

Table 5.4. Types of ECM-degrading MMPs

Family	MMP type	Substrate
Collagenase	1, 8, 13, 18	Triple-helical fibrillar collagens
Gelatinase	2, 9	Type IV collagen and gelatin
Stromelysin	3, 10, 11	Variety of ECM proteins but not collagens
Membrane type	14, 15, 16, 17	Variety of ECM proteins

ids. A conserved sequence HExxHxxGxxH (histidine–glutamic acid–xx–histidine–xx–glycine–xx–histidine) forms the zinc-binding motif, and three histidine residues mediate direct interaction with the zinc ion (Massova et al. 1998). The catalytic domain is linked to the third conserved domain, represented by a C-terminal hemopexin-like domain expressing a variable hinge region of up to 75 amino acids. The hemopexin domain seems to determine substrate specificity of the MMPs and serves as binding domain for tissue inhibitors of matrix metalloproteinases (TIMPs).

Beside the above-described intramolecular inhibition of catalytic function, TIMPs provide another regulatory mechanism controlling proper MMP function (Gomez et al. 1997; Sternlicht and Werb 2001). Four members of this family are known, TIMP-1 to -4. These inhibitors are also expressed in a tissue-specific way. They either inactivate active MMPs or inhibit the activation process. Gelatinases such as the well known MMP-2 and MMP-9 possess an additional gelatin-binding region within their catalytic domain before the zinc-binding motif. Membrane-type furin-activated MMPs contain a furin cleavage site within their prodomain and a C-terminal transmembrane domain.

In adults, the activity of MMPs is very low due to tight inhibitory regulation. This fragile balance is somehow perturbed during invasive tumor progression due to mutations in encoding MMP genes as well as inhibition and reduced expression of TIMPs. Overall, MMPs pronouncedly contribute to local tumor cell invasion and metastasis (Guo and Giancotti 2004; Sternlicht and Werb 2001).

In a variety of human cancers, the expression of different MMPs is elevated and responsible for metastatic events limiting the success of anticancer therapy (Erler et al. 2006; Jinga et al. 2006). In general, former and current efforts in targeting MMP expression and activity failed to show significant improvement or resulted in just slightly improved tumor control.

5.6 Migration and Metastasis

Elucidating the process of metastasis in the context of this chapter in more detail, clarification about the different molecules and steps involved is necessary. The set of molecular actors expressed by both tumor cells and tumor-associated normal cells like endothelial cells and fibroblasts is large and their activity is likely to depend in majority on paracrine effects. The complexity of events responsible for the intrinsic pressure driving tumor cell outgrowth and settling at distant organ sites is unclear to date. Likely, a mutual combination of autonomous cellular factors such as constitutive activation of migration-related molecules and life-threatening microenvironmental changes in oxygen and metabolite levels triggers the tumor cells to search for better survival conditions.

For the execution of the metastatic circuit of actions, a tumor cell requires a range of prerequisites of which integrins, matrix metalloproteinases, and signal transduction are crucial (Friedl and Brocker 2000; Hood and Cheresh 2002; Munshi and Stack 2006). During the course of events, cells must detach from the ECM and neighboring cells, migrate while degrading the extracellular matrix, penetrate the basement membrane, a thin ECM structure that segregates tissue compartments, invade the bloodstream, a process called intravasation, survive the shear stress in the vasculature, exit the bloodstream in the target organ (extravasation), attach, and proliferate in their new surrounding (Guo and Giancotti 2004; Hood and Cheresh 2002). These different processes depend on dysregulation of integrin and receptor tyrosine kinase signaling in metastatic cells due to activating mutations in oncogenes and loss-of-function mutations in tumor suppressor genes (Bissell and Radisky 2001; Giancotti and Ruoslahti 1999; Hynes 2003). Additionally, upregulation of integrin expression in tumor cells has been shown to enhance migration, invasion, and tumor progression (Albelda et al. 1990; Guo and Giancotti 2004; Mercurio and Rabinovitz 2001; Plantefaber and Hynes 1989). For the transition from adenoma to invasive carcinoma, cells undergo a process, which involves a modification of integrin-mediated cell–ECM interactions (see Sect. 5.3.1) and E-cadherin-mediated cell–cell contacts (see Sect. 5.3.2).

Migration events, both in normal and malignant cells, are initiated by polarization of the cell, followed by actin polymerization at the leading edge of the cell and lamellipodium formation (Raucher and Sheetz 2000). Integrins and integrin-associated proteins accumulate at the leading edge of the cell to stimulate adhesion processes and signaling in response to new contact sites (Kiosses et al. 2001; Schmidt et al. 1993). The focal contact sites at the rear end of the cell are detached by cleavage of focal adhesion proteins or modulation of integrin affinity to ECM proteins (Franco and Huttenlocher 2005; Shiraha et al. 1999). Finally, the cell moves forward due to contractile forces (Lauffenburger and Horwitz 1996). FAK is essential for migration by interaction with cytosolic part of integrin

subunits and growth factor receptors. Additional molecules involved are p130Cas, Src, Crk, and Rho-GTPases such as Rac controlling actin organization (Klemke et al. 1998). A second FAK-mediated promigratory pathway is the Grb2/SOS/Ras/ERK pathway (van Nimwegen and van de Water 2007). Integrins also participate in regulating proteases, for example MMP-2 and -9, that degrade the basement membrane, composed of ECM proteins like collagens type IV, laminins, and proteoglycans. An example for integrin–MMP interaction is the recruitment of MMP-2 by integrins on the outside of the cell, where MMP-2 degrades ECM components to facilitate migration and invasion of the cell (Brooks et al. 1996). Furthermore, integrins can associate with uPA receptors (Chapman and Wei 2001).

Conclusively, migratory and metastatic events result from concerted and complex actions that provide optimal avenues for cells to traverse from one point to another, in case of metastasis, beyond physiological borders and the intravascular phase.

5.7 Radiation and Chemoresistance of Tumor Cells Through Cell–Matrix Interactions

A current hypothesis is that malignant tumors, in general, develop areas, so-called niches (different from the "cancer stem cell" niche), which confer a high degree of resistance against ionizing irradiation or cytotoxic drugs to the tumor cells. The molecular characterization of these specific areas is ongoing but surely involves the binding of cells to the ECM as well as the binding of cells to other cells, regardless if malignant or normal.

As obvious from literature search, cell–cell contacts have been a focus of interest in cancer research for many years (Ruch and Trosko 2001; Trosko and Ruch 1998). Recently, the impact of cell–matrix interactions on tumor cell resistance was evidently demonstrated in several cell lines from different solid and hematologic tumor entities in vitro. Dependent on the characteristics, the phenomena were called "cell adhesion–mediated radiation resistance" (CAM-RR) or "cell adhesion–mediated drug resistance" (CAM-DR) (Cordes and Meineke 2003; Dalton 2003; Damiano et al. 1999). Most relevant for in vitro cancer research, this effect can easily be observed when comparing the radio- or chemosensitivity of cells plated on a conventional plastic culture dish with cells plated on different matrix proteins like fibronectin, collagen, or laminin. The contact with ECM proteins and the alterations in signaling caused by these interactions strongly support the cell to survive the treatment. A further increase in resistance can be achieved by placing the cells in a three-dimensional matrix, simulating more physiological growth conditions. There are ongoing efforts to reveal the underlying mechanisms and identify the cellular proteins involved in CAM-RR and CAM-DR, with the hope that this knowledge can add useful and successful novel therapeutic agents to cancer treatment.

It has been discovered early that integrins, the main receptors for ECM proteins, participate in the cellular response to geno- and cytotoxic stress. Irradiation, for example, leads to a dose-dependent upregulation of several integrin subunits or of specific heterodimeric integrin receptors (Cordes et al. 2003; Wild-Bode et al. 2001), while knockdown of integrin expression with small interfering RNA (siRNA) radiosensitizes numerous normal or neoplastic cells (Cordes et al. 2006, 2007; Estrugo et al. 2007). Experiments with mouse fibroblasts expressing a signaling-incompetent mutant of β_1 integrin demonstrated that not only integrin expression, but also integrin function is essential for cell survival after genotoxic injury (Cordes et al. 2006).

Unraveling the underlying molecular mechanism contributing to CAM-RR and CAM-DR, it was found that cell adhesion to fibronectin leads to an increase and prolongation of the radiation-induced G2-phase arrest in lung carcinoma cells, providing time for DNA damage repair thereby ensuring genome integrity (Cordes and van Beuningen 2003). Similar effects were found in prostate epithelial cells after irradiation (Kremer et al. 2006).

Besides the modulation of cell cycle transition and DNA repair, integrins also seem in control of drug- and radiation-induced apoptotic cell death. Small cell lung cancer cells adherent to laminin, fibronectin, or collagen type IV undergo less apoptosis after treatment with different cytotoxic drugs than cells grown on a nonspecific control substrate (Sethi et al. 1999). Subsequent to inhibition of β_1 integrin, using a function-blocking β_1 integrin antibody, this prosurvival effect of matrix proteins was pronouncedly diminished. It was further confirmed in leukemia cells that integrins play a critical role for the regulation of apoptosis. While downregulation of β_1 integrin resulted in elevated caspase-3, -9, and -8 cleavage and enhanced radiation-induced apoptotic death, treatment with β_1 integrin stimulatory antibodies had the opposite effect and reduced significantly the rate of apoptosis (Estrugo et al. 2007). Eventually, Estrugo et al. delineated a novel mechanistic model showing fibronectin-ligated β_1 integrins to efficiently block

caspases-8 cleavage upon radiation via recruitment and stimulation of Akt.

In addition to resistance against irradiation and classical cytotoxic drugs, integrin-mediated cell–ECM interactions reduce the efficacy of novel molecular therapeutics. Recently, Eke et al. (2006) showed that adhesion to fibronectin attenuates the anti-proliferative effect of a potent pharmacological EGFR tyrosine kinase inhibitor in human squamous cell carcinoma cells of the head and neck. An explanation for the antagonistic effects by cell adhesion lies in the concept of receptor transactivation where integrin–fibronectin binding cross-activates EGFR and vice versa. Moreover, as reviewed in Sect. 5.4, cytoplasmic signaling is rather organized like a network than as straight pathways of canonical order. Ongoing studies testing other molecular therapeutics are currently characterizing the more general aspects of this phenomenon, which would at least in part explain the low efficacy of targeting drugs like Erbitux (cetuximab, EGFR antibody), Iressa (gefitinib, EGFR tyrosine kinase inhibitor), or Avastin (bevacizumab, VEGF antibody) (Baumann et al. 2008; Krause et al. 2008; Zips et al. 2005).

But not only integrin-mediated cell adhesion modulates cellular radio- or chemosensitivity. Integrin downstream molecules such as ILK and FAK or integrin-associated proteins like Caveolin-1 have been reported to alter resistance against cyto- and genotoxic injury (Fig. 5.5) (Cordes et al. 2007; Eke et al. 2006, 2007; Hehlgans et al. 2007a, 2008; Kasahara et al. 2002). Most interestingly, ILK confers opposite survival effects upon irradiation as expected from the literature on drug sensitivity and ILK (Duxbury et al. 2005; Edwards et al. 2005; Persad et al. 2000). Human lung carcinoma cells, which are transfected with a constitutive active ILK mutant, are more sensitive to irradiation with X-rays than is the ILK wild type and control cells (Cordes 2004). These data could be confirmed in human squamous cell carcinoma cells of the head and neck (Eke et al. 2006, 2007). Consistent with these observations, reduction of ILK protein levels with siRNA confers radioresistance. Not only in solid tumors, but also in leukemia cells, ILK has shown antisurvival effects (Hess et al. 2007). ILK–overexpressing cells are highly sensitive to radiation-induced apoptosis, while downregulation of ILK results in radioprotection of the cells. These effects seem to be due to an interaction between ILK and different caspases. Interestingly, in mouse fibroblasts but not in tumor cells the radiosensitizing effect of ILK is antagonized when cells interact with different extracellular matrix proteins (Hehlgans et al. 2008) indicating that ILK modulates the radiation response of normal fibroblasts and cancer

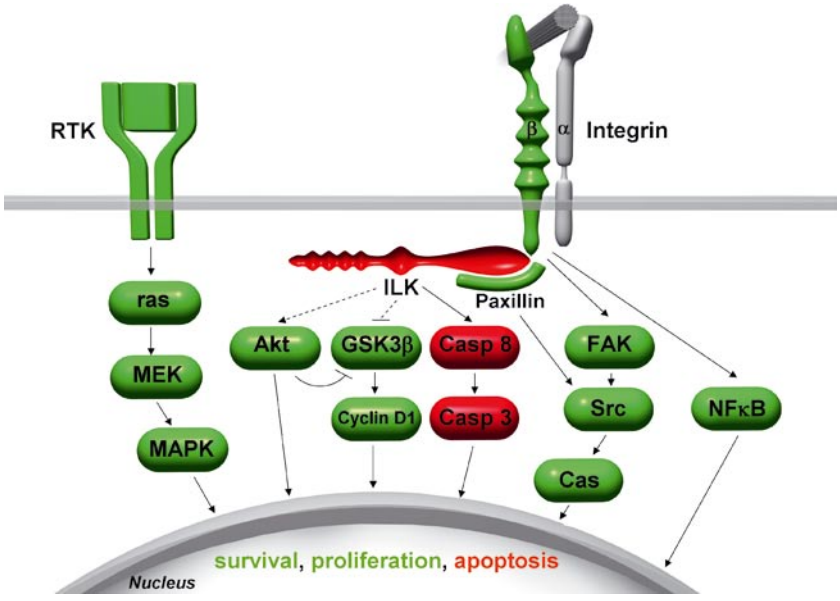

Fig. 5.5. A summary of our current knowledge how integrin signaling critically modifies certain cellular response pathways upon radiation- or drug-induced cytotoxic stress. Cascades in *green* mediate prosurvival signals from transmembrane located β integrins and RTKs via Ras/MEK/MAPK, Akt, or FAK/Src/Cas in a cell type- and/or context-dependent manner. The cascade in *red* transduces anti-survival signals via ILK, caspase-8 and caspase-3 to promote apoptosis

cells in a differential manner. Recent additional data provided evidence that ILK is strongly associated with differentiation in normal tissues as well as with redifferentiation in tumor tissues (Haase et al. 2008).

In contrast to ILK, overexpression of the prosurvival integrin signaling mediator FAK protects leukemia cells from radiation- and chemo-induced apoptosis (Kasahara et al. 2002). Silencing of FAK protein expression with siRNA mediated knockdown increases the radiosensitivity of different tumor cell lines originating from pancreatic cancer (Cordes et al. 2007), breast cancer, and colorectal cancer (McLean et al. 2005). Others have shown that human melanoma cells become more sensitive to the chemotherapeutic agent 5-fluorouracil when FAK expression is downregulated (Smith et al. 2005).

Besides the critical role of proximal integrin signaling proteins, the integral membrane protein Caveolin-1, essential for endo- and exocytosis and linking of integrins with growth factor receptors, is a critical modulator of cellular radiation sensitivity. Recently, Cordes et al. (2007) showed that Caveolin-1 expression as well as the number of Caveolin-1-positive caveolae is induced by ionizing irradiation. In reference to this chapter, Caveolin-1-overexpression in pancreatic carcinoma cells leads to a significant reduction in radiosensitivity in comparison with control cells. Consistent with these results, a knockdown of Caveolin-1 enhanced the cellular radiosensitivity. These effects are partially channeled by a strong growth delay with a concomitant rise in G1-phase cells but may be also caused by activity changes in important prosurvival signaling pathways such as the Akt cascade (Cordes et al. 2007).

5.8 Summary and Perspective

Adhesion and invasion are controlled by integrin receptors and are frequently dysregulated in cancer with disastrous consequences such as local destruction of

Table 5.5. Therapeutic agents against integrins. Data summary of approved agents and agents currently evaluated in clinical trials

Active agent	Brand name	Target	Indications (approved)	Indications (in clinical trials)
Antibodies				
Natalizumab	Tysabri	$\alpha_4\beta_7$	Multiple sclerosis, Crohn's disease	
Abciximab	ReoPro	GPIIb/IIIa	Angioplasty	
Volociximab (M200)		$\alpha_5\beta_1$		Renal cell carcinoma, melanoma
Vitaxin (MEDI-522)		$\alpha_v\beta_3$		Melanoma, colorectal carcinoma, prostate cancer, rheumatoid arthritis, psoriasis
CNTO 95		α_v		Prostate cancer, melanoma
Peptides				
Cilengitide (EMD 121974)		$\alpha_v\beta_3$, $\alpha_v\beta_5$		Glioblastoma, melanoma, lymphoma, renal cell carcinoma, colon carcinoma
Eptifibatide	Integrilin	$\alpha_2\beta_3$	Small heart attacks, angioplasty	
JSM6427		$\alpha_5\beta_1$		Macular degeneration
Non-peptides				
Tirofiban	Aggrastat	GPIIb/IIIa	Instable angina pectoris	
E7820		α_2		Colorectal carcinoma, lymphoma

Data obtained from http://www.clinicaltrials.gov and http://www.fda.gov

normal tissue, metastases, and ineffective local tumor control by anticancer therapeutics. In particular, aggravated local tumor control evolves from the combination of genetic alterations and changes in the tumor micromilieu. Being widely neglected for decades, the myriad micromilieu factors such as oxygen, lactate, and extracellular matrix are increasingly recognized as potent modulators of therapy resistance in cancer.

With respect to the content of this chapter, Table 5.5 summarizes the integrin targeting compounds current in clinical trials. Many of them are administrated in inflammatory diseases but others are given as monotherapy in a variety of human cancers. Speculatively not curative by themselves, anti-integrin agents might be potent when applied in combination with conventional radio- and chemotherapy as well as in a combination with other molecular drugs. Solving the intra- and extracellular networks a tumor cell exploits for its growth and spreading benefits in more depth may foster the diagnosis of early-stage cancer, the development of novel drugs, and eventually increased patient survival.

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