Integrins, ECM and Cancer – Should I Stay or Should I Grow?

Yaniv Erlich, Watson School of Biological Sciences, Cold Spring Harbor Laboratory, NY. 2007

I. Overview of Integrins

Integrins are cell surface receptors that bind to the ECM and integrate the extracellular environment with the cytoskeleton and signaling molecules in bi-directional manner[1]. They participate in numerous diverse biological processes such as organ morphogenesis[2, 3], angiogenesis[4, 5], and homing of leukocytes[6]. They are heterodimeric proteins and consist of two distinct type-1 transmembrane subunits that are referred to as α and β[1, 7]. They are restricted to metazoa[7], and there are at least 18 different α and 8 different β subunits in mammals[7] that can form heterodimers to produce 25 different types[8] of integrin receptors. They can be roughly categorized into four sub-groups according to their ligand[7]: (a) RGD receptors (b) laminin receptors (c) collagen receptors and (d) leukocyte-specific receptors. The last sub-group has different binding targets, since it mediates mainly cell-to-cell adhesion. Although different integrins can bind to the same ligand, each integrin has distinct binding and signaling properties[6, 7]. This specificity is apparent in knockout experiments in mouse, wherein each integrin knockout led to a distinct phenotype[6, 7].

The integrin protein is composed of three segments: extracellular, transmembrane, and cytoplasmic (Figure 1A). X-ray crystallography and electron microscopy studies revealed that the structure of the extracellular segment behaves like a "pocket knife"[1] as it has two conformations: a bent V-shape conformation found in the inactive state, and a straight conformation when in the active state[1, 7] (Figure 1B). The transition between the conformation states is rapid (<1s), reversible, and is stimulated by intra-cellular signals or the binding of certain ligands[1]. Therefore, integrins are dynamic cell adhesion molecules that response rapidly to stimulation. The allosteric transitions also 'open' and 'close' the ligand binding site[1, 7] that is comprised of both subunits and resides toward the N-terminus. This site has several cation-binding motifs, such as the MIDAS domain that is always found on the β subunit, and sometimes on the α subunit; and divalent binding loops in the seven-bladed propeller domain of the α subunit[1, 7, 8]. The presence of cations in these sites is crucial for a transition between the conformational states, and therefore these sites may serve as regulatory switches[1]. The binding-site is above two flexible stalks, one from each subunit, which crosses the plasma membrane. The cytoplasmic segment is smaller and usually contains less than 50 amino acids. An exception is the cytoplasmic tail of the β_4 subunit, which consists of approximately 1000 amino acids[1, 6-8]. The cytoplasmic domain has two functions – reflecting the binding state of the extracellular domain, and modulating the affinity of the ligand-binding site by allosteric changes. Thus, integrin transduces signals in two directions: outside-in signals that indicate the ligand binding state and inside-out signals that change its affinity[1, 6-8].

The affinity of integrins to their ligands is relatively low[9] with, K_d=~10^{-7},

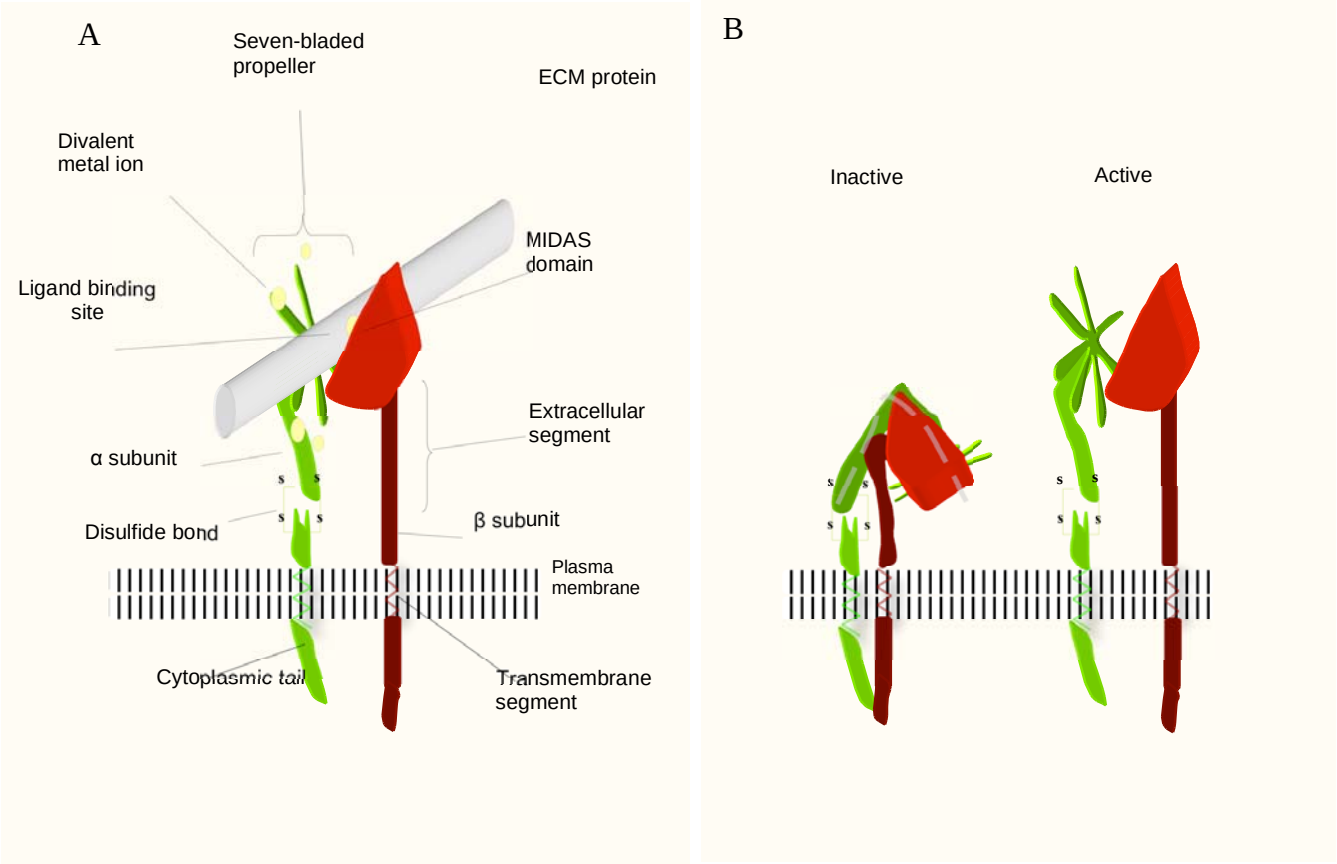

Figure 1: A schematic representation of heterodimeric integrin structure.
(A) Integrin has three segments – extracellular, transmembrane and cytoplasmic. The ligand binding site is composed of a seven bladed propeller and a MIDAS domain contributed by the α and β subunit, respectively. In half of the cases, the α subunit also has a MIDAS domain (not shown). The ligand binding site also includes several cation binding loops, and it is above on two flexible stems that cross the plasma membrane.
(B) The pocket-knife model - Integrin can switch between an inactive to an active state through allosteric changes. The inactive state has a V-shape conformation, and the stems serve as a hinge. Upon an intracellular signal (inside-out signal), or extracellular stimulation, a transition in the conformation state occurs – the stems open-up, and the ligand binding site is exposed.
Figure A is adapted from Kuphal et al.
Figure B is adapted from Hynes et al.

compared with other cell surface receptors that typically have an affinity of K_d=~10^{-10}. Nonetheless, the accumulation of thousands of integrins that bind to the ECM generates a Velcro-like effect and docks the cell reversibly to the ECM[9]. Moreover, some integrins, unlike other receptors, need both ligand occupancy and self-aggregation in order to signal to the cytoskeleton[10]. These integrin aggregations, that can include more than 50 types of proteins, are usually found in three typical forms[9, 11]. The smallest one is a focal complex that is around 1μm and is found on lamellipodia. The second type is a focal adhesion that has an elongated oval shape of 2-5 μm, and is found on the cell periphery. The biggest

type of cluster is a fibrillar adhesion that is around 10μm, and is found in the central region of cells. It was suggested that the three forms of integrin aggregations are precursors of each other[11]. All of these structures also include other adhesion molecules in addition to integrins, such as hyaluronan binding protein and cell-surface proteoglycans[11]. Moreover, all three types of clusters are connected to the actin cytoskeleton on the cytoplasmic side[11]. There is one exception that is found primarily in keratinocytes: the hemidesmosome, which connects laminin in the basal lamina to keratin intermediate filaments in the cytoplasm through $\alpha_6\beta_4$ integrins[12]. Its main role is to enhance the resistance to stress forces, and in the skin, it mediates firm adhesion of the epidermis to the dermis. Patients who have mutations in β_4 integrin that disrupt hemidesmosomes suffer from severe blistering and skin fragility[12].

The tension forces that are conveyed by integrins from the ECM to the cytoskeleton can serve two purposes: as signals, and can play a mechanical role[2, 3]. These forces can influence both cell shape and fate, and it was suggested that they induce specific gene expression patterns[2]. For instance, it was shown that different tension forces that act on endothelial cells *in-vitro* can elicit different cellular responses, such as apoptosis, differentiation or cell growth[2]. In addition, the cytoskeleton is not only an effector of integrins, but it can also react through them to change the docking properties of the cell, as it can modulate the assembly and the activity of focal contacts and maintain an isometric tension with the ECM[11]. Notably, the cytoskeleton can also mount forces that rearrange the ECM. The best characterized example is the conversion of soluble fibronectin to fibrillar fibronectin[11, 13]. During this step, the binding of $\alpha_5\beta_1$ integrin, the major fibronectin receptor, induces cytoskeleton rearrangements that mount a strong dynamic tension. This tension stretches the fibronectin four times than in its soluble state, and reveals cryptic sites that are embedded inside the protein. The cryptic sites can form interactions with cryptic sites on another fibronectin to form a fibril. In conclusion, integrins can convert mechanical forces to biochemical signals and vice versa, thereby allowing cells to communicate with their microenvironment.

II. Overview of ECM

The ECM is a complex network of proteins and polysaccharides that is secreted by, and surrounds various types of cells in multicellular organisms[9]. It protects the cells in the tissue, and provides physical support. In addition, it plays a significant role in the regulation of cellular activities and shape[3]. A growing body of literature shows that tumor cells extensively remodel their adjacent ECM[13-15]; this process includes massive degradation of the ECM, and generation of structures that resembles wounded[16] or embryonal tissues[17]. Four groups of components constitute the ECM[9]: collagen proteins, proteoglycans, hyaluronan and multi-adhesive proteins. There are several members of each group, and specific members contribute to the composition of different ECMs throughout the body, thereby creating highly diverse structures. Below I will discuss each group in more detail.

There are approximately 20 different types of collagen proteins, and they are the most abundant proteins in metazoa[18]. Their building block is an α chain, which has a long coiled structure that completes a turn every three amino acids. α chains are mainly comprised of the repeating motif of Gly-Pro-X, which provides a rigid folding[9, 18]. The collagen molecule is formed by three α chains that are wound together, and create lateral

hydrogen bonds that stabilize the triple helix[9, 18]. The collagen family can be grouped into four classes according to the overall structure that they form[18]: fibrillar-forming collagen (such as type I, II, III and V) that creates 300nm fibrils; network forming collagen (such as type IV and VII); fibril-associating collagens (such as type IX, XII) that do not aggregate, but change the global alignment of collagen fibrils, and therefore, are essential for tissue organization. The last class of collagens is a collection of collagens whose structure is yet to be determined.

Another component of the ECM are proteoglycans, which are a highly diverse family of macromolecules that are found both as secreted glycoproteins as well as cell surface receptors[9, 18]. They are comprised of a core protein and a variable number of unbranched carbohydrate chains known as GAGs. The connection between a GAG chain and a core protein is made through a covalent link between a sugar and a serine residue that resides on Ser-Gly-X-Gly motifs[9, 18]. Each GAG chain is comprised of multiple disaccharide repeats, wherein one sugar in each pair is always an N-acetyl hexosamine[19]. In the context of a proteoglycan, the disaccharide repeats contain one or two sulfate residues, whereas hyaluronan, a GAG that is not attached to any core protein, does not have any sulfate groups[19]. The extensive amount of sulfate moieties dramatically increases the negative charge of the molecule, and attracts a large volume of water molecules and cations. This ability to absorb water and the stiffness of polysaccharide that prevents globular folding, provides the gel-like properties of GAG[9].

There are four major types of GAGs[19]: (a) chondroitin sulfate / dermatan sulfate (b) keratan sulfate (c) heparan sulfate and (d) hyaluronan. However, several mechanisms dramatically increase the diversity of proteoglycans[9, 19] – the sugar monomers can undergo additional post-incorporation modifications such as epimerization and partial acetylation; the length of the GAG chain can vary; different types of GAG chains can be linked to one core protein; not all the Ser-Gly-X-Gly motifs occupy GAG; and there are several types of core proteins. Therefore, proteoglycans are found in various configurations. Cell surface proteoglycan receptors have an additional level of structural diversity as they can be bound to the plasma membrane either by a GPI anchor or by a transmembrane core protein[9, 18]. These proteoglycans mediate the docking of cells to the ECM, as they can bind collagen fibers and other ECM molecules.

In addition to their mechanical properties, proteoglycans interact with certain growth factors and cytokines[9, 18, 19], and heparan sulfate is the most highly characterized. This GAG is synthesized by various cell types, and is found both on secreted proteoglycan and on cell surface proteoglycan receptors. In its secreted form, heparan sulfate can trap various morphogens, such as Wnt, BMP and hedgehog[19]. This interaction suppresses morphogen diffusion rates, and preserves concentration gradients, which is necessary for certain developmental processes. Heparan sulfate can also trap FGF-2, a step that protects FGF-2 from proteolytic degradation, and prolongs its availability to cells[19]. Furthermore, FGF-2 interacts with heparan sulfate that resides in cell surface proteoglycan receptors[9, 18]. This interaction induces a conformational change in FGF-2, which is an essential step for its activation. In addition, certain core proteins also participate in paracrine signaling regulation. For instance, the core protein of beta glycan, a cell surface proteoglycan receptor, binds to TGF-β and can either deliver it to the TGF-β receptor, or protect it from proteolytic cleavage[9, 18].

Hyaluronan is a GAG that consists of disaccharide repeats of glucuronic acid and N-acetylglucosamine[9, 18]. As was mentioned above, it does not attach to any core proteins, and does not contain any sulfate moieties. In spite of its simple chemical composition, hyaluronan participates in various biological processes including signal transduction[20]. This activity is mediated mainly by interactions with CD44, a cell surface proteoglycan receptor. Hyaluronans can be grouped into two categories according to their size[20]: high molecular weight (2000-10000 sugars) and hyaluronan fragments (4-1000 sugars). These two groups have distinct and sometimes, opposite biological activities[20]. The high molecular weight hyaluronan occupies a very large volume, and can serve as a space-filler[9, 18, 20]. During embryogenesis it induces strong osmotic pressures that change the structure of the tissue, which can create empty spaces for cell migration[18]. Its gel-like properties are also exploited as lubricants and shock absorbers in the joints[9, 18, 20]. Although it does not form any covalent links with proteins, it can attract about 100 aggrecans - large proteoglycans with keratan sulfate chains to form a giant aggregate that occupies the same volume as bacteria[9, 18]. Besides its mechanical roles, high molecular weight hyaluronan has anti-angiogenesis and immunosuppressive activity, as it can trap VEGF and restrict antigen accessibility[20]. Several lines of evidence indicate that it also promotes tissue integrity by inducing cell cycle arrest[20]. Conversely, small hyaluronan fragments induce opposite biological activities as they participate in angiogenesis and stimulate cytokine production that mediates inflammation[20]. Generally, they serve as signals in conditions that are associated with stress. These surprising findings regarding hyaluronan activities were revealed during the last years, and changed its perception dramatically, from a passive sticky substance to a carrier of information[20].

The main function of multi-adhesive proteins is to serve as adaptors that connect different components of the ECM to adjacent cells[9, 18]. For this purpose, these proteins contain several binding domains that can interact simultaneously with different molecules such as collagen and integrin. Fibronectin is one of the best-characterized multi-adhesive matrix proteins. It is a high molecular weight dimer that has various isoforms. Its subunits are comprised of three types of repeating motifs that create binding domains for heparin, fibrin, collagen and integrin[9, 18]. Therefore, it can bridge cells and the ECM, and can bridge different molecules of the ECM. As mentioned above, fibronectin interaction with integrin in fibrillar adhesions can change its shape, and induce its aggregation. Nonetheless, fibronectin not only adheres cells to the ECM, but it can also induce cell motility. For instance, in amphibians, fibronectin-containing fibrils mark physical pathways for migration of mesodermal cells during gastrulation[9].

The basal lamina is a special type of ECM that forms a thin and flexible sheet with a thickness of 40-120nm[9, 18]. It underlies epithelial cells but also surrounds individual cells such as Schwann, fat and muscle cells[9, 18]. These cells are also the major source of molecules that comprise the basal lamina[9]. Although the basal lamina has a diverse composition, generally, these molecules fall into four groups[9] - type IV collagen, laminin, nidogen, and perlecan. The first two groups, type IV collagen and laminin, a multi-adhesive protein, can form layered structures through self-assembly, thereby providing the overall shape. The other two groups - nidogen, another multi-adhesive protein and perlecan, a heparan sulfate proteoglycan, serve as adaptors between the two layers, and glue the structure together[9]. In multilayered epithelia, the basal lamina sometimes contains an additional layer of type VII collagen that improves the strength.

In those cases, the basal lamina and the special collagen layers are referred to as "basement membrane"[9].

The main function of the basal lamina is to separate fibroblasts from the adjacent epithelial cells allowing leukocytes and nerves to pass through[9]. However, the basal lamina is more than a simple barrier as it plays a pivotal role in the establishment of apical-basal polarity of epithelial cells, and in activation of their differentiation programs. For instance, differentiated human mammary epithelial cell lines that grow on three-dimensional laminin rich ECM respond to lactogenic cues, whereas their growth on plastic substrata prevents them from responding to these cues[3]. The basal lamina also participates in regeneration[9] – in the case of neuromuscular junctions the basal lamina separates the muscle from the axon, and if the junction is destroyed the basal lamina can guide the growing axon to the original site. These roles of the basal lamina emphasize the mutual feedback model that was presented earlier – The basal lamina maintains the differentiation program of adjacent cells, and the differentiation program determines the composition of the basal lamina. Therefore, degradation of the basal lamina during tumorigenesis can be associated with loss of differentiation in addition to invasion.

III. Integrin Signaling and the mitogenic pathway

As mentioned above, besides its mechanical role, the ECM is an active participant in signal transduction cascades during several processes, such as organ morphogenesis, differentiation, and inflammation. It accomplishes this goal in two ways - directly, by physical interactions with cell surface receptors, mainly integrins; and indirectly, by interactions with growth factors and cytokines that change their diffusion or turnover rates. However, many studies showed that these two types of signals are integrated throughout various intracellular signaling cascades to form a highly intricate network of multiple interacting pathways[21-23]. This network includes compensatory mechanisms, cross-regulation, and synergistic outcomes when the two pathways function simultaneously[22]. In this section, I will focus on the main integrin signaling cascades and more specifically on the intracellular crosstalk between integrins and the mitogenic signaling cascade, and how it relates to cell growth and survival. The signaling cascades that mainly contribute to cell motility will be presented in detail in the section that discusses integrin and cancer.

The interactions between integrins and members of the mitogenic signaling cascade are accomplished to a large extent by close physical vicinity within the cell membrane between integrins and members of the pathways[22]. Some integrins are associated with caveolin-1 that promotes the assembly of lipid rafts, which are micro-domains in the cell membrane that are enriched with palmitoylated signaling proteins like H-RAS and SFK[22, 24]. Thus, lipid rafts form sites for joint signaling by bringing participants from both pathways into close proximity. In addition, co-immunoprecipitation studies have shown that integrins can form complexes with RTKs. For instance, $\alpha_v\beta_3$ integrin can associate with the VEGF receptor, the PDGF receptor, or the insulin receptor[6]. These complexes mediate a signaling flow in a bi-directional way; integrin to RTK and vice versa[22].

Most integrins send signals to RTKs through the SFK/FAK pathway[22]. This signal cascade starts upon aggregation of integrin receptors that promotes FAK

recruitment[10] and its autophosphorylation[22]. FAK then undergoes a conformational change that recruits SFK. However, some lines of evidence suggest that since FAK autophosphorylation is facilitated by the presence of SFK, SFK acts both upstream and downstream to FAK[22]. FAK-bound SFK activates p130CAS, a key player in integrin-RTK interaction, and recruits it to its SH3 domain. p130CAS attaches to the cytoplasmic domain of RTK, thereby completing the complex assembly. In the case of EGFR, the formation of the complex promotes a tyrosine phosphorylation of EGFR by SFK[22]. Notably, this phosphorylation site does not induce full activation of the RTK, but rather primes it to such activation. Moreover, FAK/SFK signaling can also promote RTK de-phosphorylation[22]. As an example, in the case of the PDGF receptor, SFK starts a cascade that localizes SHP-2, a type of PTP, to the PDGF receptor resulting in the de-phosphorylation of the PDGF receptor. Accordingly, the receptor dissociates from RAS-GAP, a negative regulator of the MAPK pathway. Several lines of evidence suggest that the SFK/SHP-2 cascade also participates in cell migration (see below).

The SFK/FAK cascade can also target certain downstream MAP kinases[22]. Active p130CAS recruits Crk, whereupon Crk forms a complex with two types of GEFs, C3G and Dock180. Each one of these GEFs initiates a distinct cascade that activates different MAP kinases. C3G activates the RAS-related small G-protein, Rap1, that activates B-raf, a MAPKKK. Hence, in cells that express B-raf, this cascade induces the phosphorylation of ERK resulting in its activation. In the other cascade, Dock180 converts Rac-GDP to Rac-GTP, which activates JNK, a MAP kinase that is associated with cellular stress, via PAK. In addition, Dock-180 dependent Rac activation plays a significant role in the formation of lamellipodia and in cell migration[11, 25].

A subset of integrins, such as $\alpha_1\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$, exploit an additional signaling pathway, SFK/Shc, to activate ERK[22]. Notably, this pathway activates RAS directly, and is not FAK-dependent. The underlying mechanism of this pathway is the ability of these integrins to recruit PTPα that de-phosphorylates palmitoylated SFK, and induces the exposure of its SH3 domain via a conformational change[22]. Shc can then bind to the exposed SH3 domain, whereupon it is phosphorylated, and serves as a docking site for the Grb2/Sos complex. This complex activates RAS via its GEF activity, and therefore activates the MAPK pathway. However, the SFK/FAK and SFK/Shc pathways do not elicit the same type of ERK activity[22]: the SFK/Shc pathway induces a transient and strong ERK activation, whereas SFK/FAK prolongs ERK activity and presumably promotes its nuclear localization via Rac.

In most cases, integrin-dependent ERK activity is not sufficient to induce the transcription of cyclin D or to force the cell to enter S-phase[22]. On the other hand, in adherent cells, normal levels of growth factors induce only a short and modest activation of ERK in the absence of integrin signaling that is also not sufficient to drive the cell to cycle. It is only the combined signaling that includes growth factor stimulation, and both SFK/FAK and SFK/Shc, that provides effective ERK activation that leads to cell proliferation[22]. Remarkably, this mechanism serves as a gatekeeper that prevents cells that lose their ECM adherence from entering S-phase, and is known as "anchorage dependence of cell division"[9]. In addition, during M-phase, cells tend to reduce their ECM adhesions and to rearrange their contacts in order to provide extra free space for daughter cells[9].

Integrins also play a significant role in promoting cell survival, as they participate with several pathways that have anti-apoptotic effects[21, 26]. Notably, the activity of these pathways can be context dependent and it varies according to cell type[26]. The FAK/PI3K pathway plays an important role in cell survival signaling[21, 26]. This pathway starts with activation of integrins that induces a conformational change in FAK[21, 26]. FAK can recruit PI3K that catalyzes the formation of PtdIns(3,4,5)P_3. This molecule serves as a docking site for Akt kinase, a versatile kinase that participates in various cellular activities[27]. One of the targets of Akt is the pro-apoptotic machinery, including caspase-9 and Bad, which Akt can phosphorylate resulting in their inactivation[21, 27].

Many types of cells, especially epithelial and endothelial cells, undergo a from of apoptosis when they do not bind to the specific type of ECM that is found in their niche, a phenomenon that is referred to as anoikis (homelessness)[26]. This process is prevented by the anti-apoptotic signaling of integrins that is induced only upon binding to specific ECM components[26]. Anoikis supports tissue integrity, as for example the laminin-rich basement membrane in the epidermis promotes melanocyte survival, whereas collagen-rich dermis does not[26].

Mitogenic signals can modulate integrin signaling, in addition to being their effectors, by influencing integrin-dependent cell survival[22, 26]. For instance, activated RAS can interact with the catalytic subunit of PI3K to create a strong signal that compensates for the absence of integrin signaling and promotes resistance to anoikis[28]. In addition, growth factors can induce a process that causes localization of FAK with integrin clusters, thereby facilitating FAK-dependent integrin signaling[22]. Presumably, this process elicits inside-out signaling and changes the affinity of integrins for the ECM. Therefore, RTK to integrin signaling plays some role in cell motility, which will be discussed in more detail in the next section.

IV. Role of integrins in cancer

One part of the tumor microenvironment that plays a major role in the development and progression of cancer is the cross-talk between tumor cells and the ECM that is mediated by integrins[23, 29]. Although integrin signaling normally serves as a gatekeeper for tissue integrity, the genetic and epigenetic changes that tumor cells undergo allows them to escape anoikis and anchorage-dependent cell growth[23]. In fact, tumor cells can exploit integrin signaling and harness it for their benefit mainly to metastasize, but also to develop resistance to apoptosis, and to promote angiogenesis[23]. Metastasis is an intricate process that allows tumor cells to grow in distant organs. It is composed of series of steps that includes a decrease in cell to cell adhesion, degradation of the basal lamina and the ECM, an increase in cellular motility that leads to invasion, intravasation, survival in the blood stream, extravasation, and formation of a new tumor site[23]. Integrins play some role in these steps, mainly in cellular motility that underlies the ability of tumor cells to escape from the primary tumor site and some of these aspects will be discussed below.

During tumorigenesis, tumor cells usually change their integrin composition[8, 23, 29, 30]. In particular, specific types of integrins such as $\alpha_v\beta_3$, and $\alpha_6\beta_4$ that support tumorigenesis are usually overexpressed in malignant tissues, whereas integrin types that antagonize it, like $\alpha_2\beta_1$ that induces p38 MAP[22], are lost. Notably, it seems that the

specific malignant composition of integrins is context dependent[30], as for instance, it was reported that the expression of $\alpha_2\beta_1$ in rhabdomyosarcoma cell line facilitated metastasis *in-vivo*[15, 30]. However, overexpressing integrins can be deleterious to the tumor cell, as unligated integrins can elicit apoptosis, a phenomenon referred to as 'integrin-mediated death'[29]. This mechanism may provide an explanation for the abundance of $\alpha_v\beta_3$ integrin, since this type of integrin can be occupied by a wide spectrum of ligands and therefore is less likely to lead to apoptosis[29]. $\alpha_v\beta_3$ integrin also supports the cancerous process by its ability to promote a specific pathway that is associated with cell motility (see below), and by its interaction with multiple RTKs and the mitogenic pathway[6]. Many studies have shown that $\alpha_6\beta_4$ integrin plays a significant role in cell proliferation, invasion and resistance to apoptosis in some types of cancer. It was found that activated $\alpha_6\beta_4$ can activate ERBB2[23] that plays a significant role in various types of cancer. Moreover, certain RTKs can induce the tyrosine phosphorylation of β_4 cytoplasmic tail that leads to the recruitment of Shc, which amplifies the mitogenic signals[22,23], and to destruction of hemidesmosomes that increases the ability of cells to move.

Cell movement is a series of coordinated steps that includes lamellipodia protrusion in the direction of the movement that is followed by a contraction of the rear side (Figure 2)[29, 31-33]. The actin cytoskeleton provides the physical forces for this process, as its polymerization pushes the lamellipodia forward, and its contraction pulls the rear side[31]. The activity of Rho GTPase proteins, Rac, Cdc42, and Rho, dictates these actin rearrangements by inducing two distinct groups of signaling cascades[31-33]. Rac and Cdc42 activate cascades that promote actin polymerization in the leading edge, mainly through activation of Arp2/3 complexes. However, Rac activity leads to lamellipodia formation, whereas Cdc42 induces cascades that promote filopodia formation, and are mainly associated with cell polarity (see below)[33]. On the other hand, Rho induces a cascade that builds tension in actin stress fibers causing contraction of the cell[31-33]. This cascade starts by activation of Rock kinase by Rho that represses MLCP, and promotes actin contraction. In addition, Rho also elicits microtubule polymerization in the direction of the movement through mDia activity. Remarkably, several studies indicate that the microtubule cytoskeleton plays a pivotal role in cell motility, as it guides actin polymerization, and facilitates focal adhesion turnover that leads to relaxation of the tension, and terminates Rho contraction[31]. Therefore, it was hypothesized that Rho activity induces actin contraction that is followed by relaxation[31].

Integrins play a crucial role in generating the traction force necessary for movement. Notably, the amount of adhesiveness requires tight regulation, since strong binding will glue the cell to the ECM, and will suppress its ability to move, whereas weak binding will not provide a sufficient reaction force for the contraction, and will reduce the efficiency of the movement[29]. Therefore, cells switch between focal complex and focal adhesion contacts through the two phases of the movement[25]. The protrusion phase is associated with focal complexes that are very small and generate less adhesion force, and the contraction phase is associated with focal adhesion clusters that are bigger and generate sufficient reaction force[11, 25]. In addition, each one of the clusters can participate in different signal cascades that induce protrusion and contraction respectively[11, 25].

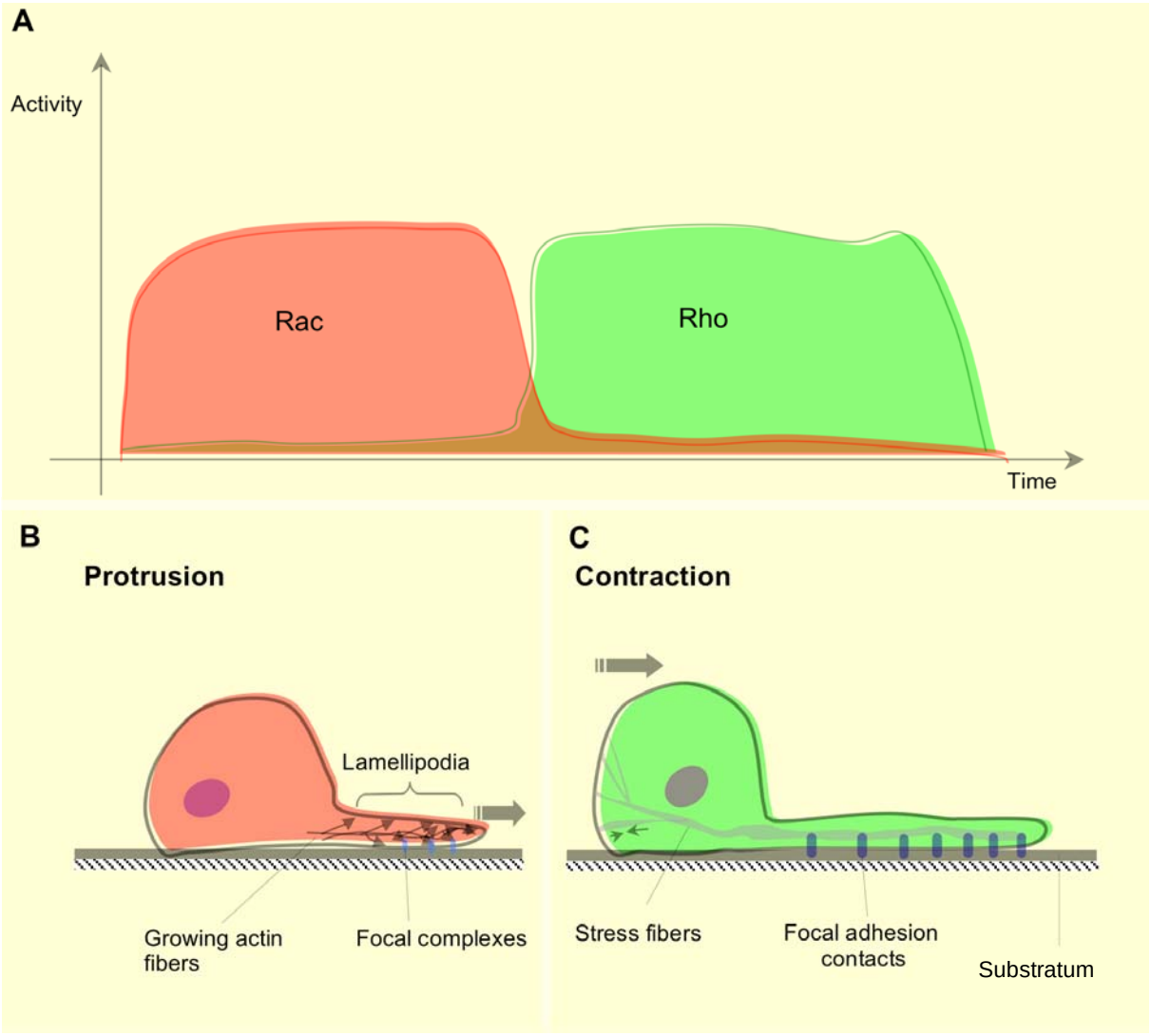

Figure 2: The biphasic activity of Rac and Rho and its affect on cell movement in cultured cells
(A) A schematic representation of Rac and Rho activity – Rac and Rho are activated during two distinct phases.
(B) and (C) A schematic cross section of a motile cell. (B) High Rac activity induces lamellipodia protrusion via polymerization of actin in the leading edge. During this stage the lamellipodia contains focal complexes. (C) High Rho activity induces contraction in actin stress fibers that pulls the rear side of the cell forward. The formation of focal adhesion contacts generates a strong reaction force that docks the leading edge during the contraction. The process terminates by polymerization of microtubules that promotes focal adhesion turnover (not shown).
(A) is based on data from Lacalle et al.
(B) and (C) are adapted from Albert BM et al.

Beside the mechanical role, integrin signaling acts upstream to the three Rho GTPases, and therefore integrin activation can stimulate cell migration[22, 29]. In the case of Rac, its integrin-dependent activation pathways include the SFK/FAK pathway that promotes Dock180 (see above) and paxillin that promotes PIX, another GEF that can activate Rac. However, the activities of the Rac and Rho pathways are antagonistic, and a transient Rho downregulation facilitates lamellipodia formation[25]. Remarkably, integrin signaling participates in this synchronization, as it switches between Rho

suppression to Rho activation[9]. DeMa Nli et al. suggested two models that are based on PTPs to describe this modulation of Rho activity by integrins[25]. According to the first model, integrins recruit SHP-2, which serves both as a positive and a negative regulator of p190RhoGAP, a Rho repressor. On one hand, SHP-2 promotes Src by removing its inhibitory phosphate, that leads Src to activate p190RhoGAP, and suppresses Rho; on the other hand, SHP2 can directly de-phosphorylate p190RhoGAP, and repress its activity, thereby inducing Rho. According to the second model, the same Src/p190RhoGAP pathway is working, but this time, with a second slow PTPα/SFK pathway (see above) that presumably promotes a specific RhoGEF. Similarly, these two antagonistic cascades first induce Rho suppression and then Rho activation. Notably, the PTPα/SFK pathway is restricted to a subset of integrins[22, 25], including $\alpha_v\beta_3$, and it may reflect their role in cell motility.

Cell polarization is essential for effective movement as it directs the exocytotic process to the leading edge, thereby providing nascent plasma membrane and integrins that facilitate the protrusion[33, 34]. Integrins participate in several positive feedback loops that polarize the cell, and specify its leading edge. Polarization of the cell toward the leading edge is based, to a large extent, on a positive feedback loop between integrins and Cdc42[33]. In this loop, active integrins in the leading edge induce and localize Cdc42, whereupon Cdc42 forms a complex with mPar6-PKCζ. This complex blocks the activity of GSK3 and promotes the accumulation of APC. APC has a dual role in cell polarization[31]: first, it binds to the plus end of microtubules, and polarizes the cytoskeleton; second, APC binds to β-catenin and therefore it may couple the actin cytoskeleton to the microtubule organization. Polarization of the cytoskeleton restricts exocytosis to the leading edge and provides more integrins to the same location[34]. These new integrins can activate the cascade again and maintain the polarization toward the leading edge. An additional positive loop is based on spatial generation of PtdIns(4,5)P_2 [25, 35]. Focal contacts recruit particular PI5Ks and activate them presumably by FAK or by talin. PI5K synthesizes PtdIns(4,5)P_2 and this promotes the binding of talin to integrin, contributing to the assembly of integrin complexes. In addition, it promotes the binding of vinculin to Arp2/3 and provides further protrusion. Another positive feedback loop is based on the spatial restriction of active Rac[36]. Rho-GDI forms a complex with cytosolic Rac-GTP that represses its activity, by binding to its geranylgeranyl tail. Integrin adhesion induces a local change of the membrane affinity for Rac that releases it from Rho-GDI, and localizes it to the integrin cluster.

An essential process that must occur prior to invasion is the controlled degradation of the ECM, specifically the basal lamina, which separates the epithelial and mesenchymal compartments[37]. This proteolytic process is achieved mainly by MMPs that can catabolize different ECM components, and by plasmin, a serine protease that plays a major role in fibrinolysis[37]. However, the amount of degradation must be tightly regulated, as extensive degradation will lead to detachment from the ECM, and will reduce the traction forces for efficient migration. Some integrins provide additional support to tumor invasion and migration through regulation of MMPs and plasmin[23]. For instance, FAK-JNK signaling enhances the expression of MMP-2 and MMP-9, type IV collagenases, in v-Src transformed fibroblasts[23]. Notably, it was shown that $\alpha_v\beta_3$ integrin participates in spatial regulation of MMP-2[23]. This integrin can bind inactive MMP-2 through its extracellular domain, and induce MMP-2 activation. MMP-2 remains active

as long as it attaches to the integrin, and its release terminates the proteolytic activity. Since this integrin resides in the lamellipodia, it localizes MMP-2 activity in the same direction as the cell movement. A similar mechanism is found in the regulation of plasmin[23], wherein uPA receptors co-localize with certain types of integrins. As a result, uPA, which activates plasmin, resides mainly in the leading edge. Interestingly, the proteolytic process exposes cryptic sites in the ECM that promotes migration via integrin signaling[13, 15, 23]. Therefore, besides removing physical barriers, ECM degradation creates additional signals to promote migration.

The process of EMT also facilitates invasion and metastasis and is characterized by carcinoma cells that gain mesenchymal markers[38]. The major underlying mechanism for EMT is downregulation of E-cadherin activity that disrupts cell-to-cell adhesion, increases the amount of nuclear β-catenin[38], and can deregulate the differentiation program[30]. A possible mechanism for integrin-induced EMT is ILK activation by integrins that facilitates the expression of Snail, a transcription factor and a strong repressor of E-cadherin expression[23]. Notably, $\alpha_v\beta_6$ and $\alpha_v\beta_8$ integrins promote EMT by a distinct mechanism that does not involve integrin signaling, as they can activate latent TGF-β, thereby initiating the Smad cascade that facilitates Snail activity[23].

In addition to the crucial role of integrins in invasion and cell motility, integrins support other aspects of tumorigenesis, particularly resistance to apoptosis and angiogenesis. Several lines of evidence showed that the adhesion of specific integrins can confer apoptosis resistance or increase cell survival, and this can potentially reduce the effectiveness of chemotherapeutic agents. For instance, overexpression of $\alpha_v\beta_3$ in a human melanoma cell line growing in three-dimensional dermal collagen increases the survival rates of the cells[23]. Other studies showed that $\alpha_6\beta_4$ integrin induces resistance to apoptosis by promoting polarization and spatial organization that is associated with NF-κB activity[23]. In addition, in certain types of cells attachment to the ECM can redirect ultraviolet stress to induce p53-dependent apoptosis, whereas in cells that detached from the ECM, this stress induces p53-independent apoptosis[15]. Since in many cases the p53 pathway is disrupted in tumors, ECM attachment can inhibit the signal and rescue the cell from apoptosis.

In addition to apoptosis resistance, several types of integrins are associated with promoting angiogenesis. For instance, $\alpha_v\beta_3$ integrin is expressed at a very low level on resting endothelial cells, but is very abundant in endothelial cells that are part of a tumor microenvironment[5]. It was suggested that this pattern of expression is mediated by stimulation of growth factors, specifically FGF and VEGF[5]. Several experiments showed that antagonists of these integrins can reduce angiogenesis by inducing apoptosis, that presumably is mediated by PKA and caspase-8[39]. However, there is still a debate regarding the role of these integrins in angiogenesis[4, 23, 39]. Notably, several endogenous anti-angiogenic factors can inhibit angiogenesis by interactions with these integrins. For instance, tumstatin, an anti-angiogenic factor, which is a fragment of collagen IV, can bind $\alpha_v\beta_3$ integrin, and downregulate angiogenesis[23].

The integrin-specific anti-cancer therapeutic efforts during recent years have been focused to a large extent on inhibition of angiogenesis[4, 5]. Until now, there are two main tools that target integrins – specific integrin antibodies and short peptides that mimic the RGD domain[4, 5, 29]. These peptides compete for integrin binding, and block its ability to aggregate. Nonetheless, several aspects complicate the anti-integrin therapy. First, in

some cases, it is hard to achieve high integrin specificity. For instance, an RGD peptide that is designed to reduce angiogenesis by blocking $\alpha_v\beta_3$ integrin, can also block $\alpha_v\beta_8$ integrin activity that normally suppresses endothelial cell growth[4]. A second aspect regarding RGD peptides is a contradiction in their activity; in high doses RGD peptides serve as antagonists of integrins, but in low dosages they serve as agonists[4]. A possible explanation is that RGD binding activates the integrins for a longer duration than its dissociation rate. Therefore, at a low concentration the net result is active integrins, whereas at high concentration, the integrins are saturated and fully blocked. A third aspect refers to the anti-angiogenesis effects of $\alpha_v\beta_3$ integrin[4]. As was mentioned earlier, tumstatin can promote anti-angiogenesis through this integrin, therefore blocking it may antagonize this anti-angiogenic effect. To conclude, integrin-based anti-angiogenic treatment can create complementary and novel therapeutic strategies, but several challenges must first be addressed to achieve a safe and effective anti-cancer treatment.

Abbreviations:

APC	adenomatous polyposis coli
Arp2/3	actin-related protein 2/3
BMP	bone morphogenic protein
CD	cluster of differentiation
Dock180	dedicator of cytokinesis-180
ECM	extracellular matrix
EGFR	epithelial growth factor receptor
EMT	epithelial to mesenchymal transition
ER	endoplasmic reticulum
ERBB2	erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog
ERK	extracellular signal regulated kinase
FAK	focal adhesion kinase
FGF	fibroblast growth factor
GAG	glycosaminoglycan
GAP	GTPase-activating protein
GDI	GDP dissociation inhibitor
GEF	guanine exchanges factor
GSK3	glycogen synthase kinase 3
GTPase	guanosine-5'-triphosphatases
GPI	glycosylphosphatidylinositol
ILK	integrin linked kinase
JNK	Jun n-terminal kinase
MAP	mitogenic-activated protein
MAPK	mitogenic-activated protein kinase
MAPKKK	mitogenic-activated protein kinase kinase kinase
MIDAS	metal ion dependent (Mg^{2+}) adhesion site
MLCP	myosin light chain phosphatase
MMP	matrix metalloproteinases
PDGF	platelet-derived growth factor
PI3K	phosphatidylinositol-3 kinase
PI5K	phosphatidylinositol-5 kinase
PAK	p21-activated kinase
PIX	PAK-interacting exchange factor
PKCζ	atypical protein kinase C zeta
PtdIns(3,4,5)P_3	phosphatidylinositol (3,4,5)-trisphosphate
PtdIns(4,5)P_2	phosphatidylinositol-4,5-bisphosphate
PTP	protein tyrosine phosphatase
Rock	Rho Kinase
RGD	Arg-Gly-Asp
RTK	receptor tyrosine kinases
SFK	Src family kinase
SH2/SH3	Src homology region 2 / 3

SHP-2	Src homology 2-containing tyrosine phosphatase
Sos	Son of Sevenless
TGF-β	transforming growth factor-β
VEGF	vascular endothelial growth factor
uPA	urokinase plasminogen activator

References:

1. Xiong, J. P., Stehle, T., Goodman, S. L. & Arnaout, M. A. New insights into the structural basis of integrin activation. Blood 102, 1155-9 (2003).
2. Ingber, D. E. Tensegrity II. How structural networks influence cellular information processing networks. J Cell Sci 116, 1397-408 (2003).
3. Nelson, C. M. & Bissell, M. J. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol 22, 287-309 (2006).
4. Tucker, G. C. Inhibitors of integrins. Curr Opin Pharmacol 2, 394-402 (2002).
5. Varner, J. A. & Cheresh, D. A. Integrins and cancer. Curr Opin Cell Biol 8, 724-30 (1996).
6. van der Flier, A. & Sonnenberg, A. Function and interactions of integrins. Cell Tissue Res 305, 285-98 (2001).
7. Hynes, R. O. Integrins: bidirectional, allosteric signaling machines. Cell 110, 673-87 (2002).
8. Kuphal, S., Bauer, R. & Bosserhoff, A. K. Integrin signaling in malignant melanoma. Cancer Metastasis Rev 24, 195-222 (2005).
9. Alberts, B., Johnson, A. ,Lewis, J., Raff, M., Roberts, K & Walter, P. The Molecular Biology of the Cell, version 4, Gerland Science, (2001).
10. Miyamoto, S., Akiyama, S. K. & Yamada, K. M. Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. Science 267, 883-5 (1995).
11. Geiger, B., Bershadsky, A., Pankov, R. & Yamada, K. M. Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. Nat Rev Mol Cell Biol 2, 793-805 (2001).
12. Litjens, S. H., de Pereda, J. M. & Sonnenberg, A. Current insights into the formation and breakdown of hemidesmosomes. Trends Cell Biol 16, 376-83 (2006).
13. Larsen, M., Artym, V. V., Green, J. A. & Yamada, K. M. The matrix reorganized: extracellular matrix remodeling and integrin signaling. Curr Opin Cell Biol 18, 463-71 (2006).
14. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. Cell 100, 57-70 (2000).
15. Lukashev, M. E. & Werb, Z. ECM signalling: orchestrating cell behaviour and misbehaviour. Trends Cell Biol 8, 437-41 (1998).
16. Dvorak, H. F. et al. Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. Lab Invest 57, 673-86 (1987).
17. Orend, G. & Chiquet-Ehrismann, R. Tenascin-C induced signaling in cancer. Cancer Lett 244, 143-63 (2006).
18. Lodish, H., Berk, A., Zipursky, LS., Matsudaira, P., Baltimore D. & Darnell, J. Molecular Cell Biology, version 3, W. H. Freeman and Company (1995)
19. Mulloy, B. & Rider, C. C. Cytokines and proteoglycans: an introductory overview. Biochem Soc Trans 34, 409-13 (2006).
20. Stern, R., Asari, A. A. & Sugahara, K. N. Hyaluronan fragments: an information-rich system. Eur J Cell Biol 85, 699-715 (2006).
21. Giancotti, F. G. & Ruoslahti, E. Integrin signaling. Science 285, 1028-32 (1999).
22. Giancotti, F. G. & Tarone, G. Positional control of cell fate through joint integrin/receptor protein kinase signaling. Annu Rev Cell Dev Biol 19, 173-206 (2003).
23. Guo, W. & Giancotti, F. G. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 5, 816-26 (2004).
24. Lacalle, R. A. et al. Specific SHP-2 partitioning in raft domains triggers integrin-mediated signaling via Rho activation. J Cell Biol 157, 277-89 (2002).
25. DeMali, K. A., Wennerberg, K. & Burridge, K. Integrin signaling to the actin cytoskeleton. Curr Opin Cell Biol 15, 572-82 (2003).
26. Gilmore, A. P. Anoikis. Cell Death Differ 12 Suppl 2, 1473-7 (2005).
27. Manning, B. D. & Cantley, L. C. AKT/PKB signaling: navigating downstream. Cell 129, 1261-74 (2007).
28. Khwaja, A., Rodriguez-Viciana, P., Wennstrom, S., Warne, P. H. & Downward, J. Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. Embo J 16, 2783-93 (1997).
29. Hood, J. D. & Cheresh, D. A. Role of integrins in cell invasion and migration. Nat Rev Cancer 2, 91-100 (2002).
30. Ben-Ze'ev, A. Cytoskeletal and adhesion proteins as tumor suppressors. Curr Opin Cell Biol 9, 99-108 (1997).

31. Etienne-Manneville, S. Actin and microtubules in cell motility: which one is in control? Traffic 5, 470-7 (2004).
32. Etienne-Manneville, S. & Hall, A. Rho GTPases in cell biology. Nature 420, 629-35 (2002).
33. Hall, A. Rho GTPases and the control of cell behaviour. Biochem Soc Trans 33, 891-5 (2005).
34. Bretscher, M. S. Moving membrane up to the front of migrating cells. Cell 85, 465-7 (1996).
35. Gilmore, A. P. & Burridge, K. Molecular mechanisms for focal adhesion assembly through regulation of protein-protein interactions. Structure 4, 647-51 (1996).
36. Del Pozo, M. A. et al. Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. Nat Cell Biol 4, 232-9 (2002).
37. Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2, 161-74 (2002).
38. Thiery, J. P. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2, 442-54 (2002).
39. Sheppard, D. Endothelial integrins and angiogenesis: not so simple anymore. J Clin Invest 110, 913-4 (2002).