Histol Histopathol (2005) 20: 593-602

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The signaling network of tumor invasion

G.K. Wang and W. Zhang

Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Summary. The ability of a cell to invade its surroundings is an important hallmark of malignant tumors and results from aberrant cell signaling mechanisms. The signal transduction that leads to tumor invasion can be broken down into major pathways. Even though the pathway systems are distinct in themselves, none of these pathways operate independently when it comes to transmitting signals that culminate in an invasive phenotype. That is, the malignant change in one receptor not only leads to malignant changes directly downstream but can also affect the molecules of many other pathways. Three major pathway systems involved in tumor invasion are discussed in this review: the integrin system, the insulin-like growth factor system, and the Rho family GTPases. Here we see that although the individual signaling systems can each contribute to invasion, each system is networked to others and should not be considered isolated. Each system is first reviewed as independent contributors to an invasive phenotype and then discussed in the context of interacting pathways that collectively result in tumor invasion.

Key words: Tumor invasion, Integrin, IGFPB, Rho, Crosstalk

Introduction

The most malignant characteristic of all cancers is their ability to grow beyond the confines of their original location. While some tumors (e.g., glioblastoma multiforme) are known for being locally invasive and remaining within the organ system and other tumors (e.g., pancreatic carcinoma) are known for metastasizing to distant sites, the spreading of cells in an uncontrolled manner is common to all cancers. Although this process can seem chaotic when observed from the phenotype perspective, a structured organization of the cell signals can be seen at the molecular level. Intracellular signaling comprises a complex and highly regulated network of pathways that are necessary for cell growth, replication, death, and survival. Players in this organized maze of pathways include ligands, receptors, kinases, adapter molecules, transcription factors, and other molecules, many with multiple roles and functions. Some molecules function strictly as agonists or antagonists, while others have dual roles in maintaining homeostasis, depending on the state of the cell. Perhaps the biggest challenge in signal transduction research is discerning the pathways that have gone awry, thereby causing cells to adopt a malignant and invasive phenotype—in other words, to become cancerous. Understanding and delineating the pathways involved in this process is particularly important because it provides us with the information needed in the pursuit of new therapy and improvements in current anticancer treatments. Today, some of the most active areas of research regarding cell invasion (to be covered in this article) include integrins, insulin-like growth factor (IGF) signaling, small GTPases, and the crosstalk between different pathways.

Offprint requests to: Wei Zhang, Ph.D., Department of Pathology, Unit 85, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030. Tel/Fax: 713-745-1103/713-792-5549. e-mail: wzhang@mdanderson.org

Integrins

Integrins are transmembrane receptors composed of an alpha and a beta subunit that are non-covalently linked. Together, the heterodimers form a receptor on the cell surface, with intracellular and extracellular domains responsible for controlling interactions of the cell with the extracellular matrix (ECM). Currently, at least 22 different integrin heterodimers have been identified (Hemler, 1999). Depending on the heterodimer composition, an integrin receptor shows high specificity for certain ECM ligands, including vitronectin, fibronectin, laminin, and collagen. In normal cells, integrins play key roles in the regulation of cell migration and attachment. These receptors also have the capacity to communicate intracellular signals that promote cell migration and survival. Defects in immune system response, specifically, defects related to cell movement (leukocyte trafficking), have been observed in mouse models lacking certain integrin function. For example, blockade or inhibition of integrin attenuates the immune response in autoimmune and inflammatory

conditions in mice (Kudlacz et al., 2002; James et al., 2003). Further, the changes in the immune response have to do with the integrin's ability to regulate leukocyte migration (Rose et al., 2001).

Integrins have been found to play an important role in tumor invasion in a wide variety of cancers. In hepatocarcinoma, inhibition of the integrins α1, α2, and ß1 significantly inhibited growth factor–stimulated cell migration (Yang et al., 2003). In breast carcinomas, expression of the α6ß4 integrin appeared to enhance tumor cell invasiveness (Chung et al., 2002). In metastatic prostate carcinoma, inhibition of the αvß3 integrin reduced cell proliferation and tumor growth (Nemeth et al., 2003). Conversely, in prostate carcinoma, expression of the αvß3 integrin correlated with an increase in cell migration (Zheng et al., 1999; Manes et al., 2003). In ovarian carcinoma, increased expression of the αvß1 integrin appeared to correlate with malignant effusions (Davidson et al., 2003). Therefore, as these observations collectively show, the functional blocking of certain integrins (e.g., via antibody antagonists) is a potential therapeutic strategy for these types of cancers.

This inactivation of an integrin and consequential decrease in cell migration and invasion would likely require the blocking of at least one of two mechanisms. First, because cell migration depends on the cell's ability to make contact with the cell's substratum and integrins control cell attachment to the ECM, it is clear that it would be necessary to prevent the integrin from attaching to the ECM. Second, because integrin receptors themselves do not have any inherent enzymatic properties and only participate in signal transduction downstream from the receptor, it would be necessary to block the pathways downstream from an activated integrin receptor, which appear to promote pro-invasive phenotypes. An example of such an integrin is the activated high-affinity integrin that is recruited to the leading edge of lamellipodia in migrating endothelial cells (Kiosses et al., 2001). We would, however, have to determine which of these two mechanisms is involved in a particular integrin's ability to contribute to cell invasion.

There is also evidence that alternatively spliced forms of integrin receptors play a role in cell invasion. These receptors have already been shown to alter normal cell migration and malignant cell proliferation. However, the expression of these alternatively spliced forms of integrins has only been found to decrease the movement of cells rather than promoting an invasive phenotype in human cell lines (Fornaro et al., 1998; Gimond et al., 2000). Nonetheless, in mice, an α6 splice variant has been linked to malignant conversion and the invasion of skin tumor cells (Tennenbaum et al., 1995). This thus shows that there are further aspects of integrin signaling that need to be understood. For example, perhaps splice variants maintain the same extracellular ligand contact but alter the intracellular signaling such that a cell becomes more invasive. Environmental conditions may also cause a particular splice variant to be dominant, thereby making a cell more invasive in certain environments. Integrins have further been shown to interact with the IGF system, which is discussed in greater detail in the next section. Indeed, interaction with this system has recently been found to play an important role in determining both normal cell migration during embryo development (Kabir-Salmani et al., 2004), malignant tumor growth, and the migration of breast cancer cells and multiple myeloma cells (Tai et al., 2003; Pereira et al., 2004). Because the IGF axis is an important modulator of cell growth, the coupling of integrin receptor pathways to this axis adds yet another dimension to an already complex signal transduction network that can produce both normal and aberrant cell proliferation. In addition, much remains to be elucidated regarding the crosstalk between integrins and the IGF system, the topic of the last section of this review.

Targeting integrin activity as a potential therapy for cancer is currently under much investigation. The most common approach to blocking integrin activity thus far is to neutralize the integrin receptor via antibodies. This method has already been shown to dramatically reduce cell migration in glioma cells (Tysnes et al., 1996; Haugland et al., 1997). However, recent advances in molecular biology, in particular RNA interference (RNAi) techniques, offer promise as ways to alter gene expression. Already, small interfering RNAs (siRNAs) have been recognized as a potential means of decreasing breast carcinoma invasion. For example, like the antibody inhibition of α6ß4 integrin function, the genetic silencing of α6ß4 integrin expression in breast carcinoma by siRNA successfully reduced cell invasion and migration (Lipscomb et al., 2003). Further *in vivo* experiments testing siRNA techniques are currently under way, and the findings will determine whether they have any potential application to cancer treatment.

A peptidomimetic agent synthesized to inhibit αvß3/αvß5 integrins also appears to be promising. The antagonist, S247, proved to be an effective inhibitor of colon cancer cell migration and invasion *in vitro* and *in vivo*. S247 appears to inhibit colon cancer cell invasion by inhibiting tumor angiogenesis and not by inhibiting the various direct downstream targets (e.g., Akt) (Reinmuth et al., 2003). Clearly, when targeting integrin receptors as a therapy for cancer, attention must be given to potential mechanisms other than the intracellular signals conveyed by the integrin receptor. Because some integrins interact with the RGD tripeptide sequence present in many ECM proteins, this interaction has been exploited as a potential target of therapy through the use of soluble RGD-peptides (Pierschbacher et al., 1987; Chen et al., 1997). Snake venom represents yet another approach to blocking integrins. In particular, lebectin, a C-type lectin contained in Macrovipera lebetina venom, has been shown to inhibit the integrin-mediated attachment and invasion of human tumor cells (Sarray et al., 2004). The snake venom disintegrin contortrostatin has been found to inhibit human glioma cell invasion by interfering with vitronectin/fibronectin adhesion

(Schmitmeier et al., 2003). Because a disintegrin interferes with an integrin receptor's ability to bind to its ECM ligand, the use of disintegrin to prevent tumor spread is currently being examined. Although the disintegrin activity in snake venom has long been known, the study and development of anticancer agents with such a property has only just begun. Such anticancer therapy holds promise.

Insulin-like growth factor (IGF) System

In recent years, high circulating levels of the peptide hormone IGF-I have been linked to an increased risk of cancer. Currently, this is becoming an increasingly investigated area of cancer research since the positive correlation of IGF-I with cancer seemed to be found throughout various cancer types (Chan et al., 1998; Hankinson et al., 1998; Ma et al., 1999; Yu et al., 1999). Yet more mechanistic explanations of the IGF and its link to cancer remain to be discovered in just about all cancer types.

The IGF system includes a family of two growth factors (IGF-I, IGF-II), two receptors (IGF-IR, IGF-IIR), and multiple binding proteins (IGFBP 1-6). The bioavailability of IGF is regulated by its binding with IGFBPs, with IGFBP-3 being the most abundant circulating IGFBP. Most of the physiological effects from IGF occur through the IGF-IR at the cell surface. However, because IGF has a greater affinity for IGFBPs than for IGF-IR, IGFBPs are known to compete with IGF-IR activated downstream signaling. IGF-I is necessary for cells to enter the G1 phase, where a new round of cell division begins. Therefore, although at first it appears the binding of an IGFBP to IGF should be growth inhibitory (clearly sequestering IGF-I from IGF-IR), there is data showing that IGFBPs may also stimulate DNA synthesis by facilitating IGF binding to its receptor (Novosyadlyy et al., 2004) and therefore potentially stimulating cell growth and division. IGF-I-stimulated receptor is linked to the downstream activation of Akt, which phosphorylates (thereby inactivating) caspase 9 (Carpenter et al., 1993; Butler et al., 1998; Brunet et al., 1999; Zheng et al., 2000). The activation of Akt also culminates in the activation of NFκB, which stimulates the transcription of pro-survival genes (e.g., bcl-2) (Khwaja, 1999; Catz and Johnson 2001; Viatour et al., 2003). Both of these examples of the downstream effects of IGF-IR signaling show how IGF-I can promote cell survival and therefore underline the importance of IGFBPs in regulating the amount of IGF-I at the cellular level. However, the regulatory processes that dictate whether IGFBPs are to either inhibit or enhance IGF pathways are very poorly understood and often confusing at present.

IGF-I also induces the expression of matrix metalloproteinases (MMPs) that participate in the breakdown of the basement membrane and remodeling of the ECM prior to invasion. In particular, IGF-I induces the expression of MMP-2, MT1-MMP, and MMP-9 (Long et al., 1998; Bredin et al., 2003; Zhang and Brodt, 2003). MMP-2 and MMP-9 are gelatinases that can cleave type IV collagen and gelatin. Through the IGF system, MMP-9 mediates the increased invasion of cells stimulated by IGF-I (Mira et al., 1999). MMP-7 affects circulating IGF-I levels by cleaving IGFBP-3 (the key regulator of free IGF-I levels), thereby increasing IGF-I signaling at the IGF-IR (Miyamoto et al., 2004). Further, the blocking of IGF-IR up-regulates IGFBP-3 in prostate cancer cells (Grzmil et al., 2004). Therefore, it is very likely that MMP-7 is also controlled (at least in part) by IGF-IR-stimulated downstream signaling, which in turn frees up more IGF-I because of its degradation of IGFBP-3. MMP-7 is also a unique MMP, in that it is only synthesized in cancer cells (Miyazaki et al., 1990; Nagashima et al., 1997). A positive feedback loop involving MMP-7 seems to explain how MMP-7 contributes to the continuous IGF-I stimulation of cell growth and invasion (Fig. 1).

IGF-II has the same structural homology as IGF-I. However, IGF-II can be bound by the insulin receptor A (IR-A) isoform in addition to the IGF-IIR. IGF-II is overexpressed in malignant tumors and not in benign or normal adrenal tissue (Boulle et al., 1998), but little is known regarding whether IGF-II is linked to any pathways that trigger tumor invasion.

The IGF-IR has become a recent focus in studies of the IGF-axis in cancer because its involvement in cancer progression goes beyond the mere transduction of oncogenic signals downstream of the receptor that result in invasion. In particular, together with IGF-IR

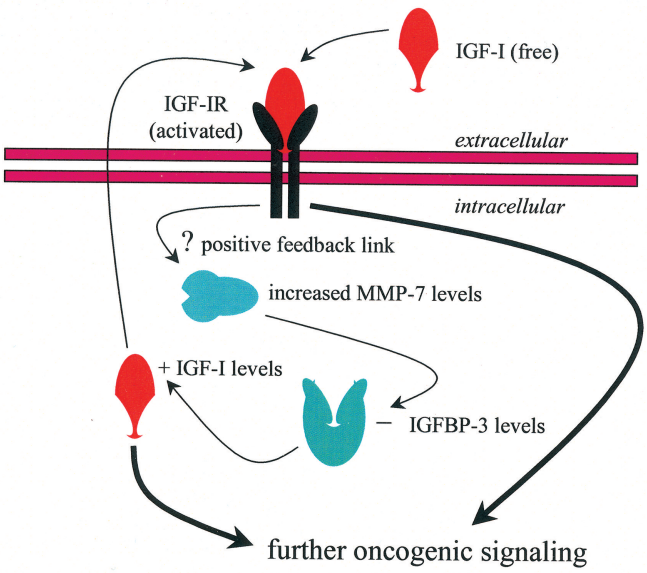

Fig. 1. Potential positive feedback loop linked to the regulation of MMP-7 levels in cancer cells. Activated IGF-IR may be linked to MMP-7 expression/activity, which causes an eventual increase in IGF-1. Increased IGF-1 levels from downstream IGF-IR signaling results in positive feedback extracellularly and thus advancing oncogenesis.

downstream signaling, IGF-IR also promotes a more invasive phenotype (Boulle et al., 1998; Brodt et al., 2001). One example is the overexpression of vascular endothelial growth factor C (VEGF-C), which is a known promoter of lymphatic metastasis in breast carcinoma that was recently found to be the result of a functional IGF-IR kinase domain (Tang et al., 2003). Once again, not only does a fully functional IGF-IR result in transduction of signals resulting in invasion, but it also triggers phenotype changes that, together in cooperation with IGF-IR downstream signaling, promote tumor spread. This therefore shows that merely studying the pathways downstream from IGF-IR may not give us a complete picture of the role of this receptor.

Consistent with the role of IGF-IR in the initiation of downstream signaling, the down-regulation of IGF-IR in a number of cancers has been shown to reduce cell proliferation and tumor dissemination (Boulle et al., 1998; Min et al., 2003; Zhao et al., 2004). For example, in a prognostic study done in patients with clear-cell renal carcinoma, the presence of IGF-IR was correlated with decreased patient survival (Parker et al., 2003), making IGF-IR a potential prognostic marker factor and therapeutic target in renal carcinoma. However, the role of IGF-IR differs in different cancers, and much remains to be known. For example, the reduced expression of IGF-IR in breast cancer was actually found to promote cell mobility and migration (Pennisi et al., 2002), possibly due to a fine balance between IGF-IR and E-cadherin complexes in maintaining a non-malignant phenotype. Therefore, simply abolishing the receptor in hopes of reducing cancer progression may not be as straightforward as initially thought from other IGF-IR studies. This adds a twist to the previously accepted perception of IGF-IR acting only as a culprit in cancer signal transduction and further underscores the importance of individualizing the roles of a receptor to each cancer system while keeping in mind the subtle but significant uniqueness of each signaling network depending on the cell type.

The IGFBPs have also recently gained attention because of their IGF-independent functions that promote cell invasion. Specifically, IGFBP-2 is up-regulated in a variety of cancers. For example, melanoma progression and the expression of IGFBP-2 were found to be linked, and the expression of IGFBP-2 was found to be increased in metastatic melanomas compared with benign nevi (Wang et al., 2003). In gliomas, IGFBP-2, which is frequently overexpressed in the most advanced-stage tumor, glioblastoma multiforme (Fuller et al., 1999), up-regulates a number of genes promoting invasion, the gene encoding MMP-2 being one of these genes (Wang et al., 2003). Western blotting, real-time PCR, and invasion studies were performed to validate the invasion-enhancing genes suspected on the basis of microarray data. Studies that afford such high-throughput data gathering are invaluable in the field of tumor signal transduction research because they identify new leads in the mapping of aberrant pathways. In prostate cancer, the overexpression of IGFBP-2 was found in over 90% of the cancers (Richardsen et al., 2003). Although IGFBP-2 is overexpressed, it has been found that IGFBP-2 plays a role in progression of cancer rather than the initiation of the disease (Moore et al., 2003; Fottner et al., 2004). Increasing IGFBP-6 mRNA levels were found to be correlated with increasingly invasive meningiomas (Nordqvist and Mathiesen, 2002). Clearly, the state of our knowledge of the roles of IGFBPs is now only observational at best, with the mechanisms by which an IGFBP acts remaining to be elucidated.

Another possibility that must be considered is that IGFBPs may also interact with other pathway molecules in order to achieve an alternate phenotype. This was pointed up in a recent study in which a yeast two-hybrid system revealed the existence of a protein antagonistic to IGFBP-2. The protein was named invasion inhibitory protein (IIp45) because of its ability to inhibit the invasive phenotype in gliomas (Song et al., 2003). It will be interesting to know the exact mechanism by which IIp45 counteracts the actions of IGFBP-2. In a similar scenario, in breast cancer, IGFBP-2 has also been found to interact with αvß3 integrin, which then reduces tumor growth and migratory potential (Pereira et al., 2004). Thus, the cross-talk between IGFBP and other pathways can alter the final phenotype presented by an IGF-independent pathway.

Some IGFBPs may also have dual roles. For example, IGFBP-3 appears to have both oncogenic and anti-proliferative cell signaling effects. In breast cancer, IGFBP-3 has growth-enhancing effects, possibly through a link with the epidermal growth factor (EGF) system (Butt et al., 2004). Yet IGFBP-3 has also been shown to induce a caspase-dependent apoptosis in breast cancer (Kim et al., 2004), and its expression is up-regulated in cyclooxygenase inhibitor-mediated apoptosis (Levitt et al., 2004). IGFBP-4 appears to inhibit cell proliferation and growth in lymphoid tissue (Zhou et al., 2004) and has also been shown to reduce anchorage-dependent colony formation (potential escape from anoikis) and invasion in colorectal cancer cells (Diehl et al., 2004).

Clearly, different IGFBPs have different effects on phenotype. As previously mentioned, since most IGFBPs also have IGF-independent functions, it is likely that many of the IGFBPs have dual-roles that can either promote or suppress cancer as the case may be.

Rho Family GTPases

The Rho family proteins consist of about 20 individual genes that encode signaling molecules, which contain a small GTPase domain (of the Rho consensus type). On the basis of amino acid sequence and protein function, these proteins have been divided into five groups: Rho-like, Rnd, Cdc42-like, Rac-like, and RhoBTB. These proteins regulate numerous cellular activities, such as cell growth, cell differentiation, cell cycling, and tumor cell invasion. To date, perhaps the

two most investigated groups of proteins are the Rho-like proteins and the Rac-like proteins.

The Rho-like subfamily of proteins in general contributes to the formation of stress fibers and focal adhesions in cells. RhoC, in particular, appears to play a significant role in the promotion of tumor invasion (Clark et al., 2000). For example, the direct overexpression of RhoC and the constitutive expression of an active mutant of RhoC were both observed to up-regulate the expression of invasion-enhancing genes in breast carcinoma cells (Wu et al., 2004). Further, the use of drug inhibitors that altered RhoC subcellular localization inhibited *in vitro* and *in vivo* spread of melanoma cells (Collisson et al., 2003). Much remains to be elucidated regarding the mechanism of RhoC's regulation of invasion. Currently, all we know is that some downstream genes are up-regulated and that this results in certain phenotypes. It is unclear which downstream genes are at the next step in the RhoC cascade leading to cell invasion. The interaction of RhoC with other small GTPases (such as the growth-inhibiting RhoB) may potentially show a balance between members of this important class of proteins.

The Rac-like subfamily appears to induce membrane ruffling and the generation of lamellipodia (i.e., protrusions from the leading edge of migrating cells), which is important for cell motility (Ridley et al., 1992). The Rac proteins are also known to stimulate growth transformation, activate Jun N-terminal kinase (JNK), and promote cell survival. Rac has even been shown to directly promote the invasion of fibrosarcoma cells by activating MMP-2 in the degradation of a collagen barrier (Zhuge and Xu, 2001). However, in renal cell carcinoma, there is evidence that Rac signaling inhibits invasion by up-regulating inhibitors of MMPs (Engers et al., 2001). And, once again, we cannot rule out the existence of a negative feedback loop that attenuates the effects of an up-regulated protein such as Rac (Fig. 2).

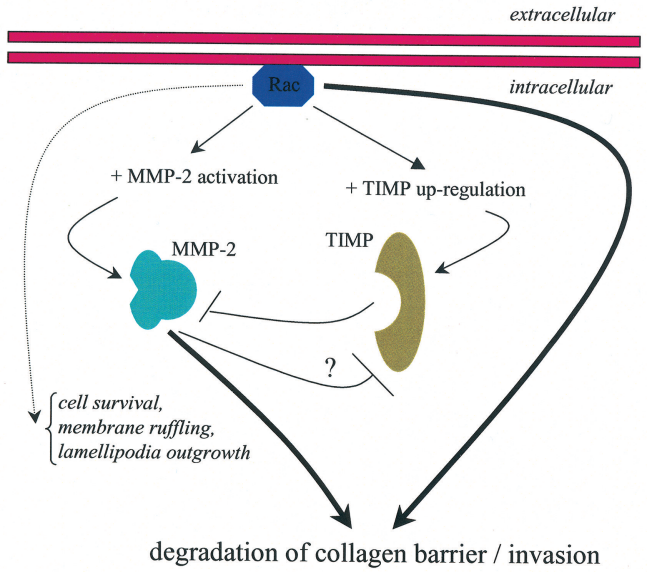

Fig. 2. In cancer cells, Rac's ability to increase and decrease MMP-2 levels may occur by means of negative feedback. An increase of MMP-2 may possibly result in a direct down-regulation of its inhibitor, tissue inhibitor of metalloproteinase (TIMP), in order for MMP-2 to degrade the collagen barrier and further promote tumor invasion.

The upstream activator of Rac, known as T-lymphoma invasion and metastasis (Tiam1), has recently received much attention in studies of Rac activity. Although Tiam1 deficiency in mice resulted in the formation of fewer Ras-induced tumors, a greater proportion of these tumors were able to convert to a malignant phenotype (Malliri et al., 2002). Here again we see the dual effects that a single protein (a gene expression regulator in this case) can have on the resulting tumor phenotype. This paradox of a decreased tumor cell number coupled with increased cell malignancy is a trend that is being observed more and more in cancer research. Therefore, targeting a protein such as Tiam1 remains a possible therapeutic strategy, yet the effects on tumor growth currently remain unclear.

Furthermore, it has been found that tumors are able to switch between different modes of invasion by using separate Rho signaling pathways (Sahai and Marshall, 2003). Indeed, it appears that some tumor cells employ multiple Rho signaling pathways that trigger the cell's invasiveness and that the cell can switch between pathways, potentially limiting the effectiveness of certain anti-cancer agents. This underlines the importance of finding a synergistic therapy that can block multiple pathways and hence completely abolish metastasis. Indeed, perhaps some of the most devastating cancers today are able to bypass current therapies simply because they can make use of multiple signaling pathways that all result in invasion. Therefore, in the search for better therapies, rather than looking for stronger inhibitors of a single pathway, the first step may be to delineate the other pathways involved that are also contributing to the same phenotype.

Pathway crosstalk

Intersecting pathways play a major role in the resulting phenotype in any signal transduction. However, currently, little is known about how pathway crosstalk leads to tumor invasion. Nonetheless, there is evidence that some phenotypes are the result of more than just the cascading events of one pathway. Indeed, the direct interaction of members of one pathway with a member of another pathway has been observed, and the resulting phenotype can either be different from the activation of one of the pathways alone or be amplified in almost a synergistic manner. In addition, as already noted, signaling pathways that contribute to tumor progression are not always strictly isolated to any one system. For example, as discussed earlier, tumor growth and invasion can be affected by the interaction of integrins with the IGF system. In particular, the IGFBPs appear to mediate

much of the crosstalk between the IGF axis with other pathways, most likely as a result of the IGF-independent functions of the IGFBPs. Interestingly, however, although IGFBP-2 upregulates invasion-enhancing genes (Wang et al., 2003), the interaction of IGFBP-2 with overexpressed αvß3 integrin appears to reduce tumor growth and migration in breast cancer cells (Pereira et al., 2004). This shows that the role of a protein can become distinctly opposite when the protein is bound to a molecule from another pathway. IGFBP-3 has been hypothesized to be linked to the EGF system, resulting in growth proliferation (Butt et al., 2004). This could establish a link between the IGF and EGF system, which would be an important link because the EGF system is a major crossroad of tumor invasion signal transduction, which will be discussed further below.

There are also data showing links between the IGFBPs with other pathways. For example, the previous finding that IGFBP-1 binds to the α5ß1 integrin (in an RGD-dependent manner) and thereby stimulates cell migration (Jones et al., 1993). The fact that IGFBP-2 also contains an RGD motif suggested that IGFBP2 may also bind integrin thus affect cellular adhesion and motility. This hypothesis was recently shown to be true – verifying that IGFBP-2 also binds to α5ß1 integrin in an RGD-dependent manner, resulting in decreased cell adhesion and proliferation (Schutt et al., 2004). These results are intriguing because the combination of these two properties does not make for a clear-cut phenotype with respect to tumor invasion (i.e., whether IGFBP-2 binding to α5ß1 integrin is pro-invasive or anti-invasive). Further studies involving invasion assays are needed to show whether the decrease in cell adhesion resulting from the interaction of IGFBP-2 and α5ß1 integrin results in an increase in tumor cell migration. Here, again, we may be seeing the juxtaposition of decreased cell growth and increased cell invasion (the result of the decreased cell adhesion), a scenario very similar to that found for the Rac-activator Tiam1 (Malliri et al., 2002), as described in the previous section. All of this together further reinforces the postulate that cells are either dividing or spreading, but never both simultaneously. The observations that lead to this belief were first seen in fibroblast cells, but are now apparent in colonic carcinomas as well as in gliomas (Varner et al., 1995, 1996; Giese et al., 1996).

The mechanism by which IIp45 counteracts IGFBP-2 (as mentioned above in the section on the IGF system) remains to be identified. It is possible that the invasion inhibitory properties of IIp45 (Song et al., 2003) manifests through the competitive binding with IGFBP-2 because both IGFBP-2 and IIp45 have an RGD-sequence that has potential to bind integrins. That is, the binding of IIp45 at the RGD domain may prevent IGFBP-2 from binding to integrin and consequently prevent the propagation of downstream signals. Another possibility may be through a conformational change induced in IGFBP-2 following its binding with IIp45. Therefore, due to steric reasons, the binding IIp45 with IGFBP-2 could possibly change the exposure of the RGD domain in IGFBP-2 and thus affect the interaction with integrins.

As mentioned above, the IGF system regulates the expression of a number of MMPs (Long et al., 1998; Bredin et al., 2003; Zhang and Brodt, 2003), which are involved in the digestion of the ECM during cell invasion. There is now also evidence of the alternative processing of integrin subunits through MT1-MMP in tumor cells (Deryugina et al., 2002; Ratnikov et al., 2002). This not only points to a link between the pathways that regulate MMPs and integrins, it also points to the possibility that some MMPs play a more broad role in the invasion of tumor cells. This also suggests that the primary function of some MMPs may be the alternate processing of proteins rather than the breakdown of the extracellular barriers. This raises the possibility that other MMPs also play alternate roles in malignant cells, contributing overall to more cancerous phenotypes. Here, again, the IGF system may interact with the integrin system, though this time via an MMP-regulated intermediate step.

The EGF system also appears to have significant crosstalk with pathways of other systems regulating cell migration and invasion. This is important because not only is EGF signaling important in producing invasive phenotypes in many cancers, it also appears to be influenced by multiple pathways. In particular, the EGF receptor (EGF-R) appears to be a focal point for many cell signals, specifically G-protein coupled receptor (GPC-R) signaling, which transactivates EGF-R, thereby promoting cell invasion (Schafer et al., 2004). Further, in

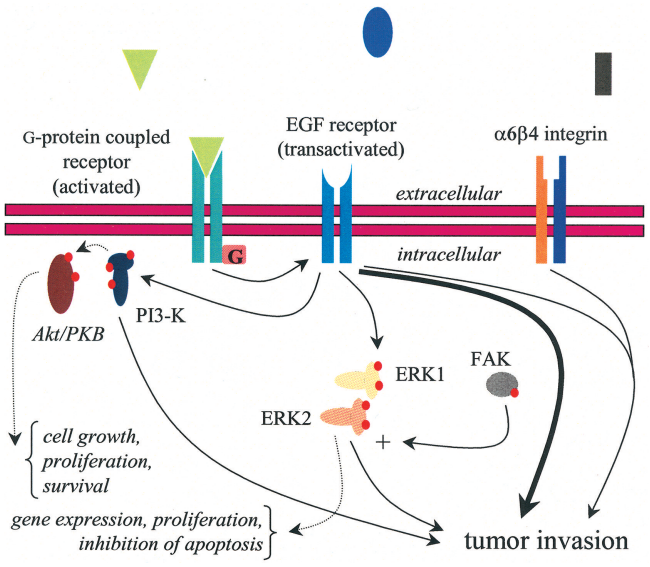

Fig. 3. The EGF system appears to be a major intrsecting point of pathways contributing to tumor invasion - an example of pathway crosstalk between varius pathways systems. Specifically, at the EGF-R, signals from multiple receptors converge that influence tumor invasion.

generating an invasive phenotype, the signals downstream from EGF-R appear to utilize both the phosphatidylinositol-3-kinase (PI3-K) and extracellular regulated kinase 1, 2 (ERK 1,2) pathways (Price et al., 2002). Focal adhesion kinase (FAK), which has been linked to an increased cell invasion potential in many human cancers, has also been found to coordinate the EGF-stimulated migration of invasive tumor cells by enhancing EGF-stimulated JNK and ERK2 kinase activation (Hauck et al., 2001). Further, the EGF-R appears to interact with the α6ß4 integrins of hemidesmosomes, leading to the migration of both normal and malignant epithelial cells (Mariotti et al., 2001). Clearly, with respect to tumor invasion, the EGF system displays by far the most crosstalk with other pathways (Fig. 3). Therefore, because the EGF system is likely a pivotal point linking multiple pathways that promote cell invasion, this makes the EGF system an excellent target for new anti-cancer therapies. Currently, clinical trials of drugs targeting EGFR are ongoing. Antibodies targeting the EGFR ligand binding site appear to be the most promising in the areas of colorectal and head/neck cancers (Crombet et al., 2004; Saltz et al., 2004; Vanhoefer et al., 2004). Clinical studies of pharmacological inhibitors are also under way. These include small-molecule kinase inhibitors selective for EGFR (Shah and Miller, 2003; Kim and Choy, 2004; Soulieres et al., 2004).

This understanding of the immense amount of crosstalk surrounding EGFR signal transduction is now translating into promising anti-cancer therapy. It will be interesting to see how the disruption of EGFR (and hence disruption of the crosstalk pathways linked to EGFR) alone decreases tumor invasion compared with the disruption of each individual pathway. Because EGFR is already an important point of pathway crosstalk, it is no surprise that synergistic mechanisms of disruption of action may come in play when targeting EGFR.

Concluding remarks

Cell migration is a fundamental feature of normal growth and development. However, the physiological signals orchestrating this phenomenon remain obscured in the network of pathways that are linked to nearly every functional activity in the cell. The complexity with which these pathways are interlinked means that normal cell migration can be disturbed by various signaling pathways if they should go awry. In general, however, tumor invasion results from the de-regulation of a signal controlling cell movement and/or cell proliferation. In reviewing the current research in this area, we have found that one or more of the multiple systems mentioned here can contribute to tumor invasion. For example, multiple pathways can be simultaneously involved (e.g., the involvement of certain integrins with the IGFBPs). Further, feedback looping may explain uncontrolled signaling (e.g., the potential involvement of MMP-7 in IGF-IR signaling). Tumors can even utilize multiple pathways to achieve the same phenotype (e.g., invasion resulting from two different Rho signaling pathways) and potentially evade current anti-cancer therapies though this mechanism. Further, the crosstalk of pathways can dramatically alter phenotypes. Thus, whereas the pathways responsible for tumor progression were once thought to be relatively isolated, we are now learning that these pathways overlap and to a certain degree may even work in a unified fashion. Clearly, therefore, the signal transduction that determines a tumor's invasive capacity is far more complex than originally thought and should not be viewed as a simple matter of cause and effect. On a positive note, our increasing appreciation and understanding of the complexity involved in this process is bringing to light new targets for therapy, which is the first step in cancer research.

References

Boulle N., Logie A., Gicquel C., Perin L. and Le Bouc Y. (1998). Increased levels of insulin-like growth factor II (IGF-II) and IGF-binding protein-2 are associated with malignancy in sporadic adrenocortical tumors. J Clin. Endocrinol. Metab. 83, 1713-1720.

Bredin C.G., Liu Z. and Klominek J. (2003). Growth factor-enhanced expression and activity of matrix metalloproteases in human non-small cell lung cancer cell lines. Anticancer Res. 23, 4877-4884.

Brodt P., Fallavollita L., Khatib A.M., Samani A.A. and Zhang D. (2001). Cooperative regulation of the invasive and metastatic phenotypes by different domains of the type I insulin-like growth factor receptor beta subunit. J. Biol. Chem. 276, 33608-33615.

Brunet A., Bonni A., Zigmond M.J., Lin M.Z., Juo P., Hu L.S., Anderson M.J., Arden K.C., Blenis J. and Greenberg M.E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96, 857-868.

Butler A.A., Yakar S., Gewolb I.H., Karas M., Okubo Y. and LeRoith D. (1998). Insulin-like growth factor-I receptor signal transduction: at the interface between physiology and cell biology. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 121, 19-26.

Butt A.J., Martin J.L., Dickson K.A., McDougall F., Firth S.M. and Baxter R.C. (2004). Insulin-like growth factor binding protein-3 expression is associated with growth stimulation of T47D human breast cancer cells: the role of altered epidermal growth factor signaling. J. Clin. Endocrinol. Metab. 89, 1950-1956.

Carpenter C.L., Auger K.R., Chanudhuri M., Yoakim M., Schaffhausen B., Shoelson S. and Cantley L.C. (1993). Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. J. Biol. Chem. 268, 9478-9483.

Catz S.D. and Johnson J.L. (2001). Transcriptional regulation of bcl-2 by nuclear factor kappaB and its significance in prostate cancer. Oncogene 20, 7342-7351.

Chan J.M., Stampfer M.J., Giovannucci E., Gann P.H., Ma J., Wilkinson P., Hennekens C.H. and Pollak M. (1998). Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 279, 563-566.

Chen X.M., Wang J.Z., Fu B. and Yu L.F. (1997). RGD-containing peptides trigger apoptosis in glomerular mesangial cells of adult cells of adult human kidneys. Biochem. Biophys. Res. Commun.

234, 594-599.

Chung J., Bachelder R.E., Lipscomb E.A., Shaw L.M. and Mercurio A.M. (2002). Integrin (alpha6beta4) regulation of eIF-4E activity and VEGF translation: a survival mechanism for carcinoma cells. J. Cell Biol. 158, 165-174.

Collisson E.A., Kleer C., Wu M., De A., Gambhir S.S., Merajver S.D. and Kolodney M.S. (2003). Atorvastatin prevents RhoC isoprenylation, invasion, and metastasis in human melanoma cells. Mol. Cancer Ther. 2, 941-948.

Clark E.A., Golub T.R., Lander E.S. and Hynes R.O. (2000). Genomic analysis of metastasis reveals an essential role for RhoC. Nature 406, 532-535.

Crombet T., Osorio M., Cruz T., Roca C., del Castillo R., Mon R., Iznaga-Escobar N., Figueredo R., Koropatnick J., Renginfo E., Fernandez E., Alvarez D., Torres O., Ramos M., Leonard I., Perez R. and Lage A. (2004). Use of the humanized anti-epidermal growth factor receptor monoclonal antibody h-R3 in combination with radiotherapy in the treatment of locally advanced head and neck cancer patients. J. Clin. Oncol. 22, 1646-1654.

Davidson B., Goldberg I., Reich R., Tell L., Dong H.P., Trope' C.G., Risberg B. and Kopolovic J. (2003). AlphaV- and beta1-integrin subunits are commonly expressed in malignant effusions from ovarian carcinoma patients. Gynecol. Oncol. 90, 248-257.

Deryugina E.I., Ratnikov B.I., Postnova T.I., Rozanov D.V. and Strongin A.Y. (2002). Processing of integrin alpha(v) subunit by membrane type 1 matrix metalloproteinase stimulates migration of breast carcinoma cells on vitronectin and enhances tyrosine phosphorylation of focal adhesion kinase. J. Biol. Chem. 277, 9749-9756.

Diehl D., Hoeflich A., Wolf E. and Lahm H. (2004). Insulin-like growth factor (IGF)-binding protein-4 inhibits colony formation of colorectal cancer cells by IGF-independent mechanisms. Cancer Res. 64, 1600-1603.

Engers R., Springer E., Michiels F., Collard J.G. and Gabbert H.E. (2001). Rac affects invasion of human renal cell carcinomas by up-regulating tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-2 expression. J. Biol. Chem. 276, 41889-41897.

Fornaro M., Manzotti M., Tallini G., Slear A.E., Bosari S., Ruoslahti E. and Languino L.R. (1998). Beta1C integrin in epithelial cells correlates with a nonproliferative phenotype: forced expression of beta1C inhibits prostate epithelial cell proliferation. Am. J. Pathol. 153, 1079-1087.

Fottner Ch., Hoeflich A., Wolf E. and Weber M.M. (2004). Role of the insulin-like growth factor system in adrenocortical growth control and carcinogenesis. Horm. Metab. Res 36, 397-405.

Fuller G.N., Rhee C.H., Hess K.R., Caskey L.S., Wang R., Bruner J.M., Yung W.K. and Zhang W. (1999). Reactivation of insulin-like growth factor binding protein 2 expression in glioblastoma multiforme: a revelation by parallel gene expression profiling. Cancer Res. 59, 4228-4232.

Giese A., Loo M.A., Tran N., Haskett D., Coons S.W. and Berens M.E. (1996). Dichotomy of astrocytoma migration and proliferation. Int. J. Cancer 67, 275-282.

Gimond C., Baudoin C. and Sonnenberg A. (2000). Defects in adhesion and migration, but not in proliferation and differentiation, of embryonic stem cells upon replacement of integrin subunit beta1A by beta1D. Differentiation 66, 93-105.

Grzmil M., Hemmerlein B., Thelen P., Schweyer S. and Burfeind P. (2004). Blockade of the type I IGF receptor expression in human prostate cancer cells inhibits proliferation and invasion, up-regulates IGF binding protein-3, and suppresses MMP-2 expression. J. Pathol. 202, 50-59.

Hankinson S.E., Willett W.C., Colditz G.A., Hunter D.J., Michaud D.S., Deroo B., Rosner B., Speizer F.E. and Pollak M. (1998). Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351, 1393-1396.

Hauck C.R., Sieg D.J., Hsia D.A., Loftus J.C., Gaarde W.A., Monia B.P. and Schlaepfer D.D. (2001). Inhibition of focal adhesion kinase expression or activity disrupts epidermal growth factor-stimulated signaling promoting the migration of invasive human carcinoma cells. Cancer Res. 61, 7079-7090.

Haugland H.K., Tysnes B.B. and Tysnes O.B. (1997). Adhesion and migration of human glioma cells are differently dependent on extracellular matrix molecules. Anticancer Res. 17, 1035-1042.

Hemler M. (1999). Extracellular matrix, anchor, and adhesion proteins. Oxford, UK: Oxford University Press. 196-212.

James W.G., Bullard D.C. and Hickey M.J. (2003). Critical role of the alpha 4 integrin/VCAM-1 pathway in cerebral leukocyte trafficking in lupus-prone MRL/fas(lpr) mice. J. Immunol. 170, 520-527.

Jones J.I., Gockerman A., Busby W.H. Jr, Wright G. and Clemmons D.R. (1993). Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. Proc. Natl. Acad. Sci. USA 90, 10553-10557.

Kabir-Salmani M., Shiokawa S., Akimoto Y., Sakai K. and Iwashita M. (2004). The role of alpha(5)beta(1)-integrin in the IGF-I-induced migration of extravillous trophoblast cells during the process of implantation. Mol. Hum. Reprod. 10, 91-97.

Khwaja A. (1999). Akt is more than just a Bad kinase. Nature 401, 33-34.

Kim D.W. and Choy H. (2004). Potential role for epidermal growth factor receptor inhibitors in combined-modality therapy for non-small-cell lung cancer. Int. J. Radiat. Oncol. Biol. Phys. 59(2 Suppl), 11-20.

Kim H.S., Ingermann A.R., Tsubaki J., Twigg S.M., Walker G.E. and Oh Y. (2004). Insulin-like growth factor-binding protein 3 induces caspase-dependent apoptosis through a death receptor-mediated pathway in MCF-7 human breast cancer cells. Cancer Res. 64, 2229-2237.

Kiosses W.B., Shattil S.J., Pampori N. and Schwartz M.A. (2001). Rac recruits high-affinity integrin alphavbeta3 to lamellipodia in endothelial cell migration. Nat. Cell Biol. 3, 316-320.

Kudlacz E., Whitney C., Andresen C., Duplantier A., Beckius G., Chupak L., Klein A., Kraus K. and Milici A. (2002). Pulmonary eosinophilia in a murine model of allergic inflammation is attenuated by small molecule alpha4beta1 antagonists. J. Pharmacol. Exp. Ther. 301, 747-752.

Levitt R.J., Buckley J., Blouin M.J., Schaub B., Triche T.J. and Pollak M. (2004). Growth inhibition of breast epithelial cells by celecoxib is associated with upregulation of insulin-like growth factor binding protein-3 expression. Biochem. Biophys. Res. Commun. 316, 421-428.

Lipscomb E.A., Dugan A.S., Rabinovitz I. and Mercurio A.M. (2003). Use of RNA interference to inhibit integrin (alpha6beta4)-mediated invasion and migration of breast carcinoma cells. Clin. Exp. Metastasis 20, 569-576.

Long L. Navab R. and Brodt P. (1998). Regulation of the Mr 72,000 type IV collagenase by the type I insulin-like growth factor receptor. Cancer Res 58, 3243-3247.

Ma J., Pollak M.N., Giovannucci E., Chan J.M., Tao Y., Hennekens C.H. and Stampfer M.J. (1999). Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J. Natl. Cancer Inst. 91, 620-625.

Malliri A., van der Kammen R.A., Clark K., van der Valk M., Michiels F. and Collard J.G. (2002). Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature 417, 867-871.

Manes T., Zheng D.Q., Tognin S., Woodard A.S., Marchisio P.C. and Languino L.R. (2003). Alpha(v)beta3 integrin expression up-regulates cdc2, which modulates cell migration. J. Cell Biol. 161, 817-826.

Mariotti A., Kedeshian P.A., Dans M., Curatola A.M., Gagnoux-Palacios L. and Giancotti F.G. (2001). EGF-R signaling through Fyn kinase disrupts the function of integrin alpha6beta4 at hemidesmosomes: role in epithelial cell migration and carcinoma invasion. J. Cell Biol. 155, 447-458.

Min Y., Adachi Y., Yamamoto H., Ito H., Itoh F., Lee C.T., Nadaf S., Carbone D.P. and Imai K. (2003). Genetic blockade of the insulin-like growth factor-I receptor: a promising strategy for human pancreatic cancer. Cancer Res. 63, 6432-6441.

Mira E., Manes S., Lacalle R.A., Marquez G. and Martinez-A C. (1999). Insulin-like growth factor I-triggered cell migration and invasion are mediated by matrix metalloproteinase-9. Endocrinology 140, 1657-64.

Miyamoto S., Yano K., Sugimoto S., Ishii G., Hasebe T., Endoh Y., Kodama K., Goya M., Chiba T. and Ochiai A. (2004). Matrix metalloproteinase-7 facilitates insulin-like growth factor bioavailability through its proteinase activity on insulin-like growth factor binding protein 3. Cancer Res. 64, 665-671.

Miyazaki K., Hattori Y., Umenishi F., Yasumitsu H. and Umeda M. (1990). Purification and characterization of extracellular matrix-degrading metalloproteinase, matrin (pump-1), secreted from human rectal carcinoma cell line. Cancer Res. 50, 7758-7764.

Moore M.G., Wetterau L.A., Francis M.J., Peehl D.M. and Cohen P. (2003). Novel stimulatory role for insulin-like growth factor binding protein-2 in prostate cancer cells. Int. J. Cancer 105, 14-19.

Nagashima Y., Hasegawa S., Koshikawa N., Taki A., Ichikawa Y., Kitamura H., Misugi K., Kihira Y., Matuo Y., Yasumitsu H. and Miyazaki K. (1997). Expression of matrilysin in vascular endothelial cells adjacent to matrilysin-producing tumors. Int. J. Cancer 72, 441-445.

Nemeth J.A., Cher M.L., Zhou Z., Mullins C., Bhagat S. and Trikha M. (2003). Inhibition of alphaVbeta3 integrin reduces angiogenesis, bone turnover, and tumor cell proliferation in experimental prostate cancer bone metastases. Clin. Exp. Metastasis 20, 413-420.

Nordqvist A.C. and Mathiesen T. (2002). Expression of IGF-II, IGFBP-2, -5, and -6 in meningiomas with different brain invasiveness. J. Neurooncol. 57, 19-26.

Novosyadlyy R., Tron K., Dudas J., Ramadori G. and Scharf J.G. (2004). Expression and regulation of the insulin-like growth factor axis components in rat liver myofibroblasts. J. Cell. Physiol. 199, 388-398.

Parker A., Cheville J.C., Lohse C., Cerhan J.R. and Blute M.L. (2003). Expression of insulin-like growth factor I receptor and survival in patients with clear cell renal cell carcinoma. J. Urol. 170(2 Pt 1), 420-424.

Pennisi P.A., Barr V., Nunez N.P., Stannard B. and Le Roith D. (2002). Reduced expression of insulin-like growth factor I receptors in MCF-7 breast cancer cells leads to a more metastatic phenotype. Cancer Res. 62, 6529-6537.

Pereira J.J., Meyer T., Docherty S.E., Reid H.H., Marshall J., Thompson E.W., Rossjohn J. and Price J.T. (2004). Biomolecular interaction of insulin-like growth factor (IGF) binding protein-2 with alphaVbeta3 negatively modulates IGF-I-mediated migration and tumor growth. Cancer Res. 64, 977-984.

Pierschbacher M.D. and Ruoslahti E. (1987). Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. J. Biol. Chem 262, 17294-17298.

Price D.J., Avraham S., Feuerstein J., Fu Y. and Avraham H.K. (2002). The invasive phenotype in HMT-3522 cells requires increased EGF receptor signaling through both PI 3-kinase and ERK 1,2 pathways. Cell Commun. Adhes. 9, 87-102.

Ratnikov B.I., Rozanov D.V., Postnova T.I., Baciu P.G., Zhang H., DiScipio R.G., Chestukhina G.G., Smith J.W., Deryugina E.I. and Strongin A.Y. (2002). An alternative processing of integrin alpha(v) subunit in tumor cells by membrane type-1 matrix metalloproteinase. J. Biol. Chem. 277, 7377-7385.

Reinmuth N., Liu W., Ahmad S.A., Fan F., Stoeltzing O., Parikh A.A., Bucana C.D., Gallick G.E., Nickols M.A., Westlin W.F. and Ellis L.M. (2003). Alphavbeta3 integrin antagonist S247 decreases colon cancer metastasis and angiogenesis and improves survival in mice. Cancer Res. 63, 2079-2087.

Richardsen E., Ukkonen T., Bjornsen T., Mortensen E., Egevad L. and Busch C. (2003). Overexpression of IGFBP2 is a marker for malignant transformation in prostate epithelium. Virchows Arch. 442, 329-335.

Ridley A.J., Paterson H.F., Johnston C.L., Diekmann D. and Hall A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 70, 401-410.

Rose D.M., Grabovsky V., Alon R. and Ginsberg M.H. (2001). The affinity of integrin alpha4beta1 governs lymphocyte migration. J. Immunol. 167, 2824-2830.

Sahai E. and Marshall C.J. (2003). Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. Nat. Cell Biol. 5, 711-719.

Saltz L.B., Meropol N.J., Loehrer P.J. Sr, Needle M.N., Kopit J., Mayer R.J. (2004). Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J. Clin. Oncol. 22, 1201-1208.

Sarray S., Berthet V., Calvete J.J., Secchi J., Marvaldi J., El-Ayeb M., Marrakchi N. and Luis J. (2004). Lebectin, a novel C-type lectin from Macrovipera lebetina venom, inhibits integrin-mediated adhesion, migration and invasion of human tumour cells. Lab. Invest. 84, 573-581.

Schafer B., Gschwind A. and Ullrich A. (2004). Multiple G-protein-coupled receptor signals converge on the epidermal growth factor receptor to promote migration and invasion. Oncogene 23, 991-999.

Schmitmeier S., Markland F.S., Ritter M.R., Sawcer D.E. and Chen T.C. (2003). Functional effect of contortrostatin, a snake venom disintegrin, on human glioma cell invasion in vitro. Cell Commun. Adhes. 10, 1-16.

Schutt B.S., Langkamp M., Rauschnabel U., Ranke M.B. and Elmlinger M.W. (2004). Integrin-mediated action of insulin-like growth factor binding protein-2 in tumor cells. J. Mol. Endocrinol. 32, 859-868.

Shah N.T. and Miller V.A. (2003). Antitumor activity and tolerability of gefitinib in patients with non-small-cell lung cancer treated in an expanded access program. Clin. Lung Cancer 5, 182-186.

Song S.W., Fuller G.N., Khan A., Kong S., Shen W., Taylor E., Ramdas

L., Lang F.F. and Zhang W. (2003). IIp45, an insulin-like growth factor binding protein 2 (IGFBP-2) binding protein, antagonizes IGFBP-2 stimulation of glioma cell invasion. Proc. Natl. Acad. Sci. USA 100, 13970-13975.

Soulieres D., Senzer N.N., Vokes E.E., Hidalgo M., Agarwala S.S. and Siu L.L. (2004). Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. J. Clin. Oncol. 22, 77-85.

Tai Y.T., Podar K., Catley L., Tseng Y.H., Akiyama M., Shringarpure R., Burger R., Hideshima T., Chauhan D., Mitsiades N., Richardson P., Munshi N.C., Kahn C.R., Mitsiades C. and Anderson K.C. (2003). Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of beta1-integrin and phosphatidylinositol 3'-kinase/AKT signaling. Cancer Res. 63, 5850-5858.

Tang Y., Zhang D., Fallavollita L. and Brodt P. (2003). Vascular endothelial growth factor C expression and lymph node metastasis are regulated by the type I insulin-like growth factor receptor. Cancer Res. 63, 1166-1171.

Tennenbaum T., Belanger A.J., Glick A.B., Tamura R., Quaranta V. and Yuspa S.H. (1995). A splice variant of alpha 6 integrin is associated with malignant conversion in mouse skin tumorigenesis. Proc. Natl. Acad. Sci. USA 92, 7041-7045.

Tysnes B.B., Larsen L.F., Ness G.O., Mahesparan R., Edvardsen K., Garcia-Cabrera I. and Bjerkvig R. (1996). Stimulation of glioma-cell migration by laminin and inhibition by anti-alpha3 and anti-beta1 integrin antibodies. Int. J. Cancer 67, 777-784.

Vanhoefer U., Tewes M., Rojo F., Dirsch O., Schleucher N., Rosen O., Tillner J., Kovar A., Braun A.H., Trarbach T., Seeber S., Harstrick A. and Baselga J. (2004). Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody EMD72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. J. Clin. Oncol 22, 175-184.

Varner J.A. and Cheresh D.A. (1996). Integrins and cancer. Curr. Opin. Cell Biol. 8, 724-730.

Varner J.A., Emerson D.A. and Juliano R.L. (1995). Integrin alpha5beta1 expression negatively regulates cell growth: reversal by attachment to fibronectin. Mol. Biol. Cell 6, 725-740.

Viatour P., Bentires-Alj M., Chariot A., Deregowski V., de Leval L., Merville M.P. and Bours V. (2003). NF- kappa B2/p100 induces Bcl-2 expression. Leukemia 17, 1349-1356.

Wang H., Shen S.S., Wang H., Diwan A.H., Zhang W., Fuller G.N. and Prieto V.G. (2003). Expression of insulin-like growth factor-binding protein 2 in melanocytic lesions. J. Cutan. Pathol. 30, 599-605.

Wang H., Wang H., Shen W., Huang H., Hu L., Ramdas L., Zhou Y.H., Liao W.S., Fuller G.N. and Zhang W. (2003). Insulin-like growth factor binding protein 2 enhances glioblastoma invasion by activating invasion-enhancing genes. Cancer Res. 63, 4315-4321.

Wu M., Wu Z.F., Kumar-Sinha C., Chinnaiyan A. and Merajver S.D. (2004). RhoC induces differential expression of genes involved in invasion and metastasis in MCF10A breast cells. Breast Cancer Res. Treat. 84, 3-12.

Yang C., Zeisberg M., Lively J.C., Nyberg P., Afdhal N. and Kalluri R. (2003). Integrin alpha1beta1 and alpha2beta1 are the key regulators of hepatocarcinoma cell invasion across the fibrotic matrix microenvironment. Cancer Res. 63, 8312-8317.

Yu H., Spitz M.R., Mistry J., Gu J., Hong W.K. and Wu X. (1999). Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. J. Natl. Cancer Inst. 91, 151-156.

Zhang D. and Brodt P. (2003). Type 1 insulin-like growth factor regulates MT1-MMP synthesis and tumor invasion via PI 3-kinase/Akt signaling. Oncogene 22, 974-982.

Zhao H., Dupont J., Yakar S., Karas M. and LeRoith D. (2004). PTEN inhibits cell proliferation and induces apoptosis by downregulating cell surface IGF-IR expression in prostate cancer cells. Oncogene 22, 786-794.

Zheng D.Q., Woodard A.S., Fornaro M., Tallini G. and Languino L.R. (1999). Prostatic carcinoma cell migration via alpha(v)beta3 integrin is modulated by a focal adhesion kinase pathway. Cancer Res. 59, 1655-1664.

Zheng W.H., Kar S., Dore S. and Quirion R. (2000). Insulin-like growth factor-1 (IGF-1): a neuroprotective trophic factor acting via the Akt kinase pathway. J. Neural Transm. Suppl. 60, 261-272.

Zhou R., Flaswinkel H., Schneider M.R., Lahm H., Hoeflich A., Wanke R. and Wolf E. (2004). Insulin-like growth factor-binding protein-4 inhibits growth of the thymus in transgenic mice. J. Mol. Endocrinol. 32, 349-364.

Zhuge Y. and Xu J. (2001). Rac1 mediates type I collagen-dependent MMP-2 activation. Role in cell invasion across collagen barrier. J. Biol. Chem. 276, 16248-16256.

Accepted November 2, 2004