DNA damage signaling networks: from stem cells to cancer

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Chapter 6

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Integrins and Oncogenes: Partners in Crime

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Abstract

Metastatic spread and acquired or intrinsic resistance to existing therapies form the two major obstacles in cancer treatment. Accumulating evidence indicates that cancer growth, metastasis, and the response to therapy are strongly affected by integrin-mediated interactions with the extracellular matrix (ECM) in the tumor microenvironment. Indeed, altered expression levels of various integrins has been associated with poor differentiation, increased metastasis, and decreased overall and recurrence-free survival after radio- or chemotherapy in different types of cancer. Recent evidence indicates that the role of specific integrins in cancer progression and treatment response depends on the spectrum of oncogenic mutations present in cancer cells. In this PharmSight, we discuss several examples of such cross talk between integrins and oncogenes that may point to new avenues for cancer therapy.

Keywords: Integrins; Oncogenes; Extracellular matrix; Tumor microenvironment; Cancer therapy

Introduction

Cellular interactions with the ECM regulate survival, proliferation, and differentiation. These interactions are mediated by members of the integrin family of transmembrane adhesion receptors (1). Out of 18 α and 8 β subunits, 24 integrins are known to be formed in humans, with the different αβ combinations defining ligand-binding specificity. Following ligand binding, integrins cluster and organize multi-protein complexes termed cell-matrix adhesions that connect to the actin cytoskeleton through a variety of cytoskeletal linker proteins. Cell-matrix adhesions also contain signaling intermediates, including protein and lipid kinases, phosphatases, and small guanosine triphosphatases (GTPases) as well as their substrates. Concentration of these proteins may explain the fact that signaling by various other receptor classes is augmented in the context of integrin-mediated adhesion (2).

Anchorage-independent growth is a hallmark of malignant cells, suggesting that cancer cells might, to a large extent, have become independent of integrin-mediated adhesion. However, specific alterations in the expression levels of integrins are associated with tumor growth, differentiation, and metastasis suggesting that integrins can in fact play a decisive role in the malignant behavior of cancers. Indeed, in vitro studies and animal models for human cancer have implicated integrins in various steps of cancer development and malignant progression (3).

In addition, the expression levels of certain integrins correlate with overall and recurrence-free survival after radio- or chemotherapy suggesting that integrins can help protect cancer cells against the cytotoxic effects of these treatments. Chemotherapy and radiotherapy relies on induction of DNA damage in cancer cells, which, if not repaired can lead to the induction of apoptosis (4). Adhesion to an ECM substrate can enhance resistance to chemo- and radiotherapy, a phenomenon known as Cell Adhesion Mediated Radio Resistance or CAM-RR, and Cell Adhesion Mediated Drug Resistance or CAM-DR (5). This process can mediate drug resistance of small cell lung cancers: these cancers are surrounded by ECM that engages β1 integrins and thereby stimulates protein tyrosine kinases that protect against

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apoptosis (6). Ligation of β1 integrins appears to play a general important role in pro-survival signaling that protects against radiation and chemotherapy in non-transformed and various types of cancer cells (7, 8, 5). Targeting such cancer cell-ECM interactions might be a realistic therapeutic avenue: antibodies against β1 integrins can significantly reduce the radiation dose needed for breast cancer growth inhibition and apoptosis in rodent models (9).

Recent studies show that oncogenes can make use of the ability of certain integrins to form signaling platforms to enhance their malignant potential and resist anticancer therapy. Here, we will we focus on such examples for two integrins: i) cross talk between integrin αvβ3 and the Src oncogene and ii) cooperation between integrin α6β4 and the VEGF-R, bFGF-R, ErbB2, and MET receptor tyrosine kinases (RTKs).

Cooperation between integrin αvβ3 and c-Src in cancer

The ability of integrins to regulate the activity of Src family kinases (SFKs) is important for the dynamic interactions between proteins clustered in cell-matrix adhesions, for cross talk between integrins and other transmembrane receptors, such as RTKs, and for downstream signaling to proliferation and survival (10). The prototype SFK, c-Src is maintained in an inactive, closed conformation through two types of intramolecular interactions: binding of the SH2 domain to the phosphorylated C-terminal tyrosine 530; and binding of the SH3 domain to the polyproline stretch in the linker region. Dephosphorylation at tyrosine 530 or competitive interactions with the SH2 domain, as well as competitive interactions with the SH3 domain contribute to the unfolding and activation of Src molecules. Integrin clustering and cellular tension drives the autophosphorylation of focal adhesion kinase (FAK), creating a binding site for the Src SH2 domain that may activate Src at cell-matrix adhesions (11). In addition, a selective direct interaction between the SH3 domain of Src and the integrin-β3 cytoplasmic domain can also contribute to Src activity (12). These interactions promote a "primed" state of c-Src that can be fully activated by cross-phosphorylation of the tyrosine 419 residue in the activation loop.

Increased expression and activation of c-Src is associated with poor prognosis in various types of cancer (13). Increased levels of c-Src and binding of overexpressed RTKs to the c-Src SH2 domain may enhance c-Src priming. In addition, C-terminal truncating mutations in the *SRC* gene stabilizing a primed conformation of c-Src have been detected in colon and endometrial cancer though such mutations appear to be rare (13, 14, 15). Increased levels of αvβ3 have also been described in those same types of cancer. To investigate if the association between αvβ3 and c-Src modulates the oncogenic potential of c-Src mutants we made use of epithelial cells expressing either H-Ras^{GV} or c-Src^{YF} oncogenes in the context of different integrin subunits. We found that the activity of primed c-Src is strongly enhanced in the presence of αvβ3, irrespective of the presence of β1 integrins. This has strong consequences for Src-induced tumor growth: while transformation by H-Ras was independent of the integrin expression profile, the primed c-Src mutant could induce anchorage-independence and tumor growth only in cells expressing high levels of αvβ3 (16). Notably, increased Src activity also leads to a migratory phenotype, characterized by reduced adhesiveness and formation of podosomes, highly dynamic cell-matrix adhesions. Such features are believed to promote the metastatic potential of cancer cells. We observed that αvβ3 can protect cells against Src-mediated inhibition of cell spreading, while leaving the formation of podosomes intact (17). Thus, the interaction between c-Src and the integrin β3 subunit not only regulates the activity of c-Src during cell adhesion but also contributes to the c-Src activity that promotes tumor growth and may drive tumor progression.

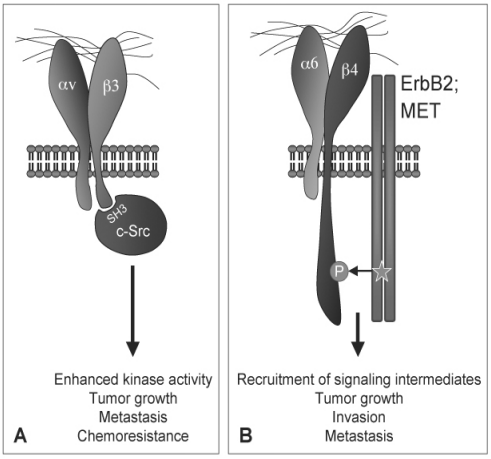

Figure 1. Schematic representation of cooperation between (A) integrin αvβ3 and c-Src and (B) integrin α6β4 and ErbB2 and MET RTKs. See text for details.

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We have used the same cellular system as described above to investigate the role of different oncogenes in the context of different integrins in sensitivity to chemotherapy. While the c-Src^{YF} oncogene sensitized the cells to a number of genotoxic chemotherapeutics in the context of β1 integrins, such sensitization was not observed in cells expressing H-Ras^{GV} or in cells expressing high levels of αvβ3 together with Src (18). Accumulation and sensing of DNA damage was similar for all investigated integrin/oncogene combinations and depletion of p53, an important mediator of the classical DNA damage response did not protect against apoptosis in the sensitive variants. Notably, besides DNA damage compounds such as cisplatin also induce an ER stress response and it turned out that Src sensitizes cells to this pathway when the expression of αvβ3 is low (18). These findings indicate that in addition to its role in normal adhesion signaling, the interaction between αvβ3 and c-Src can modulate tumor growth and chemoresistance.

Recently, it was reported that integrin αvβ3 is expressed in subpopulations of human carcinomas of the pancreas and breast where it may promote anchorage-independent growth through recruitment and activation of c-Src (19). In agreement with our findings using primed c-Src (16), cells expressing low levels of αvβ3 or endogenous c-Src failed to survive in the absence of adhesion. Moreover, silencing c-Src expression or pharmacological inhibition of c-Src suppressed spontaneous metastasis of αvβ3-expressing cells without affecting migration or invasion. Thus, the interaction between αvβ3 and c-Src supports survival signaling that can enhance primary tumor growth, chemoresistance, as well as metastatic spread. This interaction may be targeted using Src inhibitors. It is questionable if αvβ3 antibodies would be effective since this integrin supports Src activity in an adhesion-independent manner although it cannot be ruled out that binding of ligands in solution or trapped between the cells within clusters is important.

Cross talk between α6β4 & VEGF-R/bFGF-R in tumor angiogenesis

The α6β4 integrin with its unusually long cytoplasmic β4 tail is a receptor for laminin-5 within basement membranes that resides within a subtype of cell-matrix adhesions termed hemidesmosomes (20). This integrin interacts with different oncogenic RTKs and has been implicated in tumor progression and tumor angiogenesis (21). Angiogenesis, the formation of blood vessels driven by growth factors such as VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor), is a driving force behind tumor growth and metastasis, providing oxygen and growth factors to the tumor cells and an entry point for spread to distant organs (22).

It is thought that several integrins play a role in tumor angiogenesis through their function in ECM adhesion, cell survival, proliferation, and migration (23, 24). Angiogenic factors such as VEGF and bFGF increase the expression of endothelial integrins (25, 26), and inhibition of integrin function can interfere with angiogenesis (27). At the basal surface of endothelial cells of tumor vessels α6β4 and laminin-5 are expressed. A C-terminal deletion in the β4 subunit does not impair the adhesive function of α6β4 to laminin-5, nor the formation of hemidesmosomes. However, mice expressing such a deletion mutant are defective in i) bFGF-induced vascularization of matrigel plugs, ii) VEGF-mediated angiogenesis in a retinal hypoxia neovascularization model, and iii) angiogenesis towards subcutaneously injected tumor cells (28). This defect appears to be related to impaired ERK and NF-κB signaling implicating α6β4 as an important signal modifier in tumor angiogenesis. Mechanistically, it is currently unclear how β4 signaling augments the response to VEGF and bFGF in endothelial cells.

Cross talk between α6β4 & ErbB2 in tumor growth and metastasis

In mammary epithelial cells growing in three dimensional cultures, the interaction of α6β4 with the basement membrane surrounding the cysts promotes polarization and NF-κB-mediated survival signaling. This pathway can protect normal and transformed mammary epithelial cells against chemotherapy induced apoptosis (29). Invasive mammary carcinoma cells produce laminin-5 which, through ligation of α6β4 bypasses the need for basement membrane anchorage and polarity for this survival pathway (30). In an in vitro matrigel culture model the oncogene ErbB2, which is frequently overexpressed in breast tumors, could promote proliferation leading to luminal repopulation in epithelial acini where normally apoptosis occurs due to the absence of α6β4-mediated polarity as described above. Such filled acini resemble structures found in vivo in mammary carcinomas (31).

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EGF-R, ErbB2, Met, and Ron associate with α6β4 and stimulation of these RTKs leads to SFK-mediated phosphorylation of the β4 subunit and subsequent disruption of hemidesmosomes resulting in enhanced cell migration (32, 33, 34, 35, 36). Integrin α6β4 is upregulated in various tumor types (37) where it may act as a signal modifier outside of hemidesmosomes since enhanced RTK and/or SFK activity is expected to cause extensive β4 phosphorylation. ErbB2 signaling is enhanced when associated with the β4 subunit and this has been shown to underlie various aspects of breast cancer progression (36, 21): deletion of the β4 cytoplasmic signaling domain in mice led to a reduction in growth, invasion, and metastasis of ErbB2-driven mammary tumors. Importantly, this deletion sensitized existing tumors to regression by ErbB2 inhibitors (36). This suggests that breast cancer patients who fail to respond to ErbB2 inhibition might benefit from additional inhibition of β4 signaling.

Cross talk between α6β4 & MET in tumor invasion

Integrin α6β4 can interact with MET, the receptor for hepatocyte growth factor (HGF), which leads to tyrosine phosphorylation of the β4 cytoplasmic tail. This phosphorylation serves as a platform for the recruitment of Shc and PI3K and can accelerate signaling via the Ras/MAPK pathway. The constitutive physical interaction with α6β4 has been reported to promote HGF-induced carcinoma cell invasion, growth, and protection from apoptosis through a mechanism that is independent of the adhesive function of α6β4 (34). Besides the adaptor function for Shc/PI3K, phosphorylated integrin β4 can also recruit the tyrosine phosphatase Shp2, which dephosphorylates the inhibitory C-terminal tyrosine in Src (see above) leading to Src activation and subsequent stimulation of the Ras/MAPK cascade in support of anchorage-independent growth of breast carcinoma cells (38). It has also been shown that the overexpression of β4 can greatly enhance MET-mediated transformation of rodent fibroblasts in an adhesion independent manner, further defining a role for the signaling domain of β4 in cooperation with the MET oncogene (39). It should be noted that there is also evidence against a critical role for α6β4 in MET signaling. Carcinoma cells lacking β4 exhibited HGF-induced invasion that was dependent on MET and although the expression of α6β4 increased the invasive response to HGF, invasion in response to other, MET-independent stimuli was increased to the same extent, thus arguing against a specific interaction between α6β4 and MET (40).

Concluding remarks

The findings that certain oncogenes make use of certain integrins to drive tumor growth and progression to metastatic disease open up possibilities for targeted cancer therapy. Carcinomas in which αvβ3 and c-Src are (over)expressed may be more effectively treated by combining classical chemotherapy with small molecule Src inhibitors. Antibodies targeting αvβ3 may not be helpful if indeed the integrin augments c-Src activity in a fully ligand-independent manner. This would have to be further tested in animal models. Specifically targeting the physical interaction between the c-Src SH3 domain and the β3 cytoplasmic tail, although at present impossible, might be highly effective to sensitize cancers to radio- or chemotherapy. In carcinomas where the integrin β4 subunit is strongly expressed a number of RTKs that promote growth, metastasis, and therapy resistance may be dependent on the signaling platform that this integrin forms. In those cases where ErbB2 or MET activity is a driving force but patients fail to respond to inhibitors targeting these RTKs, interfering with β4 signaling or with the interaction between β4 and RTK may prove beneficial.

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Conflicts of Interest

No potential conflicts of interest to disclose.

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