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Growth Factor-Induced Cell Motility in Tumor Invasion

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Tumor progression to the invasive and metastatic states dramatically enhances the morbidity and mortality of cancer. Rational therapeutic interventions will only be possible when we understand the molecular mechanisms governing the cell behavior underlying this transformation. For invasion, a subpopulation of tumor cells must recognize the extracellular matrix barrier, modify the barrier, migrate through the barrier, and then proliferate in the adjacent but ectopic locale. Prevention of any one of these steps would prevent invasion, but determining the most sensitively dysregulated step should provide the most promising therapeutic index. In many invasive tumors, upregulation of active motility is stimulated by growth factor receptor signaling, the EGF receptor being the most frequently implicated. Two key downstream molecular switches, PLCγ and m-calpain, are required for growth factor-induced motility but not basal, matrix-stimulated motility. Inhibition of either of these enzymes blocks in vitro and in vivo invasion of prostate, breast, and bladder carcinomas and glioblastomas. These represent novel and potentially selective targets for drug development. Future advances in the imaging of tumors in animals and ex vivo organ culture systems should provide additional new targets.

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Tumor invasion into and destruction of adnexa account for significant morbidity and mortality in a variety of tumors, particularly glioblastomas and carcinomas of the prostate, bladder, head and neck, and esophagus. These primary tumors are accessible for debulking by surgical and radiological means but local extension beyond the physiological borders render these approaches impotent or engender significant adverse effects and poor outcomes in themselves. Limiting further invasion may yield palliative benefit, delay further tissue destruction, or even stabilize the disease at an advanced but manageable stage. Thus, the control of invasion per se is an appropriate goal for cancer treatment, to be employed in conjunction with other approaches that target tumor cell proliferation. However, to target this aspect of tumor progression rationally, greater understanding of the operative molecular controls is required.

Growth factor receptors originally were linked to tumorigenesis, as a number of retroviral oncogenes were found to derive from peptide growth factors and their receptors; the primary examples were the v-erbB/EGF receptor and v-sis/PDGF. In the immediate post-proto-oncogene era, a wide variety of human tumors were found to overexpress this class of signaling molecules, the epidermal growth factor (EGF) receptor (EGFR) being the most frequently identified (1). However, these early studies in all likelihood underestimated this relationship since they targeted gene amplification and steady-state protein levels. The vast majority of epithelial cells express EGFR while producing ligands for this receptor, primarily EGF and TGFα, though these are spatially segregated by cell polarization of receptor to basolateral surfaces and ligand released at the apical membranes to prevent autocrine signaling (2, 3). When the epithelium becomes dysplastic and neoplastic with weakened cell–cell junctions, the segregation is lost and inappropriate autocrine signaling is enabled (4). As receptors and ligands are actively internalized and degraded upon binding and receptor activation, misleadingly low receptor protein levels may be found at steady state for highly active signaling loops (5). This situation was the reason for earlier controversies in prostate cancers in which various reports failed reproducibly to detect EGFR; however, examination of mRNA

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species demonstrated high levels of both receptor and ligands (6). Thus autocrine signaling through the EGF receptor system is likely a common occurrence in carcinomas.

The prevalence of upregulated EGFR signaling in tumors did not initially provide insight into how this might contribute to tumor development. Upon closer examination of the clinical data, a number of studies demonstrated that EGFR overexpression correlated not with proliferation and initial tumorigenesis but, rather, with tumor progression to invasion. Sentinel examples include glioblastomas, in which half or more of the invasive tumors present upregulated EGFR compared to almost none of the non-invasive gliomas (7, 8). In one study of bladder carcinomas, most (21/24) of the invasive but few of the superficial (7/24) tumors overexpressed EGFR (9). High levels of EGFR were visualized in the regions of the tumors that were actively invading the underlying musculature while the superficial aspects of the tumors expressed lower, physiological levels of EGFR (10). In a series of gastric carcinomas, approximately one-third of the invasive tumors (38/130) overexpressed EGFR, whereas none of the early superficial tumors (0/26) similarly upregulated this system (11). Thus, EGFR signaling was hypothesized to contribute to the invasive phenotype.

To simplify matters thoroughly, in order to invade, a subpopulation of tumor cells must acquire the abilities to (i) recognize the extracellular matrix (ECM) barrier, (ii) modify the ECM barrier, including proteolytic degradation, (iii) actively migrate through the matrix space, and (iv) proliferate in the ectopic but adjacent site (12). It is not required that all of these are upregulated during tumor progression, since a basal level of the first three occurs in localized tumors and even in normal tissue. Thus, while any of these properties could be, and have been targeted to block invasion, determination of one that might be most problematically dysregulated to drive invasion could provide insight regarding which might provide the greatest therapeutic index as a target.

CELL-MATRIX INTERACTIONS

Paradoxically, the initial step of altered adhesion is both the most elucidated and least dissected to date. One reason for this is that the action of recognizing the barrier matrix is not passive but induces changes in cell behavior, often inducing the other properties needed for invasion. A second reason is that the effects of matrix may be non-monotonic or -monophasic for even a singular property, so that prediction of even the qualitative effect of altering a recognition effect is difficult. A third reason is that the cell-matrix interaction is highly dynamic. In poorly differentiated and invasive carcinomas the matrix changes, including upregulation of fetal and wound response proteins such as tenascin-C and laminin-5 (13, 14). The re-emergence of these proteins provides new sites for interactions and with different integrins and other adhesion receptors. For all these reasons, cell-matrix interactions in motility and invasion are difficult to understand even qualitatively.

Even the same cellular receptors for the matrix qualitatively alter their behavior with tumor progression. One well-documented example is the $\alpha 6\beta 4$ integrin, which normally functions as an anchor to the substratum in well-differentiated epithelia (15). In invasive carcinoma cells, $\alpha 6\beta 4$ relocalizes and concentrates at the leading edge of the cells. These receptors now drive motility forward, even signaling from laminin-5, which is soluble, rather than matrix embedded (16). Thus, this integrin changes from primarily an anchoring function to one of active signaling. Furthermore, even the same integrin-ligand pair function in qualitatively and quantitatively distinct ways depending on the geometry of ligation. It is well established that cell shape as dictated by the adhesion footprint impacts on cell function (17) and that the extent of integrin clustering dictates which signals are induced (17). We have shown that cells can adhere to individual integrin ligands, but only microclusters will support both integrin-mediated haptokinesis and growth factor-induced chemokinesis (18).

The next step in the invasion program is to alter the matrix to allow passage. The major aspect of this process involves extracellular proteases (19). Inhibitors of metalloproteinases (MMP) block tumor progression but the underlying mechanism for this is undergoing reappraisal (20). It is unlikely to be simply matrix degradation, as non-invasive tumor cells and even normal cells express high levels of many MMPs; moreover, cell migration is not necessarily enhanced by matrix breakdown since adhesive traction can be lost. Rather, the membrane-tethered MMP (intrinsic transmembrane MT-MMP and secreted MMP bound to cell surface receptors) might function itself as a motility-promoting adhesion receptor at the tip of an invadipodia (21, 22), or the MMP might function to create signals for cell motility either by cleaving autocrine growth factors (23), releasing sequestered growth factors from the matrix, or uncovering cryptic signals in matrix components (24, 25) (Fig. 1).

This last possibility is an especially exciting new avenue. It had earlier been reported that matrix components might activate receptors that are members of the family of growth factor receptors with intrinsic tyrosine kinase activity (RPTK). The most striking example of this is the discoidin domain receptors that bind fibrillar collagen (26, 27). Another matrix component, decorin, has been shown to activate EGFR (28). This concept potentially has been extended recently with the report that some of the EGF-like repeats in the onco-fetal and wound repair protein tenascin-C can signal through the EGF receptor (25). These monomers are of very low affinity with a Kd in excess of 10 μM. However, this protein is produced as a hexamer with 84 repeats clustered centrally, enabling multivalent binding (14); When expressed multivalent on

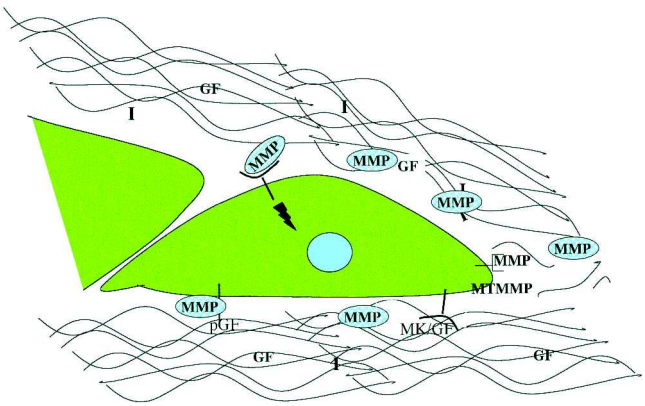

Fig. 1. Metalloproteinases (MMP) and other secreted proteases may promote motility by a number of avenues. The MMP were first hypothesized to digest the matrix (lines) at the front of the tumor edge to allow passage; this may be due to secreted and soluble MMP, secreted but receptor-bound MMP, or the membrane tethered MMP (MTMMP). Alternatively, MMP may signal through cell surface receptors (lightning bolt), liberate membrane tethered pro-growth factors (pGF), digest invasion inhibitors (I) in the matrix, liberate matrix-sequestered growth factors (GF), or uncover matrikines (MK/GF) that signal through members of the growth factor receptor superfamily.

beads the individual EGF-like repeats can form stable complexes with EGFR. This type of matricrine signaling through growth factor receptors greatly expands the regulatory functions of matrix components. However, this complexity of simultaneous adhesion, degradation and signaling confound clear analyses of the contribution of this stage to tumor invasion.

CELL MOTILITY

Cell motility should be central to tumor invasion since to grow in the ectopic site the cells must transmigrate a physiological barrier, ECM and, in the case of prostate and bladder carcinoma, enveloping muscular layer. However, in order directly to address this hypothesis, regulatory molecules need to be identified that are specific to the process of cell motility. Thus, inhibition of these signaling pathways should not affect other required cell properties such as proliferation.

Motility can be considered as a cyclic series of the biophysical processes of extension, front adhesion, transcellular contraction and rear release (29). All of this is preceded by reorganization of the actin cytoskeleton that enables normally cuboidal epithelial cells to assume the asymmetric fusiform shape that enables active locomotion (30). These steps are likely controlled by external signals acting through separable signaling pathways. Over the past decade, a large cohort of investigators has begun to identify key switches for motility in response to adhesion and growth factor signals (31) (Fig. 2). Growth factor-induced motility differs from basal adhesion-mediated motility not

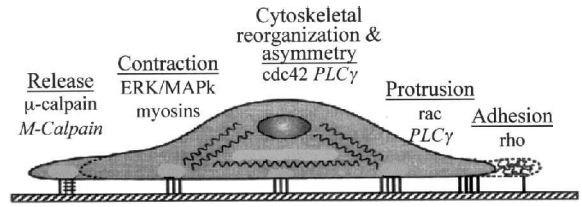

Fig. 2. Biophysical dissection of cell migration highlights key molecular switches. Cell movement can be considered as a series of distinct but concerted events, each of which involves separate signaling. As illustrated, each of these processes involves a unique set of effector molecules and signaling pathways. Required molecular switches for adhesion receptor-mediated motility are shown in block and those for growth factor receptor-mediated motility in italics, though the signaling separation may overlap somewhat. Taken with permission from Kassis et al. (57).

only quantitatively in generating a greatly enhanced rate but also in the fact that it is superimposed upon haptokinesis; while one might speculate that adhesion-signaled motility processes might be required (18), this has not yet been proven. Such a situation suggests that signaling pathways to growth factor-induced motility may have distinct switches, providing for a therapeutic window.

This has been borne out by our extirpation of two signaling pathways that appear highly selective for growth factor-induced motility. Briefly, activation of PLCγ is required for motility signaled by EGFR (32), PDGFR (33) and IGF-1R (34) but not integrins (35). PLCγ actuates motility by hydrolyzing PIP2 to mobilize gelsolin to sever and cap the actin cytoskeleton in the immediate submembrane region (36). This allows for the cytoskeletal reorganization necessary for cell polarization and continuous cytoskeletal plasticity required for the forward flow of the cell (37). Of importance for parsing events, PLCγ inhibition actually leads to increased proliferation not inhibition of growth by shunting the EGFR signaling towards that competitive cell response (38). Thus, PLCγ can be targeted as a motility-selective signal; in such a case other agents and/or approaches would need to be utilized to limit tumor cell growth.

A second key regulatory switch selective for growth factor-induced motility is the m-calpain isoform of this ubiquitous intracellular limited protease (39). Activation of this molecule is required for the lessened adhesion needed during tail retraction (40). The other ubiquitous isoform, μ-calpain, appears to be required during haptokinesis (41) and chemokine-driven motility (Satish et al. unpublished study, 2001). Currently, molecular inhibitors can differentially target these isoforms. Cell motility can be inhibited by pharmacological agents, such as ALLN, at levels that do not abrogate growth factor-induced proliferation. Thus, two switches that are selective for growth factor-induced motility provide targets to limit just this type of cell movement. This would minimize any deleterious side effects, since the integrin-mediated slow motility appears to be fully sufficient for homeostasis.

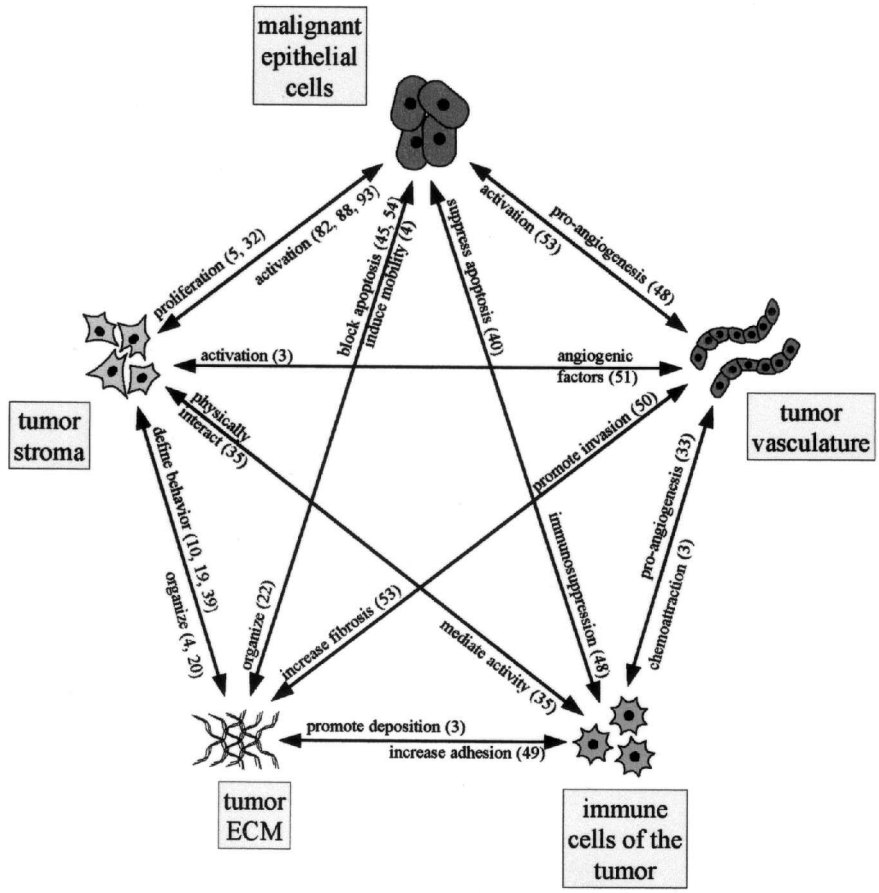

Fig. 3. Tumor progression is a multicellular field event involving matrix, and stromal and other support cells. The emerging concept of tumor multicellularity posits bi-directional effects of the neoplastic cells with the stromal cells, extracellular matrix, vasculature, and even immune response cells. Taken with permission from Radisky et al. (48).

With these tools, we could ask, at least for therapeutic targeting purposes, whether tumor invasion could be considered effectively as a disease of dysregulated EGFR-induced motility. In human prostate carcinoma, cell lines, overexpression of a motility-inducing EGFR construct but not a fully mitogenic but non-motile construct resulted in increased cell invasiveness in vitro and in vivo (42, 43). This invasiveness was due to autocrine EGFR signaling as inhibiting EGFR signaling with a selective pharmacological agent blocked the invasiveness of prostate, breast and bladder carcinomas (44). Pharmacological and molecular inhibitors of PLCγ prevented invasion of these cells into the diaphragm or other abdominal organisms when these tumor cells were grown intraperitoneally in athymic mice (43, 45). Such inhibitors also blocked invasion of glioblastoma cells into normal brain tissue in ex vivo model systems (46). This latter tumor type highlights another advantage of targeting the convergent molecular switches rather than the specific triggering receptor since it is an open question whether glioblastomas are driven to invade by EGFR, PDGFR, IGF-1R or other growth factor receptors. The glioblastoma cells' motility response to all three factors was similar prevented by inhibiting PLC. Importantly, initial studies demonstrated that inhibition of calpain also blocks tumor invasiveness in vitro. Thus, motility per se appears to be the key target rather than any particular molecule.

INVASIVE GROWTH

The definition of invasiveness is the ability of the tumor to grow outside the physiological confines. A number of threads of evidence suggest that this adjacent ectopic growth is likely to be somewhat distinct and probably less stringent than the growth needs for metastatic growth (47). In the main, it has become evident over the past few years that tumorigenesis and progression is a cell field event requiring changes not only in the cancer cell but also in the stromal cells and matrix (48, 49) (Fig. 3). Since invasive growth is contiguous with the orthotopic 'soil', it is conceivable that not only do the carcinoma cells migrate to the ectopic site but also the supporting stromal cells and matrix.

Still, growth outside the matrix barrier is critical, and may involve the same peptide factors that induce ortho-

topic growth (3, 50). These may be the same factors that also promote cell motility. Prostate cancer cell growth relies upon autocrine EGFR signaling, since blocking antibodies limit cell proliferation (45, 51). However, this is distinct from motility signaling, since inhibition of PLC does not limit cell proliferation. Such an autocrine EGFR signaling loop is also required for proliferation of a number of other tumor types such as breast cancer (52). One interesting hypothesis in this regard is that spatially restricted autocrine loops provide for physiologically appropriate cell homeostatic signaling, whereas loss of this restriction generates signals leading to motility (discussed above) and/or proliferation in inappropriate contexts (4). Thus, in this case at least, the same set of signaling elements promotes different cell phenotypes to accomplish two responses needed at distinct stages of invasion.

OPPORTUNITIES AND CHALLENGES

These studies provide a promising start to understanding tumor invasion as the first step towards defining therapies. We have focused on cell motility as a rate-limiting step since it is most clearly distinguishable, whereas the recognition and remodeling steps are intertwined also with motility. It must be emphasized that, at least in experimental models, inhibition of adhesion or extracellular proteases does block invasion (19). However, as growth factor-induced cell motility appears to be a re-creation of fetal and repair biology and distinct from homeostatic and immune response-related motility mediated by adhesion receptors, there seems to be the greatest opportunity for a large therapeutic index.

Much more needs to be understood before one can rationally prevent invasion and turn aggressive tumors into indolent life-long conditions. Advances are likely to be driven by findings that (A) utilize proteomics and post-proteomics to determine the activation status and location of key regulatory switches, (B) define the interplay between adhesion receptors and growth factor receptors in cell motility, and (C) parse the contributions of the non-carcinoma cells in tumor invasion.

These insights that may lead us forward to the next level of understanding will build on the technical advances in imaging and ex vivo organ systems. Tumor cell behavior can now be visualized in live, splayed animals (53–55). Already, such techniques have shown that progressive growth at the ectopic site appears to be the rate-limiting step in metastasis and have also shown active movement of metastatic cells towards blood vessels. Combining these initial forays with multicolored tagging of specific proteins should prove powerful in delineating key molecular functions. However, these model systems are limited by the short time span of observation in h and the difficulty in manipulating the tumors and conditions. As an intermediary step, ex vivo model systems will be key in studying invasion and metastatic seeding. An exciting possibility is a month-long viable liver system that can be visualized continually during this extended period (56). This is particularly germane to ectopic growth studies as the liver is a common site of metastatic growth.

Unfortunately, one key stumbling block that is inhibiting further developments in this field is the lack of a way to study invasion inhibition in people, in clinical trials. Currently, the accepted parameters for Phase II/III trials are geared towards tumor growth and thus are not applicable to therapies that limit progression without a concomitant effect on tumor size. Until clinical oncologists contrive means to determine effectiveness of invasion and metastasis inhibitors, we will not be able to apply this burgeoning knowledge to the benefit of our patients.

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