Spotlight on Cancer Cell Dormancy

Addressing the Role of Cell Adhesion in Tumor Cell Dormancy

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ABSTRACT

The dissemination of tumor cells prior to the surgical resection of early stage tumors poses a serious risk to the disease free survival of cancer patients. This risk arises from the latent capacity of these cells to form solid metastatic lesions after a prolonged period of dormancy, exacerbated by the fact that these cells are often refractory to adjuvant chemotherapeutic protocols. Ensuring the long term survival of cancer patients therefore necessitates an understanding of the mechanisms of tumor cell dormancy and the accompanying drug resistance. Experiments designed to compare the biological behavior of metastatic versus nonmetastatic variants of tumor cells provide evidence that there exists a phenomenon of single-cell dormancy which may depend on a reciprocal dialogue between the tumor cell and the tissue microenvironment. Through a combination of 3-dimensional cell culture technique and in vivo models investigators are now beginning to elucidate the molecular mechanisms underlying this phenomenon. Here we review the results of a series of experiments describing the role of cell adhesion events in dictating tumor cell behavior, including the balance between proliferation and dormancy, and the acquisition of drug resistance.

The diagnosis of premetastatic, stage 3 cancers, such as those of the breast or colon, is normally followed by surgical resection of the tumor burden as a first course of treatment. A subsequent period of disease-free survival, however, is often followed by a new round of tumor growth at or near the site of resection, or by the appearance of metastatic lesions in vital organs. Since relapse of malignant disease can occur after several to many years, it is assumed that there exists a prolonged period in which residual, disseminated tumor cells have the capacity to remain in a state of dormancy. Given that secondary lesions are often refractory to standard treatment protocols, the relapse of tumor growth is the single greatest cause of mortality from malignant disease. Efficacious adjuvant therapy therefore requires an understanding of the biological and molecular mechanisms underlying dormancy, as well as mechanisms of relapse and chemotherapy resistance.

One of the best characterized mechanisms behind tumor dormancy involves the lack of a tumor-promoting blood supply. In this case it is argued that a lesion will be limited to 2 mm in diameter until the activation of an angiogenic switch recruits the necessary vasculature. Experimental evidence is emerging, however, suggesting that there exist preangiogenic determinants of dormancy at the level of a single cell. For example, intravital imaging of poorly metastatic, GFP-labeled human and murine mammary carcinoma cells has revealed that these cells can disseminate to the liver of mice in spontaneous metastasis assays, yet fail to divide in the liver environment. The inclusion of a drug resistance marker in one of these experiments allowed the authors to recover dormant cells from the liver and reimplant them orthotopically into the mammary fat pad. The subsequent growth of these cells into solid tumors confirmed that they had indeed retained their proliferative and tumorigenic capacity while in a state of dormancy.

The experimental evidence that solitary, disseminated tumor cells can exist in a state of dormancy suggests that proliferation may depend on tissue context and the interplay of intrinsic cellular properties with those of the tissue microenvironment. In order to understand the molecular mechanisms underlying this phenomenon at a level facilitating therapeutic intervention, we must turn to the development and expansion of novel model systems, both in vitro and in vivo. The use of 3-dimensional (3D) cell culture systems, consisting of reconstituted basement membrane (rBM), has become a valuable tool in this regard. Through manipulation of BM constituents and cell adhesion receptors, the 3D culture matrix facilitates the investigation ex vivo of cell-matrix interactions, as well as the role of polarity in cell growth.
Using the rBM culture environment, for example, it was possible to induce a dormancy-like phenotype in spontaneously transformed human breast cancer cells. These experiments were performed on tumor cells which normally grew in the rBM matrix as disorganized aggregates devoid of polarized cell-cell adhesive structures. Through the application of an inhibitory anti-β1-integrin antibody, the authors were able to restore the differentiated phenotype of these cells. In contrast to the disorganized aggregates of transformed cells, the revertant population now formed well-defined tissue-like acini of properly polarized mammary epithelial cells. This phenotypic reversion included the restoration of cell-cell adhesive structures, the assembly of a proper BM, and restoration of the actin-based cytoskeleton. In addition, blocking the binding activity of the β1-integrin subunit resulted in a proliferative block both in vitro and following transplantation into nude mice.

These experiments provided a convincing demonstration of how altering cell-matrix interactions could "normalize" a tumor cell, regardless of the underlying genetic lesions predisposing the cell to form a tumor. By blocking β1-integrin binding activity the authors imposed a polarized phenotype on the cells which was dominant over oncogenic events such as elevated EGFR levels. In addition, the reversion of the transformed phenotype was found to be reversible, which is a hallmark of tumor cell dormancy. Following further investigation, the authors revealed a reciprocal dialogue between β1-integrin and other members of the cell signaling machinery, such as EGFR and MAPK. A role for β1-integrin in mediating proliferation through growth factor receptors such as EGFR explains in part how modulating cell adhesion events can attenuate tumor cell growth. The contribution of the 3D culture environment to a detailed understanding of proliferation and dormancy is highlighted by the fact that these reciprocal interactions were not detectable on plastic.

Further insight into the central role of β1-integrin in tumor cell proliferation was provided in a series of experiments designed to examine the behavior of HEp3 head and neck carcinoma cells in vivo. In these experiments, the authors demonstrated that the α5β1 fibronectin receptor occupies a pivotal position in a feedback loop involving uPAR expression, fibronectin matrix assembly and cellular proliferation. By recruiting the focal adhesion kinase (FAK), the α5β1 integrin heterodimer facilitates uPAR-dependent activation of the Ras-ERKMAPK pathway, inducing proliferation of the HEp3 cells in the chorioallantoic membrane (CAM) of chick embryos. In addition, β1-integrin-mediated fibrillogenesis and fibronectin matrix assembly was found to play a direct role in the inhibition of p38SAPK activity in this system. The integrin-mediated regulation of these kinases was found to have important physiological consequences, since a high ratio of ERKMAPK activity to that of p38SAPK was shown to promote proliferation of the HEp3 cells in vivo, while the converse resulted in a state of dormancy. As a result, dormancy could be induced in aggressive HEp3 cells by blocking expression or activity of uPAR, β1-integrin, FAK, or by disrupting the interaction between uPAR and β1-integrin. These observations may explain why highly tumorigenic populations of HEp3 cells express elevated levels of uPAR and α5β1, as well as exhibiting substantial fibronectin matrix deposition.

The results of these experiments provide an intriguing demonstration of how the phenomenon of cell adhesion can dictate tumor cell fate, by regulating a dialogue between the intracellular proliferative machinery and the tumor microenvironment (Fig. 1). We could envision, therefore, that variations in either integrin levels or BM constituents could alter the proliferative properties of disseminated tumor cells in human cancer patients. Tumor cells residing in a fibronectin-depleted tissue, for example, might be at a proliferative disadvantage, regardless of the activation status of growth factor receptors such as EGFR or HER2. Consistent with this hypothesis, the expression of unligated α5β1 on HT29 colon carcinoma cells results in growth suppression, both in vitro and in vivo, which can be rescued through the introduction of fibronectin. In addition, there is evidence that a basal level of fibronectin may be required for the survival of dormant human breast cancer cells which have metastasized to the bone.

Alternatively, the levels of the integrins themselves may modulate growth, even in a physiologically compatible microenvironment. A comparison of aggressive versus nonaggressive colon carcinoma cells, for example, has revealed a direct correlation between malignancy and levels of α5β1 integrin. In addition, experiments in our lab have shown that β1-integrin expression is required for the growth of mouse mammary gland tumor cells in the orthotopic environment.

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Figure 1. Experimental models suggest mechanisms by which the microenvironment can influence the fate of residual tumor cells. Dormant, solitary tumor cells have been identified in animals models through intravital imaging. Experiments performed both in vivo and in 3D cell culture systems have shown that the composition of the microenvironment, such as the presence of fibronectin (FN) or laminin (LN), can regulate the growth of the dormant cells. These extracellular matrix proteins serve as ligands for cell adhesion receptors such as β1-integrin (β1). Inhibition of β1 and associated molecules such as FAK and uPAR has been shown to override oncogenic events such as constitutive activation of the Her2/neu receptor tyrosine kinase. Inhibition of signaling through cell adhesion complexes may subsequently block tumor cell proliferation through downregulation of ERKMAPK activity and induction of a differentiation program. Growth-promoting changes in the microenvironment and/or adhesion molecules can result in resumption of tumor cell proliferation through high ERKMAPK activity, angiogenesis, and the establishment of macroscopic secondary lesions.
of the mouse mammary fat pad.\textsuperscript{25} Consistent with the role of β1-integrin described in other systems, the genetic ablation of β1-integrin from these cells did not result in cell death, but rather induced a state of non-proliferation resembling that of dormancy. As with the other experimental systems, the ablation of β1-integrin from these tumor cells resulted in a dramatic reduction in the levels of FAK phosphorylation.\textsuperscript{23} Although these cells had been transformed by the highly oncogenic polyomavirus mT oncogene, the inability of these cells to proliferate in vivo again highlights the central role of the β1-integrin/FAK signaling complex during tumor growth.

The induction of a dormant phenotype in vivo, by blocking β1-integrin activity, corroborates the results reported in the 3D cell culture system described earlier.\textsuperscript{13} The experimental approaches are indeed complimentary, whereby the 3D models offer detailed mechanistic explanations for the observations reported in the more physiological, in vivo context. In this regard, it is interesting that reversion of the tumorigenic phenotype in the 3D model involved restoration of a differentiated tissue phenotype.\textsuperscript{13} The results of those experiments suggest that dormancy in vivo may involve normalization of the transformed cells, rather than simply a proliferative block. A role for differentiation in tumor dormancy has indeed been demonstrated using a mouse model of liver carcinoma.\textsuperscript{24,25} When a conditionally activated allele of the myc oncogene is induced in the liver of these animals, they develop multiple, poorly differentiated lesions throughout the liver parenchyma.\textsuperscript{25} The attenuation of myc expression in these tumors, however, results in the rapid regression of the lesions into well-differentiated liver tissue. Importantly, the differentiated cells retain their oncogenic potential through a prolonged period of dormancy, since tumorigenesis could be rapidly restored following another round of myc expression.\textsuperscript{25}

Further experiments in the 3D culture system suggest that the role of differentiation in tumor dormancy may be problematic, since differentiated tissue structures may indeed be refractory to chemotherapeutic protocols.\textsuperscript{26} In this case, the authors demonstrate that forced expression of α6β4 integrin could restore well-differentiated growth properties to transformed human breast epithelial cells in the rBM. The phenotypic reversion of these cells again involved the transformation of irregular clusters of tumor cells into well-defined acini resembling normal breast tissue. Most importantly, however, these differentiated structures were found to be resistant to cell death by conventional chemotherapeutic agents, including cytochalasin B, paclitaxel and etoposide.\textsuperscript{26} The induction of drug resistance in these normalized cells, which was found to be due to a mechanism involving NFkB activation, was in stark contrast to the rapid cell death incurred in the phenotypically transformed parental population.\textsuperscript{26}

**CONCLUSION**

The experimental results described above suggest that a differentiated phenotype combined with latent oncogenic potential may explain the clinical phenomenon of disease relapse following chemotherapeutic adjuvant therapy. Progress in understanding these mechanisms of tumor dormancy has been made possible by the sequential addition of novel experiment approaches, combined with the complimentary nature of these approaches. Through the combined application of fluorescent labels and intravital imaging, for example, we are now able to observe dormancy at the level of the single cell, rather than the tumor burden as a whole. In addition, the manipulation of transformed epithelial cells in 3D culture systems has provided a great deal of insight into the precise mechanisms of dormancy, thereby offering potential explanations for the behavior of dormant cells in the in vivo models.

Scientifically, the results of these experiments are important in that they provide a striking demonstration of how the microenvironment can dictate the phenotype of an epithelial cell. In this regard, the ligation of integrin receptors to BM ligands has been found to be a critical determinant of tumor cell growth, regardless of underlying oncogenic events. Clinically, this phenomenon raises some very important and intriguing questions. What, for example, could be the source of the signals tipping the balance from dormancy to proliferation? Could it be as simple as elevated serum levels of uPA, which would induce proliferation through the uPAR/65β1 complex?\textsuperscript{27-29} Alternatively, could proliferation be promoted by changes in the fibrous component of the ECM, perhaps due to age or injury?\textsuperscript{30,31}

There will also be important questions regarding the timing and nature of chemotherapeutic intervention. While blocking FAK or ERKMAPK activity, for example, may inhibit the overall proliferation of a tumor, there is the risk that targeting these proteins in the adjuvant setting will drive the tumor cells into a state of latent, drug-resistant dormancy. As a result, the very timing and nature of the chemotherapeutic agent may compromise the disease-free status of the patient for whom it was designed to protect.

Finally, it is important to point out that evidence for single cell dormancy and the apparent role of cell adhesion in this phenomenon likely represents only one of several contributing factors to the clinical phenomenon of dormancy as a whole. The growth of microscopic tumors may be driven by changes in energy metabolism, the inflammatory microenvironment, as well as angiogenesis. It seems reasonable to suggest that different factors contribute to tumor growth at different stages, and that the induction of proliferation in latent tumor cells will subsequently require a vasculature to produce a tumor mass which ultimately threatens the host. The application and careful analysis of novel experimental models will hopefully contribute a great deal more to our understanding of this process.

**References**


