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# Microenvironmental Influences in Melanoma Progression

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**Abstract** An often overlooked facet of tumor biology research is the involvement of the surrounding tumor microenvironment. Increasing evidence is being presented to support a major role for stromal components in all stages of tumorigenesis including initiation, progression, and metastasis. Melanoma serves as a model for studying cellular and stromal interactions within the tumor microenvironment due to the array of cell types localized to these lesions. Here, we discuss the both the molecular mechanisms, as well as the extracellular and contextual input that contribute to melanoma progression. Special emphasis is given to the assorted cell types and their interactions with the extracellular matrix and adjacent cells. Melanoma progression also initiates development of intralesional hypoxic regions; the relative significance of hypoxia in disease is also addressed. Lastly, a number of laboratories are currently developing innovative strategies to study melanoma within a microenvironmental platform. These promising model systems and their potential for closing current gaps in knowledge of disease are reviewed. The development of such models holds translational value that cannot be achieved with most current systems. J. Cell. Biochem. 101: 862–872, 2007. © 2006 Wiley-Liss, Inc.

**Key words:** melanoma; microenvironment; hypoxia; modeling

Despite exponential advances in our understanding of the events that initiate and contribute to tumorigenesis, clinicians still lack the therapeutic tools to prevent, disrupt, or otherwise kill solid tumors. Some might argue that the success of targeted agents such as cetuximab (Erbitux™) or trastuzumab (Herceptin™) represent the progressive efforts of the basic research community; however, the truth is that for every single anticancer drug that successfully enters the clinic, there are dozens more compounds that fail to be effective in humans. Consequently, it is becoming increasingly apparent that much of the research performed in today's laboratories is inherently flawed, in terms of true translational value.

The overwhelming majority of basic research is currently performed using 2-dimensional (2D) platforms, yet solid tumors do not exist in single monolayers within the body. Is it surprising, then, that over 90% of drugs which exhibit preclinical activity are relative failures in human models? Probably not. The truth is that a solid tumor is much more than a mass of cells localized to a particular organ—they are heterogenously distributed cells that exist within an infrastructure of other cell types, as well as an array of stromal components. Together, these elements comprise a complicated network of entities, where the importance of each component is not necessarily hierarchical, but, instead, contributory towards the overall malignant phenotype.

Studies in 2D matrices undoubtedly have advanced our basic understanding of the intracellular events that support malignant transformation. However, the information garnered from such studies must be analyzed against a contextual background—the prominence of the tumor microenvironment must be considered. Taking into account the complexity of the tumor microenvironment should not only lead to the development of more effective therapeutics, but also boost the overall success rates of those drugs in the clinic.

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This review will discuss the diverse components of the tumor microenvironment and the factors that contribute to its malignant phenotype. Likewise, it is important to also provide an evaluation of some of the novel assays designed to better mimic the tumor microenvironment. Arguments will also be presented to support the use of 3D modeling systems for purposes of therapeutic development and overall understanding of tumor pathology.

## TUMOR MICROENVIRONMENT: A HISTORY LESSON

Since the discovery of oncogenes, the vast majority of basic cancer research has revolved around genetic mutations and cell signaling pathways that contribute to malignant transformation. Three decades later, the scientific community is slowly coming to the realization that neoplastic malignancies are even more complex than originally envisioned. Interestingly, the bane of modern-day cancer research may lie in our apparent disregard for studies done over a century ago; in 1889, Stephen Paget coined the "seed and soil" hypothesis based on his observations from autopsies performed from over 900 breast cancer patients. He postulated that metastatic breast cancer cells ("seed") will only colonize tissues ("soil") that are permissive to growth, while other tissues cannot support such growth and are subsequently devoid of metastases [Fidler, 2003]. As a natural extension of this hypothesis, Paget accurately predicted that the "soil" must harbor a collection of compatible factors that help potentiate settlement of the "seed" at that particular tissue locale. While Paget's study was primarily aimed at understanding the pathogenesis of metastatic behavior, he also inadvertently laid the groundwork for the studies of subsequent generations into the tumor microenvironment.

A century later, it appears that the scientific community is now beginning to realize the enormity of Paget's analyses. Modern cancer biologists generally accept the notion that cancer is not merely an accumulation of genetic mutations that lead to unharnessed cellular division, but is also largely dependent upon contextual cues from the surrounding tissue microenvironment. Consequently, it stands to reason that the collective focus of tumor biologists may be better aimed at understanding the assortment of cell types within a given tumor locus, as well as their homo- and heterotypic interactions with one another.

## MELANOMA AS A MODEL OF TUMOR MICROENVIRONMENT

Although melanoma is a disease that affects only 4% of persons afflicted with skin cancer, it accounts for nearly 80% of all deaths associated with skin cancer. Melanoma, like many other cancers, is often associated with an accumulation of genetic alterations that contribute to transformation; while these mutations are certainly instrumental in mediating tumorigenesis, there are a number of externally derived signals from neighboring cells that also must play a role. From a microenvironmental standpoint, melanoma represents a prototypical model that underscores the often overlooked aspects of stromal influences in cancer. Because there are an assortment of cell types found within a melanoma lesion (Fig. 1), it can be used to better understand the significance of the microenvironment within a solid tumor.

Within any given melanoma lesion exists several different types of cells including keratinocytes, fibroblasts, endothelial cells, and even infiltrating immunocytes. The impact of each of these cell types on tumor behavior is surely multi-faceted and, thus, merits independent discussion and analysis. The proceeding subsections begin to foray into the involvement of these cell types in mediating and substantiating melanomagenesis.

## MELANOCYTES AND MELANOMA CELLS

Melanocytes are derived from melanoblastic precursors that migrate from the neural crest to their final destination in the dermis and epidermis. Along the dorsolateral migratory pathway, the melanocyte encounters a series of microenvironmental influences which assuredly affect melanocytic gene expression and behavior. Both during and after migration, the melanocyte primarily communicates with adjacent cells through cadherin proteins. Cadherins are functionally related $Ca^{+2}$-dependent single-pass transmembrane proteins that become activated through homotypic interactions with cadherins on adjacent cells. The cadherin superfamily is comprised of classical cadherins (types I and II), protocadherins,

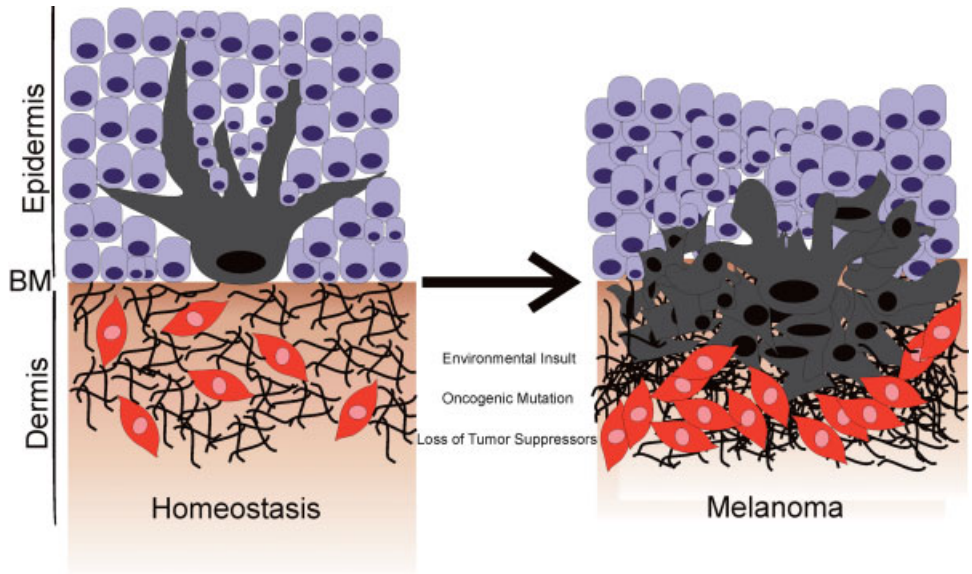


**Fig. 1.** Malignant transformation of the epidermal microenvironment. In a non-transformed state, keratinocytes (blue) modulate behavior of the resident melanocyte (black) population and the dermally located fibroblasts (red) support synthesis of the extracellular matrix. Upon malignant transformation, the melanocytes undergo phenotyping changes that enable invasion through the basement membrane (BM) into the dermal layer. The fibroblastic population also becomes activated, which results in increased growth factor production leading to a hyperproliferative microennvironment that supports growth of many cell types.

desmosomal cadherins, and other related proteins; each of these proteins harbor a number of functions including maintenance of cell-to-cell contacts, binding to the actin cytoskeleton, and induction of bi-directional cell signaling [Gooding et al., 2004]. The cadherins are comprised of three primary domains that encompass the extracellular, transmembrane, and intracellular portions of the protein. While the transmembrane domain serves to anchor the protein to the cell membrane, the extracellular domain facilitates homotypic binding with similar cadherins on neighboring cells. The intracellular motif interacts with another family of proteins, the catenins, to initiate cellular signaling. Encompassing the α-, β-, and γ- subtypes, the catenins are involved in mediating attachment to the actin cytoskeleton. Of these cytoplasmic-associated factors, β-catenin is an essential molecular switch that relays signals between the cell surface and nucleus. β-catenin, however, is part of a larger system of signaling proteins within the Wnt-signaling pathway [Larue and Delmas, 2006]. Together, this finely tuned signaling network is primarily responsible for the homeostatic phenotype observed in the non-transformed epidermal microenvironment.

The transition from melanocyte to melanoma involves a series of genetic and environmental changes, the primary of which is the loss of E-cadherin expression. In melanocytes, E-cadherin is generally associated with powerful cell-to-cell adhesion properties; as such, loss of E-cadherin leads to an invasive phenotype often linked with transformed cells [Li et al., 2001]. Not surprisingly, reintroduction of E-cadherin into melanoma cells renders them less motile, likely through a keratinocyte-regulated mechanism [Hsu et al., 2000]. The importance of E-cadherin expression in melanocytes, however, is not limited to cellular motility. Loss of melanocytic E-cadherin also leads to increased β-catenin signaling, which can initiate altered gene expression that supports malignant transformation [Heasman et al., 1994]. The biomechanistic machinery responsible for the loss of E-cadherin expression in melanocytes is reported to be via upregulation of negative repressor proteins, Snail [Batlle et al., 2000; Cano et al., 2000] and Slug [Bolos et al., 2003]; however, little is currently known about what might mediate control of expression and overall functionality of these regulators. The acquisition of such knowledge would likely provide much needed insight into the otherwise complicated pathophysiology of melanoma.

Integrins are cell-adhesion molecules that couple the extracellular environment to the cytoskeleton, while also transmitting

intracellular signals responsible for an assortment of cellular processes including survival, migration, invasion, and proliferation [Bauer et al., 2005]. Upon binding to the appropriate extracellular matrix (ECM) component, integrins form focal adhesions which contain clusters of signaling molecules that mediate the above-described processes. During melanomagenesis, melanocytes begin to display increased levels of the $\alpha_v\beta_3$ integrin concomitant with the loss of E-cadherin expression [Albelda et al., 1990]. Increased $\alpha_v\beta_3$ integrin expression is thereafter associated with the transition from RGP (radial growth phase) to VGP (vertical growth phase) melanomas. It is during this transition that melanoma cells acquire their ability to invade the basement membrane and begin to achieve metastatic potential. Likewise, $\alpha_v\beta_3$ integrin upregulates other genes associated with a malignant phenotype including Bcl-2 and matrix metalloproteinase-2 (MMP-2) [Brooks et al., 1996; Petitclerc et al., 1999]. A more recent report underscores a role for integrin signaling in conferring a rigid stromal compartment that is associated with more advanced tumors and, therefore, closely related to tumor aggressiveness [Paszek et al., 2005]. Perhaps most intriguing is the observation that normal cells placed into a foreign microenvironment initiate an integrin-dependent apoptotic response, indicating that tissue localization may override genetic profiles of various tumor cell types [Smalley et al., 2005]. Several integrin antagonists are now in development and are being evaluated in clinical trials for efficacy against melanoma and other malignancies [Tucker, 2006].

## KERATINOCYTES

Keratinocytes reside in normal skin in conjunction with melanocytes in a cellular formation known as the "epidermal melanin unit." In fact, there is a constant ratio of keratinocytes to melanocytes ($\sim$35:1) in the epidermal layer. It is thought that melanocyte homeostasis and overall number are held steady through interactions with the resident keratinocyte population. These interactions are mediated through homotypic E-cadherin binding between these cell types [Tang et al., 1994]. In vitro evidence also supports the regulatory role of keratinocytes for melanocytes; in two-dimensional culture conditions, melanocytes display altered gene expression patterns reminiscent of melanomas, including heightened expression of MCAM (Mel-CAM/CD146/MUC18) and $\alpha_v\beta_3$ integrins [Valyi-Nagy et al., 1993; Shih et al., 1994]. These genes are more often associated with melanomas, indicating that when interactions with keratinoctyes are lost, melanocytes can begin to act in a manner consistent with melanoma cells. However, co-culture assays with keratinocytes reverse these gene expression patterns, suggesting that keratinocytes possess the ability to regulate gene expression profiles of melanocytic cells. Keratinocyte-regulated expression of E-cadherin also affects the phenotypic behavior of melanocytes. One recent study outlines a regulatory role for Slug in E-cadherin expression in keratinocytes and also keratinocyte proliferation [Turner et al., 2006]; these observations demonstrate that keratinocyte-mediated control of melanocytes may be affected by the presence of regulatory molecules expressed in keratinocytes, such as Slug. Collectively, these data help underscore the importance of homotypic E-cadherin interactions between keratinocytes and melanocytes in the skin and support the notion that disease progression is controlled by variables independent of simple gene expression profiles.

## FIBROBLASTS

Fibroblasts are largely responsible for production of the extracellular matrix and are primarily localized within that ECM. Aside from providing structure in the form of the ECM, fibroblasts also supply the extracellular milieu with a steady stream of paracrine growth factors that are essential to maintenance of homeostasis in the epithelia.

For some time, the involvement of fibroblasts in tumorigenesis was thought to be minimal, playing a supportive role primarily through production of stromal components. More recent evidence, however, suggests that fibroblasts may not only support tumor formation, but might even potentiate it. In melanoma lesions, these cells produce large quantities of proliferative growth factors (i.e., IGF-1, bFGF, and TGF-$\beta$) only after the appropriate activating stimulus. In a previous publication, we outlined four primary steps required for fibroblastic involvement in melanomagenesis; those steps were (1) recruitment of fibroblasts to

the lesion, (2) activation and subsequent proliferation of the recruited fibroblasts, (3) "differentiation" of activated fibroblasts into myofibroblasts, and (4) production of ECM components that foster tumor advancement [Ruiter et al., 2002].

Fibroblastic involvement in tumor progression requires both resident fibroblasts, as well as those recruited from other tissue microenvironments. The infiltrating fibroblasts often display a phenotypic change that is marked by expression of α-smooth muscle actin; these myofibroblasts also divide more rapidly and exhibit increased ECM production [Bauer et al., 1979; Knudson et al., 1984]. The influx of myofibroblasts into the tumor microenvironment then induces a series of dynamic changes that each contribute to tumor progression. For example, myofibroblasts synthesize support matrices for the growing tumor, produce growth factors that enhance the proliferation of tumor cells, and also play a role in angiogenesis through interactions with neighboring endothelial cells [Smalley et al., 2005].

The involvement of fibroblasts in melanoma progression appears to be contingent on the stage of melanoma, as co-culture of fibroblasts with RGP melanoma cells actually represses tumor growth; conversely, the growth of metastatic melanoma cells is enhanced by the presence of fibroblasts. This observation supports the hypothesis that early stage melanoma cells are homeostatically controlled by adjacent cell types in melanoma lesions, whereas advanced melanoma cells acquire an ability to escape such control mechanisms. Another plausible explanation is that advanced melanoma cells secrete specific growth factors that then serve to activate fibroblasts, which then provide positive feedback in the form of other growth factors, such as IGF-1 [Satyamoorthy et al., 2001].

Oncogenic transformation of melanocytes leads to a cell that has distinct survival and proliferative advantages over its non-transformed counterparts. Further contributing to the malignant phenotype of melanomas are paracrine signaling loops that act to create an environmental niche that is conducive to tumor growth. Reducing the overall presence of such paracrine growth factors would likely help reduce or possibly eliminate aberrant growth of melanoma cells; however, accomplishing such an endeavor is nearly impossible when one considers the numerous cell types involved and the plethora of growth factors they can produce. The current line of thinking is that melanoma cells provide the first stimulus to activate fibroblasts via production of growth factors such as PDGF, bFGF, and TGF-β [Lazar-Molnar et al., 2000]. In turn, fibroblasts then produce a consequent series of growth factors which further supports the growth and proliferation of melanoma cells; together, these molecular events represent a bi-directional model of communication between cell types that potentiates tumor progression. A schematic of these molecular events and signaling cues is offered in Figure 2.

Interestingly, the effects of the array of growth factors which help propagate the malignant microenvironment in melanoma lesions vary from cell type to cell type. For example, melanoma-derived PDGF has no effects on nearby melanoma cells, as they do not express the cognate receptor for this ligand; however, fibroblasts are quickly activated and they soon respond by producing ECM proteins, in addition to IGF-1 [Berking et al., 2001; Satyamoorthy et al., 2001]. Not surprisingly, myofibroblasts also produce bFGF and endothelin (ET)-3, which can also contribute to melanoma progression [Ruiter et al., 2002]. These data underscore the importance of reciprocal signaling between neighboring cells within a given tumor and suggest that solid cancers result from a series of temporal cellular events, rather than merely a stepwise accumulation of mutagenic events.

Another growth factor involved in the melanoma microenvironment is transforming growth factor (TGF)-β. TGF-β is a paradoxical growth factor in that it can both suppress and promote tumor progression in the proper cellular context. TGF-β is thought to have growth-suppressive effects on epithelial and melanocytic cells within melanomas, but these inhibitory effects appear to dissipate as disease progresses [Bierie and Moses, 2006]. Another mechanism by which TGF-β may promote melanoma progression is via the production of key stromal components, such as collagen types VI, XV, XVIII, and tenascin, that are essential for tumor survival and metastatic potential [Berking et al., 2001]. Relatedly, increased ECM and related stromal components have also been associated with resistance to chemotherapeutic intervention [Sethi et al., 1999], as well as transformation of normal epithelial cells

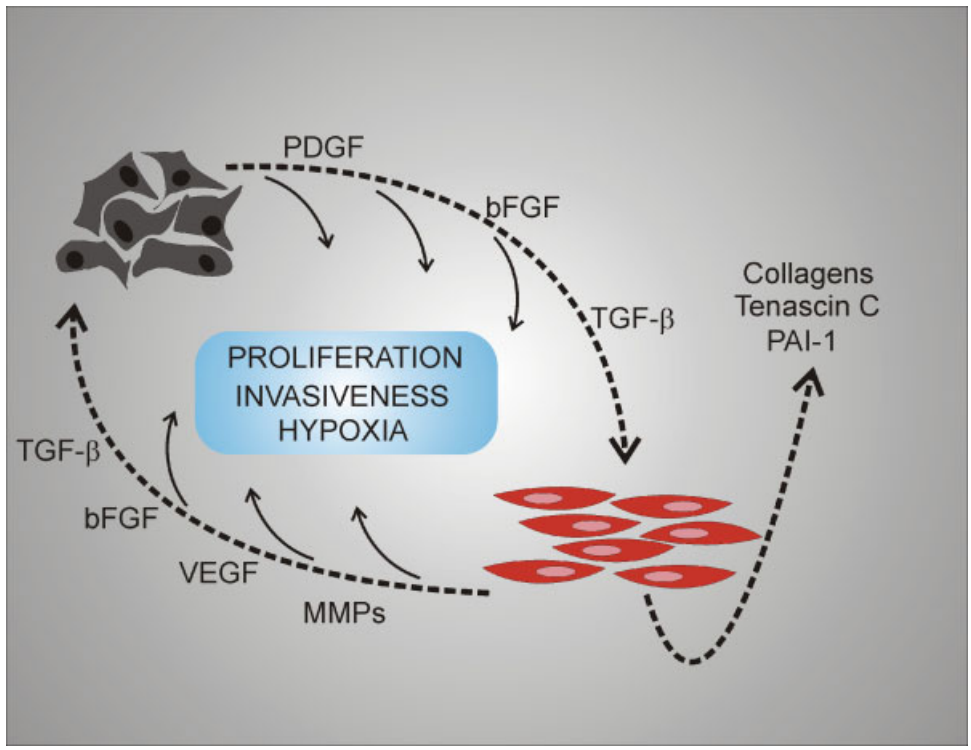


**Fig. 2.** Paracrine/autocrine-growth factor cycle relationship between melanoma cells and fibroblasts. After malignant transformation, melanoma cells (top left) acquire the ability to synthesize a number of growth factors, which are subsequently secreted into the extracellular milieu. Those growth factors then support the activation of neighboring fibroblasts (bottom right), as well as serve as a stimulus for infiltration of more distant fibroblasts. Activation of fibroblasts induces another round of growth factor production, which, in turn, enhances melanoma cell survival and proliferation. In time, this cyclical chain of events creates a favorable microenvironment for maintenance of the malignant phenotype. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Bhowmick et al., 2004]. In a prostate model, there is also evidence to support the notion that fibroblastic-mediated alteration of the ECM can cause epigenetic alterations in non-transformed epithelial cells [Pathak et al., 1997; Hayward et al., 2001]; thus, the effects of fibroblasts on the immediate microenvironment may not be limited to production of paracrine signaling factors.

## HYPOXIA IN THE TUMOR MICROENVIRONMENT

While the contribution of oncogenic mutations and growth factor production by diverse cell types cannot be overstated, these are certainly not the only factors that contribute to malignant progression. At a given tumor locus, the repertoire of resident cell types collectively create a microenvironment that becomes increasingly devoid of molecular oxygen; the appearance of these hypoxic regions further promotes the malignant phenotype by initiating neovascularization, supporting cell survival, and enhancing metastatic potential [Pouysségur et al., 2006]. The molecular events that regulate these processes are slowly becoming apparent, although much remains to be elucidated regarding the relative contributions of different cell types to the overall hypoxic response in solid tumors.

To reach growth dimensions of beyond 1 millimeter in diameter, a developing tumor requires the formation of a nascent vasculature which would potentially supply the tumor with oxygenated blood. However, the regulatory circuitry that govern such events are not always sufficiently operational within an early tumor; as such, the fledgling vasculature produced by growing tumors rarely is sufficient to support their metabolic requirements. In the epidermis, where conditions are already mildly hypoxic [Stewart et al., 1982], even the most minor of environmental influences can render a major tissue response. The cells of this tissue accommodate this apparent deficiency via a number of mechanisms, including through induction of hypoxia-inducible factor (HIF)-1 gene expression.

The HIF-1 heterodimer is often regarded as "the master regulator of oxygen homeostasis" [Semenza, 2003]. Regulation of HIF-1 begins at the level of the oxygen-sensitive α-subunit, which is rapidly degraded via ubiquitination under normoxic conditions. Depletion of molecular oxygen, however, causes stabilization and subsequent nuclear translocation of the α-subunit. Once localized to the nuclear space, the α-subunit dimerizes with the oxygen-refractory β-subunit; together, these protein partners bind to hypoxia-response elements (HREs) in the promoter region of various genes, including vascular endothelial growth factor (VEGF), whose conditional expression is associated with low oxygen.

Investigations into the role of hypoxia in melanomagenesis are only recently achieving significant advances. For example, the aforementioned loss of E-cadherin gene expression in epithelial tumors remained somewhat enigmatic before the discovery of the negative regulatory molecules, Snail and Slug [Cano et al., 2000; Bolos et al., 2003]. Since those initial discoveries, other reports have established that hypoxic regions, particularly those with high HIF-1 expression, are inversely correlated to E-cadherin levels [Imai et al., 2003; Esteban et al., 2006]. Relatedly, induction of HIF-1 transcriptional regulation was also associated with heightened expression of an ECM-modifying enzyme, lysyl oxidase (LOX) [Erler et al., 2006]. Collectively, these data provide correlational evidence that hypoxia negatively regulates E-cadherin levels via LOX-induced activation of Snail (Fig. 3). This signaling cascade, therefore, offers several attractive points of intervention for therapeutic purposes; providing proof of principle into this premise is that abrogation of LOX activity is sufficient to block metastatic behavior in breast carcinomas [Erler et al., 2006]. Moreover, HIF-1 activity is also associated with altered regulation of more than 30 genes, including a variety of genes involved in cell motility and invasion such as fibronection, vimentin, matrix

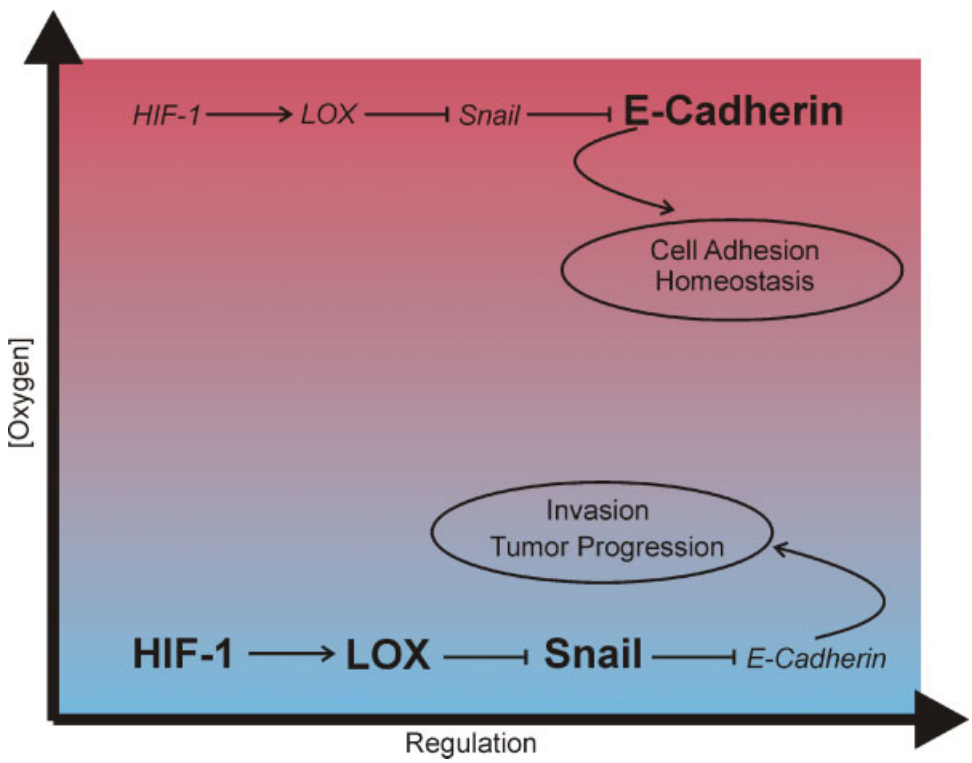


**Fig. 3.** Oxygen-regulated expression of E-cadherin. The immediate microenvironmental levels of oxygen affect melanoma behavior through modulation of E-cadherin expression. Under normoxic conditions (top), HIF-1 is quickly degraded by the proteosome and thus, is unable to act as a positive regulator of lysyl oxidase (LOX) transcription. When LOX levels are low (bottom), Snail activity is dormant and E-cadherin levels remain unaffected. However, as oxygen levels become limiting, HIF-1 is stabilized to upregulate many genes, including LOX. LOX can potentiate Snail activity, which acts to downregulate E-cadherin expression. In this diagram, catalytic activity and/or expression levels are reflected by the size of the font. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

metalloproteinase (MMP)-2, keratins-14, 18, and 19, urokinase plasminogen activator receptor (uPAR), and cathepsin D [Semenza, 2003]. Together, these data strongly argue that the hypoxic microenvironment that is established in advanced cancers contributes to tumor invasiveness.

The hypoxic microenvironment of the epidermis appears to affect melanocytic function at several levels. First, elegant studies demonstrate that constitutive Akt activity, a commonly encountered occurrence in melanomas due to loss of the PTEN tumor suppressor, can transform melanocytes only under hypoxic conditions; physiological levels of oxygen and/or inhibition of HIF-1 activity were reported to prevent this phenomenon [Bedogni et al., 2005]. The same study showed that hypoxia was also supportive of N-Ras-mediated melanocyte transformation, thus strengthening a role for normal oxygen levels in maintenance of skin homeostasis. Next, in addition to acting as a transcriptional regulator in the hypoxic response, HIF-1 activation also serves as a prognostic indicator of advanced stage disease in malignant melanomas [Giatromanolaki et al., 2003]. The preliminary data regarding the hypoxic nature of the melanoma microenvironment suggest that further exploration and experimentation is merited; such studies will certainly require more advanced and well-constructed experimental strategies to ensure an accurate recapitulation of physiological and architectural parameters of disease.

## MODELING THE MELANOMA MICROENVIRONMENT

It is becoming increasingly evident that research performed in traditional 2D matrices does not adequately take into account the microenvironmental influence of solid tumors. After decades of largely ignoring the stromal component of tumorigenesis, significant efforts are now underway to experimentally mimic the tumor microenvironment in melanoma [Berking et al., 2004]. Of particular note is the considerable progress made in modeling tumors of the skin in the past few years [Khavari, 2006]. Here, we discuss some of the more innovative experimental systems being used to study the complex intercellular interactions in melanoma.

One fairly straightforward approach to beginning to understand the behavior of melanoma cells in a microenvironment is through the use of multicellular tumor spheroids [Mueller-Klieser, 2000]. This strategy exploits the ability of many tumor cells to adhere to one another through the expression of various cell adhesion molecules. Under the appropriate experimental conditions, melanoma cells will form concentric, spherical structures that contain large proliferating cells in the periphery and smaller quiescent cells on the interior of the sphere. These spheroids can be implanted into a collagen-based matrix where they exhibit an invasive phenotype that is indicative of the stage of melanoma (i.e., RGP, VGP, metastatic melanomas) (Fig. 4) [Smalley et al., 2006].

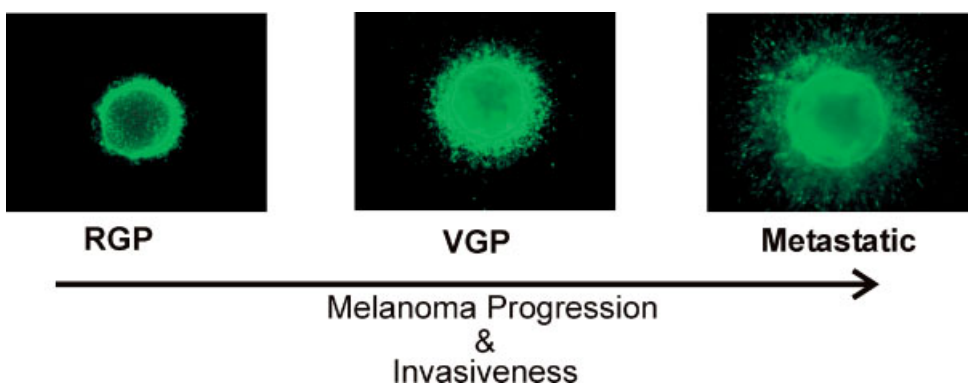


**Fig. 4.** 3D-modeling of melanoma spheroids accurately reflects invasive phenotype. Radial-growth phase (RGP), vertical-growth phase (VGP), and metastatic melanoma cells can be used to support the use of 3D models which best reflect in vivo behavior of melanoma. Spheroids were made from three different cell lines (WM35 = RGP, WM793 = VGP, and 1205Lu = metastatic) and subsequently embedded into collagen. After 72 h, each spheroid displays representative invasiveness that reflects the motile phenotype from the original tissue from where it was isolated.

This system has been further explored by interspersing fibroblasts into the collagen before imbedding a spheroid; fibroblasts generally respond by infiltrating the spheroid, as well as increasing proliferation and production of ECM proteins [Smalley et al., 2005]. This model system is also being heavily investigated for its improved predictive value for preclinical development of novel anticancer formulations [Santini et al., 1999]. Lastly, the Hendrix group has exploited a similar method using matrigel or collagen I, which resulted in the theory of vasculogenic mimicry, which describes the ability of melanoma tumor cells to form vascular channels independent of endothelial cells or angiogenic processes [Maniotis et al., 1999].

Another innovative melanoma modeling system that closely emulates the tumor microenvironment is through the use of tissue reconstructs. In this model, specific cellular types, such as keratinocytes, fibroblasts, and melanocytes (or melanoma cells) are isolated and subsequently redistributed within an appropriate assortment of matrix and cellular components to form "synthetic" human skin in a cell culture dish. When cultured over a period of time, the resultant synthetic skin can be orthotopically transplanted onto immunocompromised mice for in vivo studies. The advantages of such a model are multifaceted: first, it is possible to investigate the genetic events that are sufficient to transform normal cells (i.e., melanocytes) in the context of human tissue [Lazarov et al., 2002; Dajee et al., 2003]. Next, this system incorporates all cellular aspects of the tumor microenvironment, as well as allowing for formation of proper ECM and stromal components. Lastly, studying skin tissue reconstructs grafted onto recipient mice eliminate many of the complexities associated with interpreting data from more antiquated skin-related mouse experiments [Khavari, 2006]. It is also worth noting that such xenograft models will likely be exploited for validation of experimental therapeutics in the near future.

To date, attempts to closely mimic melanoma progression in mouse models has been subjective, at best. There are a large number of differences between murine and human skin that complicate most mouse models including distribution of hair follicles, localization of melanocytes, and overall tissue architecture [Khavari, 2006]. As such, it becomes difficult to extract accurate, species-relevant conclusions from animal models that do not account for these discrepancies. More recent attempts to develop better animal models, such as the tissue reconstruct xenografts, begin to take into account these problems and are quickly closing the gaps in knowledge. For example, an extremly innovative system was recently described which "humanizes" mouse skin, such that melanocytes become epidermally localized and thus, the skin is capable of achieving a tanning response [D'Orazio et al., 2006]; such a model will likely yield further insight into the cellular events that mediate conversion of melanocytes to melanoma in the near future. Transgenic mice have also been developed to conditionally express oncogenes or tumor suppressor knockouts exclusively in melanocytes upon systemic exposure to tetracycline-derivatives [Chin et al., 1999]. The results from these particular studies underscore the requirement for N-Ras in development and maintenance of melanoma lesions; analogous results from a similar model have also shown that B-Raf possesses this capacity, which further implicates the MAPK pathway in melanomagenesis [Hoeflich et al., 2006]. Despite the promising nature of these animal systems, they are not without limitations. For example, these models are primarily performed in immunocompromised mice and therefore preclude the involvement of any immunological cell types in pathogenesis and/or prevention of disease, which is certainly not representative of human melanomas [Lizee et al., 2006].

## CONCLUDING REMARKS

Melanoma is the most malignant of all skin cancers. While incidence is low compared to some other tumor types, the mortality rates of those afflicted with metastatic melanoma are among the highest of all cancers. The dismal 5-year survival rate is directly attributable to the lack of available therapeutic treatments for these patients. While a number of compounds are currently in preclinical and clinical trials [Flaherty, 2006], it is likely that the vast majority of them will fail to achieve significant clinical responses in most patients. This supposition begs the question, "Why do the overwhelming majority of anticancer drugs show preclinical promise, yet fall short of antitumor effects in patients?" The answer likely lies in the superficial nature by which many basic

biologists model disease. More technically advanced approaches, such as those discussed above, begin to address the shortcomings of the more traditional experimental strategies by accounting for the multiple cell types localized at a given melanoma lesion. Validating lead compounds in such models will likely lesson the number of failed therapeutic endeavors, while also acting as a stimulus for future drug formulations.

The microenvironmental influence on melanoma development can no longer be ignored in experimental strategies. The early data surrounding the impact of both stromal and cellular components of the tumor microenvironment on melanoma progression suggests that these factors drastically affect pathophysiological parameters of disease. While many questions regarding tumorigenesis still remain unanswered, it is without doubt that melanomas do not exist as singular cellular entities; as such, it becomes increasingly obvious that one must experimentally investigate the tumor as a heterogeneously comprised tissue rather than a mass of clonogenic cells. This paradigmatic shift will likely require time, however, as the complexities of efficiently modulating the tumor microenvironment preclude those scientists unwilling to part ways with their more traditional, less complicated experimental models.

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