

Review

Selective evolutionary pressure from the tissue microenvironment drives tumor progression

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Abstract

Cells grow within defined environmental niches and are subject to microenvironmental control. Outside of their niche, the environment is hostile, the normal cells lack appropriate survival signals which leads to anoikis. During tumor development and progression, malignant cells must escape the local tissue control and resist anoikis. The inherent genetic instability of tumor cells makes their phenotype very plastic, which changes under continuous environmental selection pressure. In this way the microenvironment drives the somatic evolution of the tumor. In the current review, we assess how this environmental selection pressure fits into the classical scheme of tumor progression.

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Keywords: Melanoma; Progression; Microenvironment; Stroma

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1. A classical view of tumor evolution

The classical approach to oncogenic development views cancer as being a solely genetic disease, whereby genomic mutations are acquired in a step-wise fashion, leading to uncontrolled cell growth, invasion into surrounding tissues and eventual metastasis. This model was based on the clinical observation that most tumors tend to become more aggressive over time, and that this process, termed “tumor progression”, tends to occur in a step-wise fashion (reviewed in [1]).

Cancer cells typically acquire somatic mutations in either oncogenes, tumor-suppressor genes or those genes responsible for maintaining genetic stability [2]. The consequences of mutations in either oncogenes or tumor suppressor genes are physiologically equivalent, in that they either stimulate cell growth or inhibit cell death, leading to an overall increase in tumor cell number [2]. With the exception of those individuals with germline mutations in these genes, mutations are normally acquired during a person’s lifetime, as the result of environmental exposure to carcinogens and certain chronic inflammatory conditions. The first acquired somatic mutations afford the early transformed cell some survival or growth advantage, allowing it to thrive at the expense of its neighbors [1,2]. The initial clones then undergo

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a round of clonal expansion, where the initial genetic changes are “fixed” into the genome of the tumor cells. The model then posits that further mutations are acquired through time, and that each mutation affording a survival or proliferative advantage is also fixed. It is thought that the multiple acquisitions of successive mutations ultimately allow the tumor to invade into local tissues and eventually to metastasize.

There is currently some debate over whether cancer progression proceeds in a linear fashion, through the sequential acquisition of mutations described, or whether the mutations leading to metastasis are acquired at an early stage in the oncogenic process [3]. The classic genetic model proposed by Vogelstein and colleagues would suggest a step-wise acquisition. However, a recent conceptual analysis seems to suggest that there are inconsistencies with this argument [3]. Instead, Weinberg and colleagues argue that the mutant alleles required for metastatic spread are in fact the same as those required for uncontrolled growth, and these are acquired at an early stage. If this new model is true it would mean that the eventual clinical course of a cancer is determined from the earliest stage of malignancy, and would have profound consequences for the clinical management of cancer. Current experimental data suggests that in human cells, perturbations in six genetic pathways (which variously control proliferation, cell cycle regulation, senescence, nutrient availability and genetic stability), are required for oncogenic transformation [4].

This reductionist view of oncogenesis sees the tumor cells existing in isolation, steadily acquiring mutations, with little interaction with their surrounding environment. Tumor cells are instead embedded in a matrix of structural extracellular proteins, surrounded by other cells, such as endothelial cells, fibroblasts, inflammatory and immune cells [5–7]. These multiple cell types make up the tumor tissue, and are in continuous dynamic interactions with their neighbors. Together, the cells generate myriad adhesive and chemical signals which converge to determine the metabolic, growth and survival behavior of the tumor cell. There is also evidence that this communication is bi-directional, and that the tumor can re-model the stroma to suit its changing needs [6]. Whereas, the stromal microenvironment is only directly responsible for acquisition of mutations in malignant cells under certain circumstances, such as chronic inflammation [8], it is undoubtedly responsible for determining the course of progression once these initial mutations are acquired. It is through these mechanisms that the microenvironment, in initially trying to suppress the growth of the early malignancy, exerts evolutionary pressure and may aid the selection of tumor cell clones which are ideally suited to survive in different, more hostile, tissue environments (Fig. 1). During the process of tumor initiation and progression, the relationship between the malignant cells and the microenvironment is known to change. As we shall discuss, the nascent cancerous cell must escape from the tight environmental control of the surrounding healthy tissue and through time can later recruit

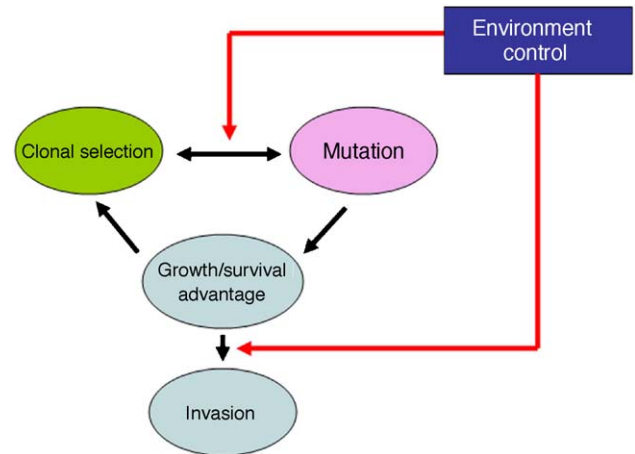


Fig. 1. Proposed non-linear model of tumor progression. In this model, the microenvironment exerts evolutionary pressure to select for clones that can escape mechanisms of control, leading to local invasion and eventually metastasis.

components of the stroma to aid and support its growth, survival and invasion.

2. The tissue microenvironment: the master controller of cell behavior

Under normal conditions, cells exist in a tightly controlled environmental niche where homeostatic processes control whether a given cell remains quiescent, proliferates, differentiates or undergoes apoptosis. This control is mediated by extracellular communication through growth factors and intracellular second-messenger signaling networks, as well as by physical interactions between a cell and its neighbors through cell–cell adhesion and cell–matrix adhesion [5–7,9]. In these normal tissues the cellular phenotype is quite plastic, though tightly controlled. During early development, melanoblasts (the precursor of melanocytes) migrate from the neural crest, along the dorsolateral pathways to colonize the dermis and epidermis [10]. Throughout this migration process the developing melanocyte experiences changing microenvironments, which require a strict regulation of adhesion protein expression and cellular phenotype to proceed [11,12]. One of the major adhesion-based mechanisms of cellular control responsible for these processes is the cadherin/catenin system. The cadherins are a group of functionally related Ca^{2+} dependent cell–cell adhesion glycoproteins, which contribute to tissue organization by forming homotypic adhesions between cells with similar cadherin profiles [13]. The organizing role for cadherins in tissue architecture is well demonstrated in the skin, where local cadherin expression patterns determine the spatial location of various epidermal cell types. The cadherin super-family is subdivided into classical cadherins (types I and II), desomosomal cadherins, protocadherins and other related proteins [13–15]. Of the type I cadherins, E-cadherin is expressed in

most epithelial tissues, N-cadherin is expressed in endothelial, neuronal, fibroblasts and muscle cells and P-cadherin is expressed mostly in embryonic tissues and the placenta. The cytoplasmic tail of the cadherins interacts with another family proteins – the catenins. Three prominent members of the catenin family are α - β - and γ -catenin, which are involved in linking cadherin-based adhesion to the actin cytoskeleton [16]. In addition to these adhesion functions, β -catenin plays an important role in cell signaling, shuttling between the cell membrane and the nucleus, as part of the Wnt signaling pathway [17,18]. Once in the nucleus it regulates the transcription of matrix metalloproteases [19–21], ECM components [22] and other cell–cell adhesion receptors [23].

Under normal homeostatic conditions, resting melanocytes form the “epidermal melanin unit” with keratinocytes in the dermis. The balance between keratinocytes and melanocytes is maintained at a constant ratio through tight regulation of melanocyte division via homotypic E-cadherin adhesion [24–28]. Evidence of this tight keratinocyte regulatory control via E-cadherin is readily demonstrated *in vitro*. Melanocytes grown in monoculture express a number of adhesion molecules which are more in common with melanoma cells, such as MCAM (Mel-CAM, CD146) and $\alpha_V\beta_3$ integrin. The introduction of keratinocytes into melanocyte cultures leads to loss of this phenotype and restriction of melanocyte growth [25,29]. In addition to this, E-cadherin expression is critical for maintenance of homeostasis of the epidermal melanin unit in two other ways. Firstly, E-cadherin provides strong cell–cell adhesion, suppressing any potential cell motility or invasion. Secondly, expression of E-cadherin at cell–cell contacts retains the mobile pool of β -catenin at the cell membrane, preventing its nuclear translocation and the initiation of transcription. Studies have shown that overexpression of cadherins antagonizes β -catenin signaling activity [30–32] and that cadherin depletion enhances β -catenin signaling [33].

Integrins are the primary means by which cells sense and respond to their microenvironment [34]. One of their most important roles is the transduction of specific survival signals, which allows the cell to determine whether it is occupying the correct environmental niche [35,36]. Following binding of integrins to their correct substrate, they become activated and form clusters known as focal adhesions. Engagement of integrins triggers the activation of cellular survival pathways, including focal adhesion kinase (FAK), integrin-linked kinase (ILK) and the two major effectors of Ras; PI3-kinase and Raf/MEK/ERK [37]. Introducing a normal cell into the wrong microenvironment leads to the incorrect integrin engagement, the correct survival signals are not received and the cell undergoes a specialized form of programmed cell death, known as anoikis [37].

The ability of integrin engagement with the correct microenvironmental substrates to suppress anoikis suggests that the restricted expression of integrins is an important feature of stromal control. Overcoming anoikis is therefore a critical step in the malignant transformation process.

All of the above mechanisms described ensure that normal cells proliferate within tightly controlled limits in a limited environmental niche. In order for an early-stage tumor to become locally invasive and eventually metastasize, these control mechanisms must be overcome. The pressure from the microenvironment to control the cells is therefore a major driving force for selecting which of these control mechanisms are inactivated. The next section examines how cells escape from environmental control and what the consequences of this loss of control are for tumor progression.

3. Genetic instability and microenvironmental selection pressure: a recipe for disaster

If we see the first stage in oncogenic transformation as being the acquisition of somatic mutations to increase cell growth, a second phase – the progression stage, must involve some degree of escape from cellular control. Not all transformed cells can do this, and it follows that not all transformed cells progress. There are well documented cases of neoplasia, which whilst undergoing enhanced proliferation, do not escape from environmental control. In these instances, the pre-malignant lesions may harbor oncogenic mutations but never progress. This phenomenon is most striking in the thyroid gland. Incidence of clinically apparent thyroid cancer in people between the ages of 50–70 is about 0.1%, however careful microscopic analysis of the thyroid gland at post-mortem reveals a 36% incidence of small thyroid tumors in the same age-matched population [38]. Furthermore, in normal sun-exposed skin, persistent oncogenic mutations in the tumor suppressor p53 are readily identified in keratinocytes, but these lesions also never progress [39]. Elsewhere in the skin, nests of hyperproliferative melanocytes, called nevi, are very common, but rarely develop into melanoma. The findings in nevi are made more interesting by the fact that these same benign nevi harbor the V600E mutation of BRAF in 21–80% of cases [40,41]. There is strong *in vitro* evidence for the role of mutated BRAF in melanomagenesis [42–45]. The presence of this potent oncogene in nevi, coupled with the low levels of nevi progression to invasive melanoma would suggest that in these instances the level of microenvironmental control is very strong. However, microenvironmental control is often lost with disastrous consequences.

The loss of cellular control is aided by two key features of the oncogenic phenotype; genetic instability and rapid proliferation. The grossest manifestation of genetic instability in tumor cells is aneuploidy (the alteration in chromosome number), which is manifested as the gain or loss of whole or parts of chromosomes [46]. In addition to tumor cells having excess chromosomes (often 60–90 per cell), other structural abnormalities in chromosomes also occur such as deletions, inversions, duplications and translocations. It is likely that these chromosomal aberrations occur as the result of defects in mitosis, leading to incomplete disassociation of chromosomes and breakages [47,48]. At the molecular level

chromosome losses are usually manifested as a loss of heterozygosity in a particular gene. In an average tumor, about 25–30% of the normal alleles are lost, and in some cases the allelic loss can be as high as 75% [49]. The loss of these alleles benefits the expanding clonal populations of tumor cells as they allow cells to rapidly adapt to the changing microenvironment, through the sequential loss of tumor suppressor and cell–cell adhesion control genes.

The effects of environmental evolutionary pressure upon the malignant behavior of tumor cells can be demonstrated in experimental models. The sequential passage of melanoma cells through a reconstituted basement membrane (which mimics invasion into the dermal microenvironment) or in serum-free media selects for clones which are 10-fold more invasive, metastatic and grow more rapidly in both serum-free and growth factor depleted media [50]. The selected invasive melanoma cells required continuous evolutionary pressure to maintain their phenotype and reverted to the parental phenotype when the environmental stimuli were removed [51]. In this instance, it appeared that although the effects of environmental selection pressure pushed the parent cells toward a more aggressive phenotype that these changes were not fixed in a genetic manner. Under certain circumstances this phenomenon is also observed *in vivo*. Well-differentiated colorectal adenomas and carcinomas have clear distinctions between the phenotype of the cells at the centre of the tumor mass and those at the invasive edges [51]. Cells in the centre of the tumor mass are well-differentiated with a polarized, epithelial phenotype, whereas the cells that form the invasive front appear to have undergone an epithelial-to-mesenchymal transition (EMT), characterized by loss of E-cadherin expression and upregulated expression of fibronectin and vimentin [51]. Surprisingly, these changes, whilst favoring invasion and metastasis, are also not genetically fixed. Lymph node metastases of these same colorectal carcinomas were found to have the same phenotype of those cells at the centre of the tumor mass, not that of those at the invasive edge. This indicates that metastatic tumor cells can re-differentiate and undergo a phenotypic switch after becoming established at a secondary site. In other examples there is selective loss of the transcription factor Cdx2 at the invasive front of colorectal carcinomas, but not the centre of the tumor mass [52]. The downregulation of Cdx2 is directly linked to the interaction of β 1 integrins at the invasive front with the collagen I of the tissue microenvironment. Again, these changes are transient, and Cdx2 expression is re-established following metastatic spread.

These findings argue against a linear model of tumor progression and instead suggest that the tumor has a more plastic phenotype which can respond to the changing microenvironment and progresses in a non-linear fashion. However, in other studies, the progressive genetic evolution of tumor cells, leading to “fixed” somatic changes is also demonstrated. The introduction of normal copies of chromosome 10 into melanoma cells lacking the tumor suppressor protein PTEN, leads to a progressive loss of the growth suppressing genes on

this chromosome [53], and a progressively more transformed phenotype.

4. Mechanisms of microenvironmental escape

The combination of genetic instability of tumor cells coupled with selection pressure to contain a growing malignancy work together to select malignant clones which are able to escape from local control. The clones that manage to escape their local environment have lost strong cell–cell contact with their neighbors, and are no longer reliant upon exogenous environmental cues to regulate their behavior. In most cancers, a critical first step in this local escape is through down-regulation of E-cadherin expression or function [54]. In melanoma, the first step in the transformation is the escape from keratinocyte control. Given that keratinocytes regulate melanocyte growth very tightly, loss of keratinocyte–melanocyte interaction at an early stage permits the earliest rounds of clonal expansion. Indeed, it appears that E-cadherin inactivation or loss is required for escape of the earliest tumor cells from the primary focus [55]. E-cadherin expression is lost in the majority of radial growth phase (RGP) and all vertical growth phase (VGP) and metastatic melanomas [56]. Even in these early melanoma cells, which still express some E-cadherin at the protein level, immunofluorescence experiments reveal that the protein has a cytoplasmic localization, suggesting functional inactivity (Fig. 2). Melanoma cells also undergo a cadherin switch, and upregulate expression of N-cadherin [56]. There is evidence that the upregulation of N-cadherin rather than loss of E-cadherin is more important for the invasive and metastatic behavior of cancer cells, with studies showing that anti-N-cadherin blocking antibodies inhibit the migration of melanoma cells [57,58]. Upregulation of N-cadherin brings with it a number of survival advantages, such as reducing the rate of apoptosis, through the increased activation of the PI3-kinase pathway and down-regulation of the pro-apoptotic factor BAD [59]. The switch to N-cadherin is also associated with less strong cell–cell adhesion, permitting greater cell motility. Indeed, endogenous N-cadherin expression in breast cancer cell lines has been correlated with increased motility and invasion, whereas forced expression of E-cadherin in these cell lines can suppress this motility [60]. Similar results have been reported in melanoma cells, where over-expression of E-cadherin can re-establish contact with skin keratinocytes, reducing growth and invasion of the melanoma cells [61].

The switch in cadherin profile of melanoma also leads to a switch in cell–cell binding partners, keratinocyte contact is lost and increased N-cadherin expression facilitates the interaction of melanoma cells with other stromal cells, such as endothelial cells and fibroblasts, which may aid tumor matrix remodeling and angiogenesis [6,7]. During tumor progression, melanomas develop a number of autocrine growth factor loops which contribute to their oncogenic behavior through increasing cell survival and reducing cell–cell adhesion. In

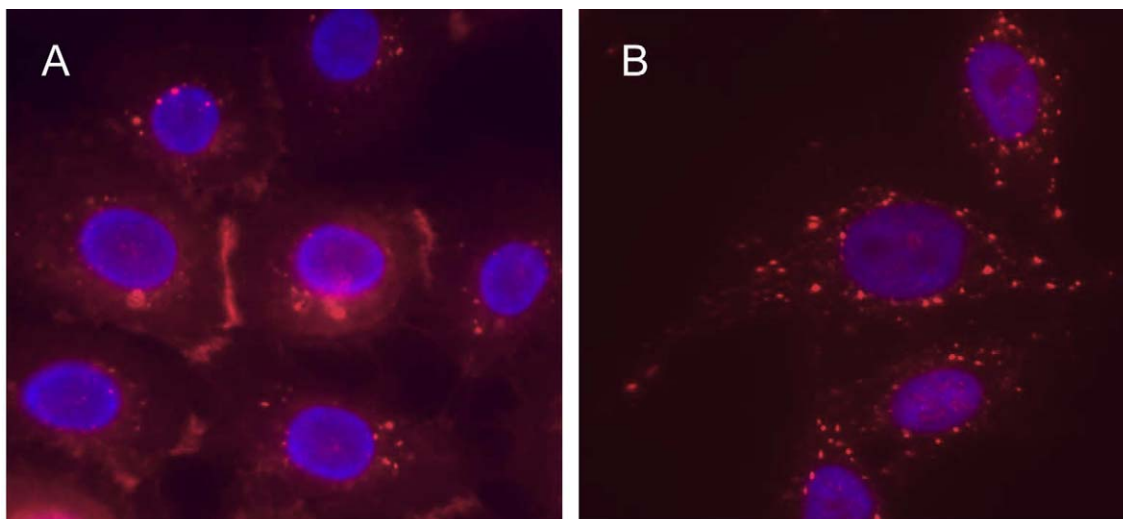


Fig. 2. E-Cadherin is located in the cytoplasm of early-stage melanoma cells. (A) In FK-126 human skin keratinocytes, E-cadherin (red) is located mostly at sites of cell–cell adhesion. (B) In WM35 RGP-melanoma cells, E-cadherin has a mostly cytoplasmic location, indicating that E-cadherin is functionally inactive ($\times 60$).

particular, the autocrine secretion of hepatocyte growth factor/scatter factor (HGF/SF), downregulates both E-cadherin and desmoglein [62].

One of the most important things a cell must do after escaping the cadherin-mediated local cell–cell control is to be able to survive outside of its own microenvironmental niche, and in doing so the nascent malignant cell must evolve mechanisms to overcome anoikis. In melanoma, this first becomes important where nevus cells invade into the more hostile dermal compartment. One method for early-stage tumor cells to circumvent anoikis is to acquire constitutive activity in the pathways responsible for cell survival, such as PI3-kinase, MEK/ERK and NF κ B. There are a number of ways in which this can happen, either through the constitutive activation of the pathways themselves, or the loss of the pathway inhibitors. In some cases, tumor cells acquire autocrine growth factor loops which activate these survival pathways. In melanoma, one of the earliest of these mechanisms is the acquisition of an autocrine basic fibroblast growth factor (bFGF) loop [63]. Primary human melanocytes show enhanced survival and proliferation in the hostile microenvironment of the dermis when transduced with bFGF [63]. Other growth factors, such as HGF, interleukin (IL)-8 and platelet derived growth factor (PDGF)-A also act in an autocrine manner to aid proliferation, survival and migration of melanoma cells [64]. The acquisition of growth factor pathways in early melanoma cells can also act in a paracrine fashion to influence the surrounding microenvironment, and is discussed in more detail in the following section [64].

Another strategy to avoid anoikis is to change the pattern of integrin expression, so that the correct environmental survival signals are received. Only transformed melanocytes can survive in the altered environment of the dermis, this survival is due in part to the expression of the correct integrins. Melanoma cells express $\alpha_V\beta_3$ integrin, which binds

to fibronectin, vitronectin, collagen and laminin, and integrin $\alpha_5\beta_1$, which selectively binds fibronectin [65]. However, β_3 integrin subunit expression is not exclusively a marker on oncogenic transformation as it is also expressed in otherwise benign Spitz nevi [66]. Expression of the β_3 integrin subunit cannot be detected in melanocytes or early melanoma, but it correlates with progression to later stage melanoma [67–70]. $\alpha_V\beta_3$ is important for adhesion of melanoma cells to dermal collagen and the suppression of apoptosis [70,71], most likely by altering the Bcl2:Bax ratio [71]. Other studies have also shown that expression of α_V or β_3 integrin increased metastatic potential of melanoma cells [72].

5. Active participation of the stroma in tumor progression

Escape from keratinocyte microenvironmental control conveys other evolutionary benefits to the nascent melanoma cells in permitting the direct interaction and communication with other cells of the stroma. It has long been appreciated that stromal changes accompany tumor development. The hard, fibrotic nature of most solid-tumors is the direct result of increased expression of collagen and fibroblasts throughout the lesion. The involvement of stromal fibroblasts in tumor progression requires the attraction and migration of fibroblasts from either the local tissue environment or circulating mesenchymal precursors/stem cells [6]. In our own studies, we have found evidence of direct chemo-attraction between melanoma cells and fibroblasts, with skin fibroblasts invading into collagen-implanted melanoma spheroids (Fig. 3, [6]). This fibroblast infiltration is often accompanied by a phenotypic switch within the fibroblast population to that of a myofibroblast, characterized by expression of α -smooth muscle actin [6]. The fibroblasts which grow in

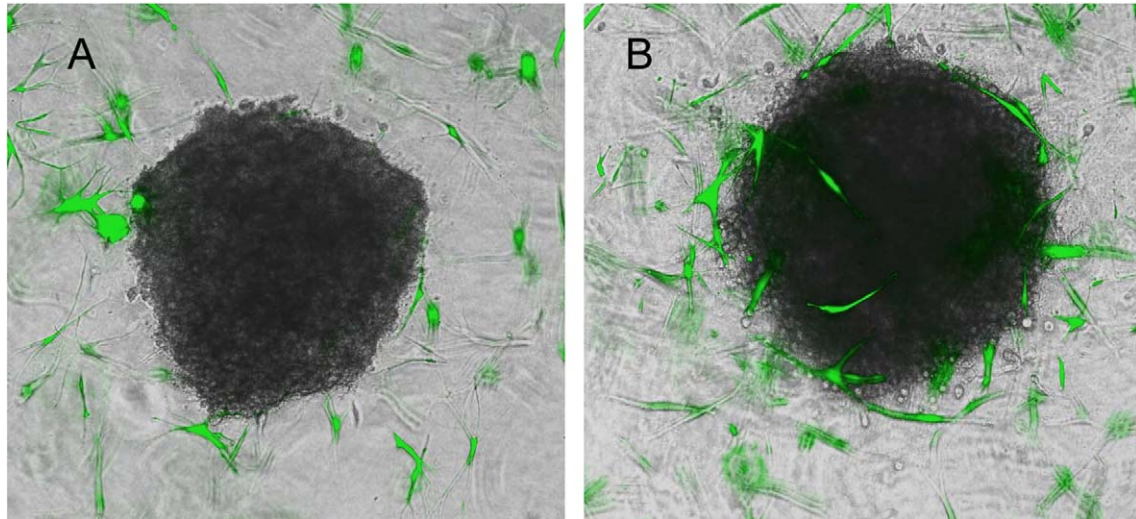


Fig. 3. GFP-tagged human skin fibroblasts infiltrate collagen-implanted melanoma spheroids. WM1366 melanoma cells were grown under non-adherent conditions until they formed spheroids they were then implanted into a collagen I gel containing GFP-tagged human skin fibroblasts (green). (A) One day after implantation. (B) Nine days after implantation the fibroblasts are attracted towards the melanoma spheroid and start to infiltrate ($\times 10$).

the vicinity of a tumor often have altered properties, such as much enhanced proliferation and increased ECM production [73–76]. Fibroblasts can also differentiate into other phenotypes, such as pericytes, which aid vasculature formation. It is known that tumor-infiltrating fibroblasts (TIFs) play a number of roles in tumor progression. They provide structural support for the tumor in terms of ECM deposition, they are a rich source of melanoma growth factors, and they aid angiogenesis via interaction with endothelial cells. In other studies, it was demonstrated that the tumor stage is an important determinant of the effects of the stroma on tumor cell growth [76]. Co-culture of early-stage melanoma cells with skin fibroblasts leads to a repression of tumor cell growth, whereas the presence of fibroblasts can enhance the growth of later stage melanoma cells [76]. This again demonstrates that at the early stages of melanoma growth, there is some degree of suppression coming from the stroma. As this control is lost there is a switch in the role of the surrounding fibroblasts, which release soluble growth factors which can stimulate tumor growth [76]. These paracrine growth factor loops are in some cases bi-directional, and can involve the release of factors from the melanoma to stimulate the stromal fibroblasts, which in turn release growth factors to activate the melanoma [7]. Melanoma-derived growth factors, such as PDGF, TGF- β and bFGF, act in a paracrine manner to stimulate the growth and activation of the surrounding endothelial cells and fibroblasts [77]. PDGF lacks autocrine effects in melanoma, as they do not express the PDGF- β receptor [78]. In addition to growth stimulatory effects upon stromal cells, PDGF can also induce fibroblasts to produce extracellular matrix proteins, such as laminin, collagen and fibronectin [79,80]. There is also evidence that PDGF stimulation releases IGF-I from fibroblasts, which then in turn stimulates the melanoma cells in a paracrine manner [81,82]. The IGF-I receptor is expressed on all melanocytic cells, and expression increases

upon malignant progression [81]. Secretion of IGF-I from stromal fibroblasts plays an important role in the survival of early-stage melanoma cells, through activation of pathways to suppress anoikis. However, the role of IGF-I becomes less important as the melanoma progresses as constitutive activity is acquired in other signaling pathways. In addition to IGF-I, activated stromal fibroblasts also release bFGF, and endothelin (ET)-3, which stimulate melanoma growth [6].

Transforming growth factor (TGF)- β is produced by melanoma cells, and is known to be growth inhibitory for epithelial cells and normal melanocytes [83]. However, as melanoma progresses, cells become resistant to these growth inhibitory effects. The paracrine secretion of TGF- β may instead aid stromal remodeling, which aids the structural integrity of the tumor and enhances survival [80]. It has also been shown that adhesion of tumor cells to ECM proteins such as fibronectin, laminin and collagen IV makes them more resistant to chemotherapeutic drugs, such as adriamycin, cisplatin and etoposide – an effect which could be reversed by anti- $\beta 1$ integrin blocking antibodies [84]. Likewise, tumor cells selected for drug resistance in adherent cell cultures also display altered integrin expression and adhesive properties [84,85].

Recent studies have suggested that under certain circumstances genetically altered stroma can drive the oncogenic transformation of normal epithelia [86]. In these instances, the fibroblasts are not innocent bystanders under the control of the tumor, but the driving force behind oncogenesis. The selective knockout of the TGF- β receptor subtype II in stromal fibroblasts leads to the formation of invasive squamous cell carcinomas in the forestomach of mice [86]. The inactivation of TGF- β signaling in the stromal fibroblasts led to the establishment of an HGF/c-met paracrine loop between the stroma and the forestomach epithelia, resulting in epithelial hyperplasia and carcinoma development. In other studies,

TIFs from human prostatic carcinoma were found to promote carcinogenesis in immortal but non-tumorigenic, BPH-1 prostatic epithelial cells [87]. Further experiments showed that neither the TIFs nor the BPH-1 cells were carcinogenic when grown alone. Furthermore, no tumors were formed when BPH-1 cells were grown with normal prostatic stromal cells, demonstrating a direct role for TIFs in carcinogenesis [87]. Interestingly, the role of TIFs in promoting carcinogenesis is not just through paracrine mechanisms and can also involve genetic changes within the epithelial cell population [88].

6. Does increased knowledge of microenvironmental control provide opportunities for therapeutic intervention?

The process of oncogenesis can be viewed as a series of discrete processes, which occur in a non-linear fashion (Fig. 1). In the first instance, cells need to acquire a competitive growth advantage through either enhanced activity in oncogenes, or the deletion of tumor suppressor genes. These rapidly growing cells undergo waves of clonal expansion, but must be able to escape the control of their immediate environment in order to progress. It seems that a combination of genetic instability within the tumor cell population and selective pressure from the surrounding environment work together leading to the loss of physiological control mechanisms and resistance to anoikis. This results in the eventual selection of tumor cell clones well suited to local invasion and enhanced survival at distant organ sites. The question still remains whether the initial oncogenic changes in melanoma, such as increased MEK/ERK activity, through the mutation of BRAF, are also those responsible for the microenvironmental escape. Constitutive MEK/ERK activity in melanoma is responsible for the upregulation of pro-invasive integrins such as $\alpha_V\beta_3$, and increased activity of the matrix degrading enzyme MMP-1 [90,91]. The fact that the tumor environment can dramatically alter phenotype, coupled with the fact that tumors with the same oncogenes have different clinical courses when expressed on different genetic backgrounds [92], suggests that this may be an oversimplification.

A greater understanding of how microenvironmental control is deregulated is important from a therapeutic standpoint. Many of the mechanisms by which tumor cells adapt to hostile microenvironments are similar to those which convey resistance to chemotherapeutic drugs [93]. One possible future therapeutic approach could be to restore the physiological methods of control to malignant cells, ensuring that any tumor cells could be contained. One approach would be to develop the “virtual keratinocyte” as a microenvironmental controller of melanoma cells. Studies with E-cadherin re-expression in melanoma have already demonstrated that this is a feasible approach [61]. New data suggests that the stroma is an active player in oncogenesis, and can help to drive tumor progression through release of paracrine growth

factors and induction of genetic changes [88]. It is possible that an alternative approach to cancer therapy could involve targeting the stroma providing another possible mechanism to regain environmental control. Other approaches could be to target the pathways responsible for resistance to anoikis with small molecule inhibitors, again rendering the tumor cells susceptible to apoptosis in the harsh microenvironment. It is clear that tumor cells do not exist in isolation, and the continual interaction of the tumor cells with the stroma and its surrounding tissue environment plays a critical driving role in progression. Further research in this important area will bring us closer to more effective treatments for this deadly disease.

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