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Are stem cell niches shared for skin cancers?

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Demonstrating the link between normal tissue stem cells and cancer stem cells (CSCs) and tracing the origins of cancer at the very earliest stages is now a key goal in the treatment of many types of cancer. Linking tumors back to the precise cell of origin has strong implications for our view of tumors and how to treat them. Normal tissue stem cells are capable of both self-renewal and differentiation, the integration of which is essential to tissue maintenance and function. Stem cells may cycle between quiescence or proliferation and self-renewal according to the natural rhythms of the tissue or following various external stimuli (e.g. tissue injury). Neighboring cells and extracellular matrix play a supporting role in maintaining stem cell identity and this microenvironment, or niche, may regulate the transition between stem cell proliferation and quiescence. Interestingly, the stem cell origin model of cancer predicts that cancer stem cells retain certain properties of the tissue stem cell, including unlimited self-renewal, the production of many daughter cells that make up the tumor bulk (i.e. CSCs are very rare within the tumor), cycling through quiescence and active cell division depending on changes in the tumor microenvironment, and creation of new niches during the metastatic process.

The specific behaviors of the tissue stem cell of origin and of the derived cancer stem cell are likely to dictate tumor pathogenesis and have a major bearing on any chemotherapeutic approach. For example, it is hypothesized that the identification of key factors that regulate stem cell cycling and quiescence may yield new insights into methods to render cancer stem cells susceptible to chemotherapeutic agents, or to permanently block cancer stem cell cycling in the presence of certain drugs. Indeed, the tumor stem cell model predicts that recurrence after chemotherapy is due to CSCs that are thought to be chemotherapy-resistant due to their quiescent nature and increased expression of drug efflux transporters. Here, we briefly examine progress in linking skin cancers including squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanomas to a tissue stem cell of origin, and we discuss the likelihood that these tumor types fit within these somewhat idealized views of the maintenance and propagation of cancer from cells with stem cell characteristics.

In the epidermis, these observations may be applied to an interfollicular keratinocyte stem cell (IFKSC) involved in normal skin regeneration, or to a follicular stem cell (FKSC) in the bulge that plays a role in regenerating the lower portion of the hair shaft during the hair cycle and in regenerating the epidermis after wounding. Through lineage tracing and molecular profiling, the characteristics of FKSC niches have been well studied, in particular that of the bulge niche. In both human and mouse, the bulge niche has been defined by expression of such markers as Tenascin C and Keratin (Krt) 15 and 19, expressed on KSCs residing in the bulge. However, the niche that supports IFKSC remains poorly characterized, although $\beta 1$ integrin and Lrig1 or the newly discovered Lgr6 (Snippert et al., 2010) are candidate IFKSC markers. In comparison, the bulge region of the hair follicle is presently

the only accepted location, or niche, of melanocyte stem cells (MSCs), which are required for repopulating hair bulb melanocytes during the Anagen phase of the hair cycle (Nishimura et al., 2002). The cycling adult hair follicle undergoes three distinct phases including Anagen, where the stem cells in the lower permanent portion of the follicle are activated and transit amplifying cells of the keratinocyte lineage generate growth into the dermis. As Anagen progresses, follicle growth ceases and a nascent hair shaft is formed that in turn pushes out the preceding hair shaft. Upon cessation of hair growth, the follicle enters Catagen, where programmed cell death causes regression of the follicle and upwards retraction. During Telogen, the hair follicle essentially rests until initiation of the next Anagen phase. It is interesting that both KSCs and MSCs reside in the lower permanent portion of the follicle, in the bulge region defined by the connection of the arrector pili muscle. Furthermore, MSCs are stimulated to proliferate in Anagen II, shortly after FKSCs are initiated to begin downward follicular growth. Thus MSCs and FKSCs exist in a shared niche and shared signals may elicit similar responses from these two stem cell types. In support of this idea, Nishimura et al. (2010) have shown that TGF β signaling in the mouse hair bulge region inhibits MSC differentiation and thus is involved in MSC maintenance, and it plays a further role in re-establishing MSC quiescence after early Anagen proliferation. In this study, the authors observed mild coat graying in mice with a melanocyte-specific deletion of the TGF β type II receptor (TGF β RII), with concomitant progressive increase in differentiated melanocyte content within the hair follicle bulge. The authors also provide evidence that TGF β signaling promotes re-entrance of MSCs into their quiescent state, which is characterized by diminished proliferation, expression of pigmentation markers, and reduced size and dendricity. These observations provide key insights into the regulation of the MSC in the niche; given the observation that bulge FKSCs exhibit similarly high levels of TGF β signaling, it is tempting to speculate that some degree of coordinated regulation may exist in the shared FKSC/MSC follicular niche.

Linking cancer stem cells back to their normal origin is essential to understand and characterize their behavior, and to elucidate the earliest stages of tumor emergence. Murine skin cancer models employing chemical carcinogenesis, xenografts and genetically engineered strains have shown that the cells of origin for SCC are KSCs that reside within the interfollicular epidermis and the hair follicle (Figure 1) (Gerdes and Yuspa, 2005). More recently, it has been shown that SCCs contain rare sub-populations of cells with enriched tumor-propagating ability that express KSC markers (e.g. CD34 in mice; Malanchi et al., 2008), among others. IFKSCs or FKSCs may have a different potential for transformation and appear to result in tumors that are more benign (IFKSC) or more malignant (FKSC). However, this remains a point of contention given studies that show a certain level of plasticity in KSCs isolated from various parts of the hair follicle and interfollicular skin, indicating that the niche where KSCs reside plays an important role in determining malignant potential. Moreover, SCCs often show a pattern of differentiation within the tumor that is reminiscent of the skin-differentiation program, from undifferentiated and quiescent stem cells to rapidly dividing progenitor cells that feed the tumor mass to a progressively cornified and hardened exterior surface. These observations fit well with the notion that SCCs are a classic stem cell tumor consistent with its tissue stem cell of origin.

Similar insights into the cell of origin of BCCs can be drawn from genetically engineered mouse cancer models. BCCs have been previously defined by the deregulation of Hedgehog (HH) signaling through acquisition of mutations in *PATCHED* (*PTCH*) or *SMOOTHENED* (*SMO*), and are thought to arise from FKSCs in the bulge niche due to the indistinguishable cytokeratin-staining patterns observed between nodular BCCs and trichoblastomas, another hair follicle-derived tumor, and the staining patterns observed in developing fetal hair follicles (Roop and Toftgård, 2008; Schirren et al., 1997). Recently, this follicular centric view of the cellular origin of BCC has been challenged by a study suggesting that long-term

KSC progenitors residing in the interfollicular epidermis (IFE) or upper infundibulum are the origin of BCCs (Youssef et al., 2010). Through the use of lineage-tracing experiments with compartment-specific targeting of a constitutively active Smo mutant (SmoM2) in mouse skin, tumors with histology reminiscent of human nodular BCC were shown to develop only in the interfollicular epidermis of the ear and tail skin. BCCs were not evident when SmoM2 was targeted to the hair bulge. However, *Ptch*^{+/-} mutant mice spontaneously develop trichoblastomas (Aszterbaum et al., 1999) that switch to BCCs upon UV exposure and mice overexpressing Gli2 developed BCCs, which upon transgene inactivation regressed, leaving quiescent remnant tumor cells that expressed Krt 17, a follicular keratinocyte marker (Hutchin et al., 2005). In addition, overexpression of SmoM2 using a truncated Krt 5 promoter instead of the Rosa26 promoter used by Youssef and coworkers did not result in BCCs, but instead in benign tumors resembling human basaloid follicular hamartomas (Grachtchouk et al., 2003). These disparate results suggest that IFKSCs can give rise to BCCs (Figure 1) and, secondly, that the niche (e.g. follicular versus inter-follicular) may determine the fate of tumors arising from mutations in HH signaling molecules. This latter point is intriguing given the fact that deregulated HH signaling in KSCs produces BCCs, not SCCs, although both tumor types have a similar spectrum of secondary mutations (e.g. UV-induced p53 mutations), and suggests that certain mutations are dominant in a niche-dependent manner. Clearly, future experiments are needed to clarify the contribution of IFKSCs to BCCs and whether different levels of mutant SmoM2 in a niche-dependent manner can sway tumor outcome (e.g. hamartomas versus nodular BCCs).

The relationship between SCCs, BCCs and a stem cell of origin highlights the complexity of the relationship between putative melanoma stem cells and the location of a possible MSC of origin. While there have been a number of high profile studies that report the identification of melanoma stem cells, these have been somewhat contradictory, with opposing suggestions that melanomas contain a low frequency of ABCB5⁺ cells that are responsible for maintaining the tumor (Schatton et al., 2008) or a very high number (~25%) of cells that perform this role (Prasmickaite et al., 2010; Quintana et al., 2008). Thus, the true nature of a putative melanoma stem cell is in dire need of confirmation.

With respect to the histological site of origin, melanoma is a very diverse tumor type, and some of its subtypes may show evidence of different locations for the originating cells (Zalaudek et al., 2008). It has been proposed that superficial spreading melanoma (SSM) may have an IFE origin, where a clear junctional component is a dominant feature of the tumor and it is rarely found in association with hair follicles. On the other hand, lentigo malignant melanoma (LMM), invariably associated with chronic UV exposure, is very frequently associated with the upper hair follicle, and some evidence suggests that nodular melanoma (NM) may arise in the dermis. Unlike the cancer studies that have illuminated the role of KSC as the origin for SCCs and BCCs, the use of lineage-tracing murine models to clarify the melanocyte reservoirs that may give rise to melanoma are complicated by the lack of interfollicular melanocytes on mouse back skin, and the lack of a junctional melanoma phenotype in mouse melanoma models that re-capitulate the genetic events that occur during human melanomagenesis. With the exception of the mouse Mt-Hgf model, virtually all others develop melanoma with a dermal origin reminiscent of human NM rather than the SSM that is more often observed. Nevertheless, the clear dermal origin of these mouse tumors does suggest that an infrequent dermal origin of human melanoma should be considered as a possibility.

While the established wisdom is that melanoma has an interfollicular epidermal origin, much work remains to pinpoint the possible variable sources of melanoma, and the relationship to any underlying stem cell. The discovery of the bulge niche for MSCs begs the question whether follicular MSCs can give rise to melanoma. Perhaps the most

compelling evidence for the role of the MSC comes from the observation that vitiligo patients may re-pigment after UV light therapy. The pathogenesis of vitiligo involves an auto-immune response, often to differentiated melanocytic antigens such as tyrosinase, with obliteration of all interfollicular melanocytes in the affected area. Repeated narrow band UVB therapy promotes proliferation of MSC of the bulge region and the first signs of interfollicular epidermal re-pigmentation are observed with the presence of new melanocytes in the IFE around the hair shaft (Grichnik, 2008). We note that both the bulge region of the hair follicle and the lower permanent portion of the outer root sheath (ORS) contain undifferentiated melanocytes (TYR⁻/TYRP1⁻/PMEL17⁻/DCT⁺/PAX3⁺), whereas no such melanocytes are detected in the interfollicular epidermis (Medic and Ziman, 2010). These data argue against a simple model of a normal IFE MSC that gives rise to SSM.

Clearly, there is a great need for experimental evidence that may assist in linking SSM with its cell of origin. We present below a few possible scenarios that might account for the appearance of SSM in the IFE (Figure 1).

1. IFE melanocytes themselves give rise to melanoma without need for an initiating MSC. However, in most cancers it is now accepted that cancer is less likely to arise from differentiated cells, and stem or progenitor cells are almost invariably the initiating cells.
2. An unknown epidermal MSC and niche give rise to melanoma. It should be noted that the IFKSC has defied identification, and may well have different marker properties to the FKSC. Interestingly, variable populations of melanocytes in the IFE with different patterns of differentiation markers have begun to be identified and after UV exposure, a population of proliferating IFE melanocytes has been identified (Medic and Ziman, 2010).
3. Differentiation of IFE melanocytes is reversible in certain micro-environmental conditions. There is in vivo evidence that vacant niches may attract or co-opt lineage-specific differentiated cells and induce full reversion to a stem cell. Perhaps such a mechanism could play a role in establishing a melanoma at the earliest stages (reviewed in Voog and Jones, 2010). We note that all current evidence suggests that the interfollicular KSC niche does not usually have an identifiable MSC partner, whereas the bulge niche is defined by a shared KSC/MSK environment. In this model, the interfollicular KSC niche is a candidate 'latent niche' in the earliest stages of de novo melanomagenesis.
4. An errant hair follicle MSC or TA cell gives rise to IFE SSM. Evidence from vitiligo repigmentation and irradiated mouse skin (Walker et al., 2009) shows that epidermal repopulation from follicular MSCs might occur after repeated UV exposure.
5. An early life-initiated melanoblast (e.g. carrying a UV-induced *BRAF* or *NRAS* mutation) may be able to create an artificial niche to maintain itself in an immature form in the IFE until later in life, where secondary mutations may enable it to progress to melanoma. It should be noted that early-life UV exposure is strongly associated with later life melanoma. We anticipate that overlapping aspects of some of these concepts may be true for development of SSM, and experimental dissection is desperately needed.

As we have highlighted, the three major forms of skin cancer may arise from common or shared niches found within the interfollicular epidermis and hair follicle. It will be very informative if, like the follicular niche shared by KSCs and MSCs in the bulge, a shared niche is also present in the interfollicular epidermis which might nurture putative cancer-initiating cells. In addition, we don't understand how mutations acquired through UV

exposure or other environmental mutagens affect how precancerous KSCs or MSCs interact with their niches to determine the severity and/or type of tumor (e.g. BCC versus SCC). Understanding the molecular factors that regulate proliferation and quiescence of MSCs of the bulge, and the relationship of these MSCs to the properties and behaviors of a putative melanoma stem cells is lagging behind the advances of KSC biology. Hence, there is a dire need for better/more fitted models to address the pressing questions of MSC biology, which could give rise to new therapeutic approaches aimed at limiting the ability of any single cancer cell to escape its niche, or to establish new niches during metastatic spread or recurrence. Moreover, discerning the cellular origin of melanoma or other major skin cancers may elucidate differences in pathogenesis for different types of skin cancers, as well as enable better methods of detection for high risk cells.

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Figure 1.

Location of niches for melanocyte (MSC) and keratinocyte (KSC) stem cells in human skin and tumors that arise from these locations. MSCs and KSCs reside in a common niche located in the bulge region. The interfollicular niche for KSCs is poorly characterized, and a speculated interfollicular niche for MSCs has not yet been identified. Anatomical locations shown: arrector pili (AP), sebaceous gland (SB), stratum corneum (SC), infundibulum (IF), melanocyte (MC).