Melanoma, Nevogenesis, and Stem Cell Biology

James M. Grichnik[1]

It is now well established that a subpopulation of tumor stem cells (TSCs) are present within cancer tissues. This suggests that tumors evolve from stem cells; however, the exact cell of tumor origin, the potential role of dedifferentiation, and the role of plasticity in tumor development are largely unknown. A model cancer for the study of the oncologic process is melanoma. The developmental biology of melanocytes is relatively well understood, the cells pigment as they differentiate making them easy to identify, and benign and malignant tumors develop on the skin surface allowing direct observation of growth features, early detection, and removal. This ready access to early-stage tumors will facilitate study of the early oncologic processes and the role of tissue stem cells. Melanomas, like other cancers, include a subpopulation of TSCs. These TSCs have access to embryologic developmental programs, including the capacity to differentiate along multiple cell lineages. For example, melanomas can activate germ–cell pathways with major implications for TSC self-renewal through the activation of telomerase and genomic instability through the collision of meiotic and mitotic pathways (meiomitosis). The TSC model is still evolving, but the existence of TSCs has significant ramifications for tumor development, diagnosis, prognosis, and treatment of melanoma and other cancers.

Journal of Investigative Dermatology (2008) **128,** 2365–2380; doi:10.1038/jid.2008.166

Introduction

Cancer development, progression, and therapeutics remain at the forefront of medical research. The recognition of a subpopulation of tumor stem cells (TSCs) in solid cancers has reinvigorated the field. These cells have the capacity to self-renew and give rise to more differentiated cell forms (Reya *et al.*, 2001). The existence of these TSCs implicates a pluripotent stem cell as the cell of origin for cancer. How-

Editor's Note

Malignant melanoma has long been identified as one of the most important and inscrutable of all skin diseases. In his 1941 review DeCholnoky concluded ''Radical surgery is the treatment of choice, and should consist of wide local excision... followed by regional lymph node dissection....'' While the recommended extent of surgery may have decreased over the last 60+ years, it is remarkable that our knowledge of the basic biology of malignant melanoma has not resulted in significant changes in treatment; indeed, metastatic melanoma remains one of the malignancies most resistant to treatment. In the next two issues of the JID, recent advances in our understanding of the biology of malignant melanoma are featured. In this issue, Grichnik discusses how melanoma stem cells may play a critical role in the pathogenesis of melanoma and its resistance to conventional therapy. Zaidi and co-workers explore the role of UV light in the pathogenesis of melanoma and how animal models can advance our understanding of both initiation and metastasis. Next month, Hocker and co-workers review recent advances in the genetics of melanoma and how they may lead to new specific therapeutic interventions. Finally, Fang and co-workers discuss how recent advances in immunology have resulted in new strategies for using the immune system to treat life-threatening metastatic disease. In 1930 Dr James Ewing wrote, ''The problems of melanoma maintain their position as the most interesting and complex of any department of oncology..... Possibly there are other important data lying within easy reach of the alert observer.'' While the ''problems of melanoma'' persist, these Perspectives demonstrate the significant progress and challenges that characterize our understanding of melanoma in the 21st century; I hope they will present new directions for study by the ''alert observer''.

Russell P. Hall, III, Deputy Editor

[1]*Departments of Medicine and Cell Biology, Duke University Medical Center, Durham, North Carolina, USA*

Correspondence: Dr James M. Grichnik, Division of Dermatology, Department of Medicine, Duke University Medical Center, 4032 Hospital South, Box 3135, Durham, North Carolina 27710, USA. E-mail: grich001@mc.duke.edu

Abbreviations: bFGF, basic fibroblast growth factor; DCT, dopachrome tautomerase; EPI-NCSC, epidermal neural crest epidermal stem cells; ET-3, endothelin 3; ESC, embryonic stem cell; SCF, stem cell factor; SKP, skin-derived precursor; TA, transiently amplifying; TSC, tumor stem cell

Received 31 October 2007; revised 9 January 2008; accepted 10 January 2008

ever, ''dedifferentiation'' from a mature tissue cell has not been completely excluded. Although the cell of origin can still be questioned, there is no doubt that the TSCs exist and play a critical role in tumor maintenance. TSCs, although only a small fraction of the tumor bulk, have been shown to be the cells with the capacity to give rise to new tumor nodules. Tumorigenic (TSCs) versus non-tumorigenic populations were initially defined in hematologic malignancies (Lapidot *et al.*, 1994; Bonnet and Dick, 1997) and have recently been identified in breast (Al-Hajj *et al.*, 2003), pancreatic (Li *et al.*, 2007), colon (Dalerba *et al.*, 2007; O'Brien *et al.*, 2007; Ricci-Vitiani *et al.*, 2007), squamous cell (Prince *et al.*, 2007), brain (Singh *et al.*, 2003; Kondo *et al.*, 2004), and lung cancers (Ho *et al.*, 2007). TSC populations have also been shown to be able to differentiate along multiple cell lineages; for example, in glioma, TSCs have been shown to differentiate along both the neural and glial pathways (Kondo *et al.*, 2004). The findings suggest that TSCs have access to embryologic developmental programs, including the capacity to differentiate along multiple cell lineages.

Melanoma, like other cancers, includes a subpopulation of TSCs. The tumor often visibly develops on the surface of the skin, and with the advent of dermoscopy, and developing technologies such as confocal microscopy, the early development of these lesions will be able to be better studied. In addition to the malignant lesions, benign nevi also develop on the skin surface and may provide additional critical information about the role of tissue stem cells and/or mature melanocytes in the early stages of tumor evolution. The relatively well-defined developmental biology of melanocytes, as well as the ready access to normal and diseased tissue, makes melanoma a model system for the study of stem cell biology and carcinogenesis. Tumorigenesis is now best viewed as an aberrant developmental process. This review examines selected aspects of normal embryologic and melanocytic development, homeostasis, nevogenesis, and tumorgenesis, as they may apply to melanocytic TSC biology.

Normal embryologic development

The fertilized egg is the ultimate ''stem cell''. During cell division, the protoplasm is asymmetrically distributed due to the influence of cellular and extracellular signal gradients. As cell numbers expand and the cells are exposed to local environmental signals, selective differentiation occurs and a vast array of tissue structures is formed. During the cellular developmental process, nuclear DNA is modified (alteration of histones and DNA methylation) and segregated within the nuclear matrix structure controlling gene expression and cell fate.

The developing cell is dependent on accurate DNA primary sequence, and therefore DNA mutations are actively identified and repaired. If mutations cannot be repaired, the cell is dependent on the intact apoptotic pathways for elimination, as otherwise a mosaic disorder would develop. Once a defective cell has been eliminated, its position in the developmental gradient will be occupied by a neighboring cell, which will differentiate appropriately as long as it has sufficient plasticity. Early embryonic cells have remarkable plasticity as up to one-fourth of the 8-cell embryo can be removed without appreciable long-term sequella (Hardy *et al.*, 1990; Pierce *et al.*, 1997). With further development, the degree of plasticity retained by cells is most likely to be dependent on its ultimate function. Some cells would be expected not to maintain the capacity to differentiate along other cell lineages. Obvious examples would include cells in which the nucleus has been reabsorbed such as red blood cells or corneocytes, cells that have undergone meiotic events such as egg or sperm cells, or cells in which DNA recombination has occurred such as T and B immunoregulatory cells. However, to sustain and repair tissue, a considerable number of cells must maintain significant plasticity into adulthood. Presumably, these cells are retained as specialized tissue stem cells, but it is possible that some well-differentiated cells retain intrinsic plasticity. The fact that some cell nuclei can be used in cloning experiments suggests that nuclear changes in some cells are sufficiently reversible to generate a new embryo (Oback and Wells, 2007).

The human developmental process proceeds quite quickly, and within 9 months a single cell gives rise to more than a trillion individual cells with a total mass of more than 3 kg. This cellular mass is elegantly organized, including a multitude of different cell types appropriately interacting with tightly controlled proliferation/apoptotic and mitotic/meiotic mechanisms, with the capacity and plasticity to grow and repair tissues for about a century. A TSC inherently has access to all these capacities.

Embryonic development of melanocytes

Melanocytic cells appear to be primarily derived from the developing neural crest. The migration of the neural crest has been elegantly studied through the utilization of a reporter system that labels cells with an activated dopachrome tautomerase (DCT) promoter, which is expressed in melanocytic and neural progenitors (Mackenzie *et al.*, 1997; Jiao *et al.*, 2006). In the murine system, melanocytic precursor cells can be first recognized in the neural crest at approximately embryonic day 8.5, these cells migrate between the dermatome and the overlying ectoderm in the dorsolateral pathway, migration continues ventrally through the developing dermis at day 10.5, the cells begin to insert into the epidermis and the developing hair follicles at day 14.5, these cells proliferate and differentiate with pigment synthesis observed around postnatal day 4 (Wilkie *et al.*, 2002).

The melanocytic developmental process is very dependent on stem cell factor (SCF) and its receptor, KIT. Distinct waves of SCF/KIT dependence and independence play a role in melanocyte survival, migration, and epidermal insertion (Nishikawa *et al.*, 1991; Okura *et al.*, 1995; Yoshida *et al.*, 1996). These waves of KIT dependence and independence are also seen during the hair cycle (Nishikawa *et al.*, 1991). In addition, endothelin 3 (ET-3) and the endothelin B receptor play a critical role in melanocyte development, as loss of

these pathways also results in the loss of cutaneous melanocytes (Baynash *et al.*, 1994; Hosoda *et al.*, 1994). A number of other cytokine pathways have also been documented to play a role, including α-melanocyte stimulating hormone, basic fibroblast growth factor (bFGF), nerve growth factor, endothelin 1, granulocyte macrophage-colony stimulating factor, and hepatocyte growth factor (for review see Halaban, 2000; Hirobe, 2005).

Melanocytic development can also be driven *in vitro* through the use of embryonic stem cells (ESC). Using mouse ESCs cultivated on a feeder layer of ST2 cells, dexamethasone was noted to induce the formation of melanocytes (Yamane *et al.*, 1999). Inhibition of the KIT pathway (with ACK2) blocked melanocyte production, whereas the addition of ET3 enhanced the number of melanocytic cells. Another group noted that the addition of retinoic acid to the ESC culture increased the production of melanocytes (Motohashi *et al.*, 2007). KIT+ cells developed were isolated by cell sorting and were shown to be multipotent with the capacity to give rise to melanocytes, glia, and neurons. The development of melanocytes could be antagonized by an ET-3 inhibitor (BQ788). A third group has demonstrated the need for Wnt3a for the development of melanocytes in their ESC system (Fang *et al.*, 2006). In the presence of WNT3a and ET-3, melanocytes could be produced. Melanocyte production was further augmented by the addition of SCF.

Thus, there is a well-orchestrated set of events within the embryonic mass that exposes embryonic cells to the appropriate factors to differentiate the proper number of melanocytes in the correct location. Theoretically, this specific series of developmental exposures reorganizes the DNA nuclear matrix to initiate the appropriate expression of melanocytic genes. The melanocytic differentiation process can also be induced *in vitro* by exposing ESCs to the appropriate cytokines, potentially allowing a more straightforward study of nuclear changes. Although this melanocytic developmental process is normally driven by external environmental signals, it is most likely that the internal abnormal activation of intracellular signaling pathways, such as the activation of B-Raf, in a stem cell could also drive melanocytic differentiation.

Adult melanocytes

Melanocytes integrate into the epidermis and function to provide melanin as a UV protectant to neighboring keratinocytes. The melanocytes may also perform other functions, including endocrine (Slominski *et al.*, 2007; Takeda *et al.*, 2007), metal chelation (Farmer *et al.*, 2003; Meyskens *et al.*, 2004; Hong and Simon, 2007), and immune functions, yet to be fully explored (Burkhart and Burkhart, 2005). In performing these duties, the melanocyte is susceptible to UV and oxidative stress, which may create genetic mutations. Ideally, damaged melanocytes would be eliminated. Although normal melanocytic turnover is generally imperceptible, melanocytes being discharged through the epidermis (''pagetoid'' melanocytes) can be seen after acute sun exposure, over nevi, and melanomas (Pharis and Zitelli, 2001; Petronic-Rosic *et al.*, 2004).

The number of melanocytes in the epidermis is tightly controlled and this is primarily due to their interaction with local keratinocytes. A major homeostatic factor in the adult skin is SCF. Epidermal keratinocytes produce SCF in both a membrane bound and soluble form, but local fibroblasts may also contribute to SCF levels. SCF/KIT stimulation or inhibition has been shown to drive proliferation or loss, respectively, of melanocytes in human skin (Grichnik *et al.*, 1995, 1998). Expression of SCF in the mouse epidermis results in the retention of melanocytes when they would have otherwise been lost (Kunisada *et al.*, 1998). Signaling through SCF/KIT and other pathways provides the homeostatic signaling to maintain the appropriate number of epidermal melanocytes in adult skin.

Melanocytes can be cultured from isolated epidermal sheets. Multiple growth factors or chemical stimulants are necessary to promote and maintain melanocyte proliferation *in vitro* (Halaban, 2000). The response of the cells to SCF *in vitro* can be modulated, with SCF functioning as a survival factor under low bFGF conditions, a proliferative factor with intermediate levels of bFGF, but having no additional proliferative effect over bFGF when high levels of bFGF are present (Lin *et al.*, 2000). Thus, the homeostatic role of SCF can be partially recapitulated *in vitro*.

Melanocytes obtained from neonatal foreskins grow robustly while melanocytes from older human skin samples grow poorly. Eventually, melanocytes will senesce in culture (Bennett and Medrano, 2002). This suggests that these epidermal melanocyte cultures either do not include a stem cell component or the existing culture conditions do not promote or maintain melanocytic stem cells. However, the melanocytes in culture may have some plasticity, as ''fibroblast'' outgrowth is not uncommon in melanocyte cultures. Although this is assumed to be due to contamination, it is possible that these cells are derived from melanocytic precursors.

As the human ages, the capacity to make and retain melanocytic cells appears to diminish. The most obvious example of this is the graying of hair. Although this loss of melanocytes is generally considered permanent, it is interesting to note that there are case reports describing spontaneous hair repigmentation (Etienne *et al.*, 2002; Kavak *et al.*, 2005).

Thus, epidermal melanocytes clearly play a critical role in UV protection. As a consequence of this function, these cells may be particularly susceptible to mutation and need to be regularly turned over to prevent the accumulation of damaged DNA. Presumably, these damaged cells are discharged through the epidermis. The number of epidermal melanocytes is tightly controlled by local factors. With aging, the total number of melanocytes gradually diminishes.

Stem cell populations in adult skin

Melanocytes, like cells in many other tissues, are turned over and replenished from immature precursors. Disease processes such as vitiligo give us a window into this process. In active vitiligo, differentiated melanocytes are

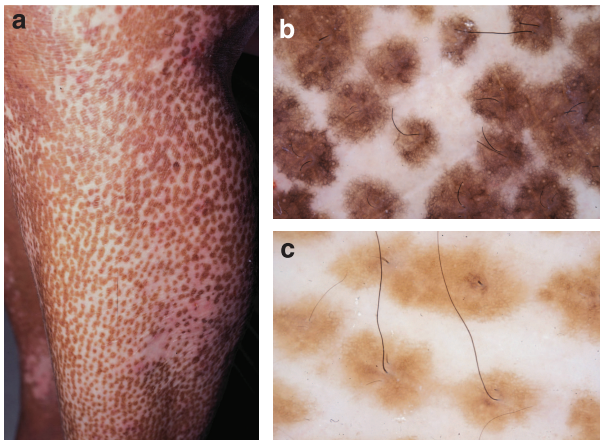

Figure 1. Repigmentation during recovery from vitiligo. During the active phase of vitiligo, cutaneous melanocytes are destroyed. During recovery, as seen in this clinical image of a patient's leg (**a**), the destroyed melanocytic cells can be replaced. These new melanocytes appear to be derived from a protected population of cells within the hair follicle as shown in these dermoscopic images (**b** and **c**) from two patients recovering from vitiligo.

destroyed. During recovery, new melanocytes migrate into the epidermis from a protected population of cells (Figure 1). A similar phenomenon can be driven experimentally in zebrafish. Treatment with 4-(4-morpholinobutylthio)phenol results in the death of differentiated melanocytes, and these cells are replaced by cells derived from a precursor population (Yang and Johnson, 2006). Thus, differentiated melanocytes are replaced by cells that differentiate from an immature precursor population.

A major reservoir for melanocytic precursors is the hair follicle. Precursor cells have been identified in this location through the expression of KIT (Grichnik *et al.*, 1996). These cells also expressed Bcl-2, which may play a role in the survival of the stem cell compartment, because mice defective for Bcl-2 loose melanocytic stem cells (Nishimura *et al.*, 2005). Melanocyte isolation from the hair follicle has revealed the presence of heavily pigmented mature and amelanotic immature cell populations (Na *et al.*, 2006). Consistent with stem cell biology, the amelanotic population was shown to have a slower doubling rate but enhanced a long-term proliferation capacity compared with the pigmented cell population. With age and graying of the hair, residual melanocytic stem cell populations have been noted to be decreased (Nishimura *et al.*, 2005).

The melanocytic stem cell compartment in the murine hair follicle has been studied using a DCT reporter system to identify the melanocytic lineage cells (Nishimura *et al.*, 2002). These cells gave rise to transiently amplifying (TA) population of melanocytes that populated the hair bulb; however, some of the cells were able to return to a quiescent state in the hair bulge area. KIT receptor expression appears to be downregulated in a quiescent subpopulation but upregulated on migratory melanocytic cells during the hair cycle (Botchkareva *et al.*, 2001; Peters *et al.*, 2002). In the quiescent cells, although a few pathways were upregulated including the Notch pathway, there appeared to be a global suppression of transcription, including decreased expression of melanocytic genes (Nishikawa and Osawa, 2007). At the end of each hair cycle, differentiated melanocytes in the hair bulb are presumed to undergo apoptosis (Tobin *et al.*, 1998).

It would be easier to model the stem cell system if it were only unidirectional. However, the capacity for a TA cell to become a quiescent stem cell suggests that some degree of dedifferentiation is possible. A dramatic example of the potential for a melanocyte to dedifferentiate *in vitro* has been demonstrated in the quail system (Real *et al.*, 2006; Dupin *et al.*, 2007). Quail skin melanocytes have been shown to give rise to Sox-10, Pax3, and slug-expressing stem cells. Furthermore, the stem cells were pluripotent, that is they had the capacity to give rise to glia, myofibroblasts, and melanocytes. Because stem cells are present in the skin, the extent to which melanocyte dedifferentiation occurs *in vivo* is unclear but clearly the potential exists.

Stem cells are present in normal skin. A number of groups have focused on epidermal stem cells and their role in giving rise to keratinocytes (Bickenbach and Grinnell, 2004; Blanpain and Fuchs, 2006; Cotsarelis, 2006). The seven groups presented below have examined skin stem cell populations capable of producing melanocytes and/or other neural crest derivatives.

One group isolated a cell population of small size and low Hoechst 33342 staining from green fluorescence protein (GFP)-labeled murine epidermis and injected the GFP+ cells into blastocysts. They were able to show that the GFP+ cells gave rise to ectodermal, mesenchymal, and neural crest-derived tissues (Liang and Bickenbach, 2002).

A second group isolated cells from human hair follicles in conditioned human embryonic stem cell media (Yu *et al.*, 2006). These ''hair follicle stem cells'' were noted to express nestin, slug, snail, twist, sox-9, and bmp4. Focal Nanog and Oct4, a totipotent embryonic stem and germ cells marker, expressions were also noted. This group was able to differentiate these cells into melanocytes, neuronal, and smooth muscle cells.

A third group using a nestin-GFP construct was able to isolate cells from the bulge area of the murine hair follicle (Amoh *et al.*, 2005). The GFP+ cells were found to be CD34+ and keratin 15−. They were able to differentiate these cells into keratinocytes, melanocytes, neurons, glia, and smooth muscle cells. *In vivo*, the formation of blood vessels and neural tissue was shown. The ability to generate melanocytes and keratinocytes from the same bulge stem cell population is quite intriguing.

A fourth group identified sphere-forming cells from skin culture (the epidermis and the dermis) that were

noted to express P75 and Sox-10 (Wong *et al.*, 2006). These cells also produced multiple lineages but not keratinocytes. With the addition of SCF and ET-3, they were able to induce the formation of a few melanocytes. It was noted that the skin from the face produced spheres with more potential, whereas spheres formed from the trunk skin grew less copiously and were more restricted in their cell production yielding only glial and melanocytic lineages. A further experiment utilized a murine line with GFP expression in cells in which the DCT promoter was active. These mice made GFP+ spheres that lacked pigmented melanocytes, suggesting that the DCT promoter was active in p75 and Sox-10-positive stem cells.

A fifth group has isolated multipotent stem cells that they term ''skin-derived precursors'' (SKPs) from the rodent skin (Toma *et al.*, 2001). The SKPs were derived from the dermis, but could not be derived from the epidermis, and were found to express nestin and fibronectin but not vimentin or cytokeratin. The SKPs were shown to differentiate into neurons, glia, smooth muscle, and adipocytes. A similar population of cells from the adult human scalp skin was also derived (Toma *et al.*, 2001). Further work on these SKPs has revealed that they also express snail/slug, twist, and Pax3 (Fernandes *et al.*, 2004). Based on localization of expressed markers, they suggest a major niche for the SKPs is in the dermal papilla rather than in the hair bulge.

A sixth group attempted to determine the molecular signature of ''neural crest epidermal stem cells'' (EPI-NCSC) isolated from the hair bulge area (Hu *et al.*, 2006). Nestin protein expression was present in 2- to 7-day culture with KIT expression present at day 5. At day 7, autonomic neurons, Schwann cells, melanocytes, bone/cartilage cells, and myofibroblasts could be identified. EPI-NCSCs were defined as the cells emigrating from hair bulge murine whisker while in culture. The gene pattern of the EPI-NCSC cells (day 2 in culture) was shown to be different than that of the epidermal stem cells (Tumbar *et al.*, 2004) and the stem cells that may be increased in the dermal papilla (Toma *et al.*, 2001; Fernandes *et al.*, 2004). Their work suggests at least three different stem cell populations may be present in the skin.

A seventh group has identified CD133+ (also Thy-1+ and CD34 low) cells in human skin. These multipotent stem cells have the capacity to differentiate into neurons, astrocytes, and oligodendrocytes (Belicchi *et al.*, 2004). The CD133+ cells constitute about 6% fetal skin cells but are only about 1% of adult cells. Cell proliferation and plasticity was more attenuated in adult cells than in the fetal counter parts. These cells expressed c-kit and CD34 (by reverse transcription-PCR) (Belicchi *et al.*, 2004). CD133+ cells have also been previously noted in the human epidermis (Yu *et al.*, 2002) and in a subpopulation of cells in cultures human foreskin melanocytes (Frank *et al.*, 2003).

The CD133+ cells are particularly interesting, as they have been noted to identify neural crest stem cells (Uchida *et al.*, 2000), endothelia precursor cells (Gehling *et al.*, 2000), and mast cell precursor cells (Dahl *et al.*, 2002). In bone marrow transplantation experiments, the precursors for mast cells have been noted to insert into the hair follicle (Kumamoto *et al.*, 2003). Although difficult to comprehend given our current models, it is possible that both melanocytes and mast cells are replenished from a common circulating CD133+ precursor. Furthermore, it is possible that these are the cells responsible for the spontaneous repigmentation of gray hair.

Thus, the skin contains cells with stem cell characteristics, and many of these defined populations have the proven capacity to give rise to melanocytes and/or at neural crest lineage cell populations. Although it could be argued that, in some of these experiments, the cells dedifferentiated from a more mature precursor, clearly in others, the stem cells expressed markers defining them as present prior to culture manipulation. The interrelationships between these different stem cell populations, their differentiation state in tissue, and the extent to which the differences experimental techniques modify the cells have yet to be fully defined. However, it is clear that there are several potential stem cell populations in human skin with the capacity to give rise to melanocytes. It is even possible that melanocytes could be replenished from a circulating CD133+ pluripotent precursor.

Nevogenesis

Origin and direction of melanocytic nevus growth. Nevi are benign clonal proliferations of cells expressing the melanocytic phenotype (Robinson *et al.*, 1998; Hui *et al.*, 2001). In congenital nevi, it is presumed that a precursor cell is mutated, possibly by the activation of N-Ras (Bauer *et al.*, 2007), and an excessive number of daughter cells result. The migrating cells populate subcutaneous, dermal, and epidermal structures, as they attempt to complete normal migration to the skin surface. Some of the excess melanocytes completing migration may be discharged through the epidermis as ''pagetoid'' cells in early congenital nevi (Figure 2). Transepidermal elimination of nevus cells may also play a role in nevus involution (Kantor and Wheeland, 1987). The nevus cells in the epidermis or in the superficial dermis are more likely to be larger and express melanocytic differentiation antigens compared with the deeper cells consistent with developmental models. A similar antigen expression pattern is seen in acquired nevi.

Although there is consensus that congenital nevi grow along developmental pathways toward the epidermis, for acquired nevi there is an active debate as to the direction of migration. Unna described the process of Abtropfung, the dropping off of melanocytic cells into the dermis from the epidermis; whereas Cramer (1991) has proposed the process of Hochsteigerung, migration toward the epidermis. He proposes that the precursor cells are associated with nerve tips and that there are four discrete migration/differentiation stages that may be involved in normal melanocytic turnover, nevus, and melanoma formation (Cramer, 1984).

One of the ways to determine the direction of growth is to evaluate nevi as they develop. The majority of the

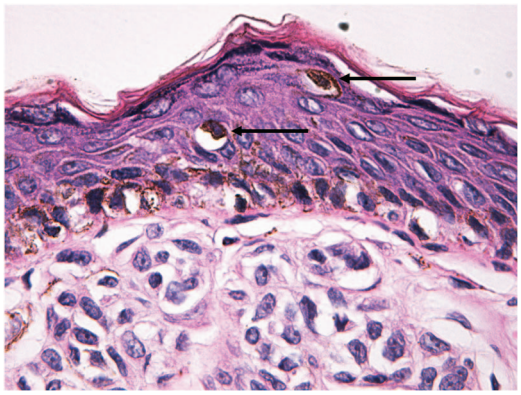

Figure 2. Pagetoid melanocytic cells in congenital nevus. Photomicrograph of hematoxylin and eosin-stained section of a congenital melanocytic nevus excised from the left buttock of a 10-month-old female child. Two pagetoid melanocytes are clearly visualized in the mid-spinous and granular layers of the epidermis (black arrows).

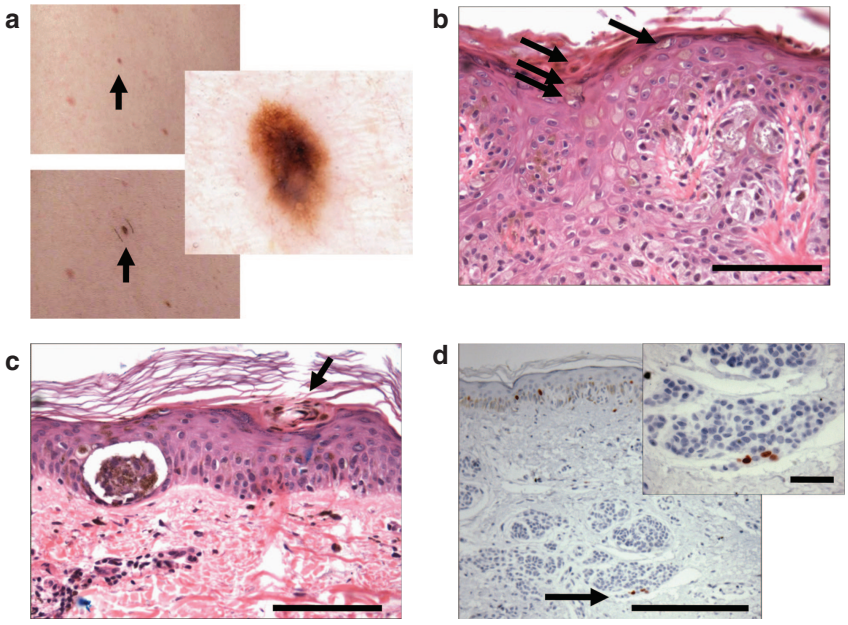

Figure 3. Growing melanocytic neoplasms have features suggesting epidermal tumor loss and dermal proliferation. Growing melanocytic neoplasms can be detected clinically (**a**) by comparing the patient's lesion on a follow-up visit (lower left panel) with photographs from a prior visit (upper left panel). This particular lesion was approximately 5 mm in longest diameter (inset) and was pathologically interpreted as an atypical (dysplastic) nevus with severe atypia. In growing melanocytic neoplasms, melanocytic cells could be detected in the upper layers of the epidermis and in the stratum corneum (**b**, arrows, size bar = 100 μm). In other lesions, melanocytic nests of cells could be identified in the stratum corneum (**c**, arrow, size bar = 100 μm) and proliferating dermal cells could be identified with immunohistochemical staining (red) for Ki67 (**d**, arrow, size bar = 200 μm, 50 μm (inset)).

nevi removed from children clearly include a dermal component (Worret and Burgdorf, 1998). Dermoscopically, nevi developing in children often have a globular pattern, which is also most consistent with nevi that include a dermal component (Zalaudek *et al.*, 2006). Thus, if these nevi do not evolve in the dermis, dermal migration from the epidermis must be a very early process.

In our own studies on early growing melanocytic neoplasms, utilizing only the residual tissue in the block, an obvious dermal component was detectable in 58% of the lesions, Ki67 + cells were rare but could be identified in the dermal component, and upwardly mobile ''pagetoid'' cells could be identified in 32% of the growing lesions (Molino *et al.*, 2003). Large junctional nests (>75% of the epidermal thickness) were present in 42% of the lesions, and some of these nests were clearly being expelled through the epidermis (Figure 3). These findings were consistent with dermal origin and epidermal migration. Given the presence of pagetoid cells in growing nevi, we need to be careful not to overinterpret growing benign nevi as melanoma. Ever improving surface microscopic technologies, such as confocal, will be able to give us a clearer picture as to the features to expect during normal nevus growth.

The direction of melanocyte migration has been evaluated in skin grafts. It has been demonstrated that KIT-activated melanocytic cells injected into the dermis of a graft migrate into the epidermis (Alexeev and Yoon, 2006; Grichnik, 2006b). This ability of melanocytic cells to migrate could also explain how nevus cells washed into a lymph node could migrate into the lymph node's capsule. Although developmentally this direction of migration makes sense, it is important to point out that experiments with transformed cultured melanocytes have demonstrated that these cells can penetrate into the dermal component of the graft (Chudnovsky *et al.*, 2005); therefore, migration from the epidermis into the dermis is also possible.

The direction of melanocytic migration during nevus development will eventually be definitively determined through sequential imaging. Confocal microscopy and optical coherence tomography are likely to play critical roles in defining this process. Dermoscopy may also provide information based on color features. Owing to light scattering, blue coloration represents dermal melanin. Both decreasing blue features suggesting upward growth and increasing blue features suggesting downward growth have been noted (Pizzichetta *et al.*, 2006; Zalaudek *et al.*, 2007). However, because amelanotic cells in the dermis could become more pigmented as they mature, increasing blue pigmentation does not necessarily reflect migration into

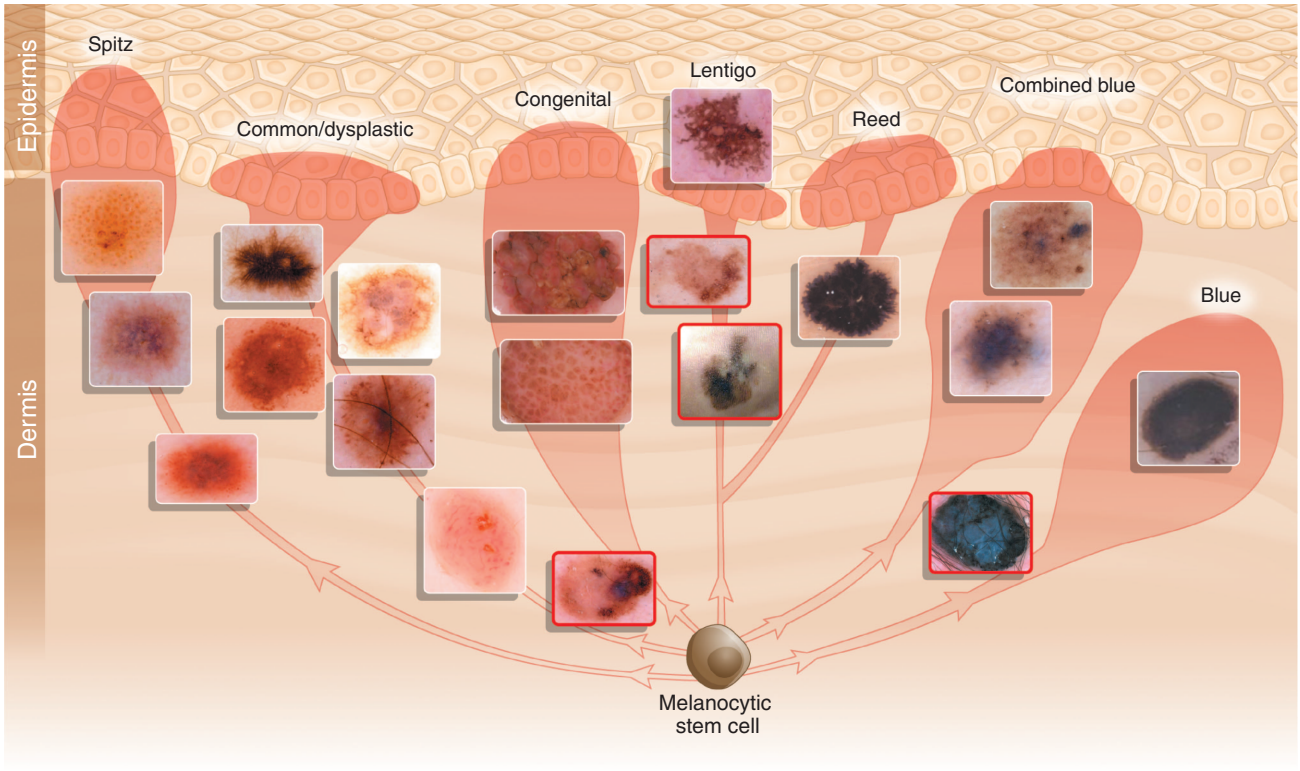

Figure 4. Melanocytic neoplasm growth patterns. Melanocytic neoplasm demonstrate numerous different repeatable patterns on patient's skin surface. Presumably, each of distinct patterns represents an underlying distinct mutation, developmental phase, or local signaling environment. Repeatable distinct patterns can be noted for acquired nevi (common and dysplastic), Spitz nevi, ''congenital'' nevi, lentigines, Reed nevi, blue nevi, and combined blue nevi. Furthermore, distinct patterns can be noted for different melanoma types, indicated here in the red boxes, superficial spreading, lentigo maligna, acral lentiginous, and others. Theoretically, these lesions could be derived from a common precursor (brown oval) that follows normal developmental pathways to the epidermis. Dependent on the mutation and local environmental factors, the neoplasm would expand and migrate in the dermal and the epidermal compartments.

the dermis, and thus further studies will be required. Beyond just direction, these imaging techniques will allow further study into the melanocytic developmental process. There are many different patterns of nevi, and theoretically, the growth pattern should be a reflection of the underlying mutation and the local environmental influences on cell growth and survival (Figure 4). B-Raf and N-Ras mutations have been associated with acquired nevi but specific dermoscopic patterns have yet to be delineated.

B-Raf-activating mutations are commonly seen in acquired nevi and N-Ras mutations are commonly seen in congenital nevi (Ichii-Nakato *et al.*, 2006; Bauer *et al.*, 2007). N-Ras not only activates a number of pathways, including phosphoinositide 3 kinase and Ral, but also activates B-Raf, leading to a common activation of downstream mitogen activated protein kinase and ERK pathways (Arbiser, 2003), reducing the need for stimulatory cytokines. In culture, nevus cells are also less dependent on growth factors when compared with normal melanocytes (Mancianti *et al.*, 1993; Alanko *et al.*, 1999). This suggests that although the underlying mutations are not sufficient to drive malignancy, they are sufficient to allow some independence from the normal homeostatic mechanisms.

Thus, nevi develop due to mutations in a precursor cell that activate proliferative pathways, or suppress apoptotic pathways, allowing for the accumulation of melanocytic cells in the skin. The specific pattern of this accumulation is anticipated to be a reflection of the underlying mutation in the precursor cell. The direction of nevus growth is still in debate; however, similar to congenital nevi, it is reasonable to assume that acquired melanocytic nevi (with a dermal component) develop from a mutant dermal precursor and migrate and differentiate along normal melanocytic developmental pathways toward the epidermis. Once in the epidermis, there may be an increased proliferative response due to local growth factors and excess melanocytic cells would be shed through the stratum corneum.

Melanoma

Role of nevi in melanoma development. The development of melanoma has been modeled as a stepwise process from a cutaneous melanocyte through nevus and dysplastic nevus stages to *in situ* and eventually invasive melanoma. However, approximately three-fourths of melanomas develop in normal skin and less than one-half of the nevi associated with melanoma are dysplastic (Bevona *et al.*, 2003). Thus, it is not clear that a dysplastic nevus is really any more likely to develop a melanoma than any other type of nevus, and further it is quite clear that a nevus precursor is not required for the majority of melanomas. This phe-

nomenon is difficult to explain utilizing current dogma. However, it is relatively straightforward to explain based on the stem cell theory. Melanoma could develop directly from a quiescent precursor cell that had accumulated a malignant complement of mutations. The one-fourth of melanomas associated with nevi may be due to a secondary mutation developing in a growing stem cell population giving rise to a benign nevus.

Increased mole burden clearly increases melanoma risk. For patients with large congenital nevi, the melanoma risk appears to be correlated with the volume of the nevus and malignant transformation often appears to occur within the nevus at a young age (Krengel *et al.*, 2006). For patients with numerous acquired nevi, the bulk of the risk may be in the otherwise normal appearing skin. It is not clear what drives this risk but there are a number of possibilities. First, the patient's nevus precursor (stem) cells may be increased in number, have had increased exposure to mutagenic events, or increased sensitivity to mutagenic events. Second, the patient may have less effective immunosurveillance (or other mechanisms) to inhibit the growth of melanomas and/or nevi from the mutant precursor cells. Finally, the precursor cells may have an inherent increase in self-renewal capacity, leading to an increased window of time for mutations to occur. Interestingly, it has been noted that the telomeres are longer in the white blood cells of patients with numerous nevi (Bataille *et al.*, 2007), suggesting increased longevity and proliferative potential.

Another interesting issue is difference in age for nevus versus melanoma development. Most nevi develop during late childhood and early adulthood, whereas melanoma incidence continues to increase in the later years of life (Banky *et al.*, 2005). It is not clear what drives this phenomenon but there are a number of possibilities. First, the longer the period the precursor (stem) cell remains quiescent, the more the mutations it may accumulate, resulting in a greater chance for malignancy. Second, mutant stem cells with anti-apoptotic mechanisms may be retained and expanded with age. Additional mutations in these cells would be more likely to create a malignancy. Third, melanomas that are slow growing may simply be easier to detect at older ages. Fourth, as the patient ages, they may lose effective immunosurveillance (or other mechanism) for inhibiting the growth of melanoma. Finally, it is possible that somehow the body recruits normal stem cells first and only later relies on the mutated stem cells.

Thus, there is a clear association of nevi with melanoma risk. Some of this risk is due to the potential for secondary mutations within nevi. However, the majority of the risk may be due to the inherent properties of the stem cell population in individuals with numerous moles.

Origin and direction of malignant melanoma growth. Similar to acquired nevi, the traditional model suggests that melanomas initially develop in the epidermis and then invade the dermis. This is largely based on pathologic features in which the lowest risk *in situ* melanoma tumors are noted to be present entirely in the epidermis, whereas high-risk tumors are present in deeper dermal tissues. Thus, it is reasonable to assume based on progression models that the tumor must first arise in the epidermis. These models also suggest that the cell of origin for the tumor is the epidermal melanocyte. This is also supported by research that reveals that melanocytes can be transformed with oncogenes and acquire malignant/invasive characteristics (Chudnovsky *et al.*, 2005). Furthermore, the tyrosinase promoter, expressed in melanocytes, has been shown to induce melanoma when driving oncogenic proteins SV40E (Kelsall and Mintz, 1998) and N-Ras (Wong and Chin, 2000). Thus, it is possible to generate a melanoma from a cell expressing melanocytic proteins. The extent to which these melanocytes are fully differentiated and the degree of plasticity maintained in the cell undergoing the transformation event have yet to be fully clarified.

The stem cell model is essentially the converse of the traditional model. Although it does not rule out epidermal evolution of melanoma, which could very well be the case especially for lentigo maligna, it favors migration to the epidermis from a dermal precursor. In this model, low-risk, *in situ* or thin invasive, melanomas would be highly attracted to the epidermis and migrate toward this location and thus minimizing spread from the tissue. As additional mutations accumulated in the tumor (due to genomic instability), the melanoma cells would become less growth factor-dependent and gain an increased capacity to grow in deeper levels of the dermis and other tissues. It is important to note that on review of *in situ* tumors, 29% were found to have an intradermal component (Megahed *et al.*, 2002), so it is not entirely inconceivable that many of these tumors have a dermal derivation. As the dermal TSC accumulated more mutations, the capacity to produce cells that could continue to differentiate along normal melanocytic pathways would be reduced, giving the impression of dedifferentiation when actually it would be due to the unmasking of the immature malignant stem cell population.

Although early melanomas are difficult to grow in culture, a significant proportion of metastatic melanomas can grow readily in culture without the need to add exogenous growth factors (Halaban *et al.*, 1986). A few melanomas do exist that have KIT-activating mutations and, therefore, may be sensitive to KIT-inhibiting agents (Curtin *et al.*, 2006), but most have downregulated this pathway presumably because its homeostatic role could be detrimental to the viability of the tumor. Inhibition of the ET-3/endothelin B receptor pathway has been reported to inhibit the growth of melanoma lines (Lahav *et al.*, 1999). However, for the most part, melanomas are largely growth factor-independent, presumably due to mutations, such as N-Ras activation or B-Raf/PTEN, that promote proliferation and survival (Tsao *et al.*, 2004) or from autocrine signaling loops due to activation of pathways leading to the production of their own growth factors. This independence from homeostatic pathways allows the tumors to thrive in non-epidermal environments.

Thus, at this point, whether melanomas develop in the epidermis and migrate into the dermis or develop in the dermis with migration to the epidermis is still a matter of debate. However, in either scenario, the deeper the tumor in the dermal tissue, the lower the requirement for epidermal factors and the greater the risk that tumor will be able to proliferate outside of the skin surface and be potentially deadly.

Stem cell/developmental behavior. Regardless of origin, it is clear that a subpopulation of TSCs exist in melanomas (Figure 5). Studies on metastatic melanoma lines have revealed that the cultures are heterogeneous even when derived from a single cell (Grichnik *et al.*, 2006). The different cell populations within these cultures appear to have diverse characteristics. A small-cell phenotype has been noted that appears to enter the cell cycle at a lower rate but has an increased capacity to expand the culture. The small cells had the capacity to give rise to larger, more proliferative cells (TA cells). The TA cells then eventually gave rise to still larger, more melanized cells. Furthermore, some of the larger, more melanized cells appeared to have terminally differentiated and were eventually lost from culture. These experiments suggested a general forward developmental flow of the process. However, even after double cell flow purification of the larger cell forms, some small cell forms reappeared in culture. This could have been due to incomplete purification, but it could also be due to the possibility that some of the TA cells were able to re-occupy the stem cell niche, reverting to a small-cell phenotype. Thus, this suggests that there may also be a degree of backward flow in the culture system to achieve the appropriate embryonic developmental balance. It is also important to note that the small cell forms were very weakly adherent and were readily released from the plate with simple media removal. Despite being relatively free floating, these cells were very effective at establishing new cultures. Tumor spheroids reminiscent of those described for stem cells also readily developed under dense culture conditions. A small subpopulation of nestin (pluripotent stem cell marker)-positive cells could be identified in dense melanoma cultures, suggesting the presence of a stem cell phenotype.

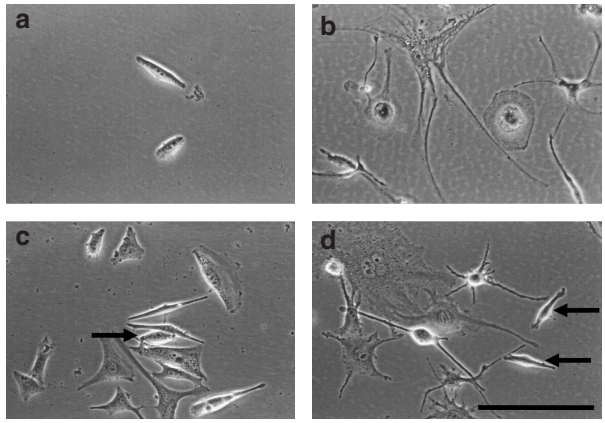

Figure 5. Metastatic melanomas include cells with stem cell-like features. Some melanoma cultures display a heterogeneous population of tumor cells, including small weakly adherent cells (**a**, line DM3N) and larger adherent phenotypes (**b**, line DM3N). Although some of the larger cell phenotypes (**b**) do not appear to be significantly proliferative, low-confluence cultures of the small weakly adherent cells (**a**) have the capacity to perpetuate heterogeneous colonies in culture (**c**, line DM3N) while maintaining a subpopulation of small weakly adherent cells (arrow). Melanoma line DM1N (**d**) plated at low confluence also give rise to heterogeneous colonies after transfer as well as maintaining a subpopulation of small weakly adherent cells (arrows). Magnification is the same for all photos; size bar = 200 μm.

Other groups have also identified potential TSC markers. Fang *et al.* (2005) noted that non-adherent spheroids developed in approximately 20% of melanomas cultured in ESC media. Cells from these spheroids could be differentiated into melanocytic, adipocytic, osteocytic, and chondrocytic lineages. Compared with the adherent cells, the spheroid cells were more tumorgenic in animal models. The non-adherent spheroid cells were found to include CD20 B-cell markers. The sorted CD20 fraction were found to be more spheroidogenic (more likely to make sphere or make larger spheres), and had more potential for mesenchymal differentiation. The CD20 marker was also noted to be present in approximately 20% of human metastatic melanomas by immunohistochemisty.

A second group has identified ABCB5 in melanomas (tissue sample and in cell lines) as preferentially marking a subset of CD133+-expressing cells in melanomas (Frank *et al.*, 2005). The ABCB5+ cells were also CD166+. Inhibition of the ABCB5 pump significantly reversed resistance to doxorubicin. Cells double labeling for ABCB5 and CD133 were approximately 2% of the G3361 line. These markers also stained melanoma tissue samples (positive in all tissue samples—overall staining in each sample <50% six tumors examined).

A third group has reported that CD166, CD133, and nestin are immunohistochemically expressed at significantly higher levels in melanoma compared with banal nevi (Klein *et al.*, 2007). Further, nestin was found to be significantly increased in metastatic melanoma compared with primary melanoma.

A fourth group has specifically isolated CD133+ cells from human melanomas (Monzani *et al.*, 2007). This group noted that the CD133+ cells were present at levels ranging from 0.2 to 0.8% of the total cells in seven melanoma specimens. The CD133+ melanoma cells all grew tumors in NOD-SCID mice whereas CD133− melanoma cells failed to grow.

Melanoma cultures are quite dynamic and antigen expression can be erratic. In our hands, the greatest degree of heterogeneity exists in long-term dense cultures with partial but frequent media changes (Grichnik *et al.*, 2006). We have noted waves of patterning between nestin and KIT

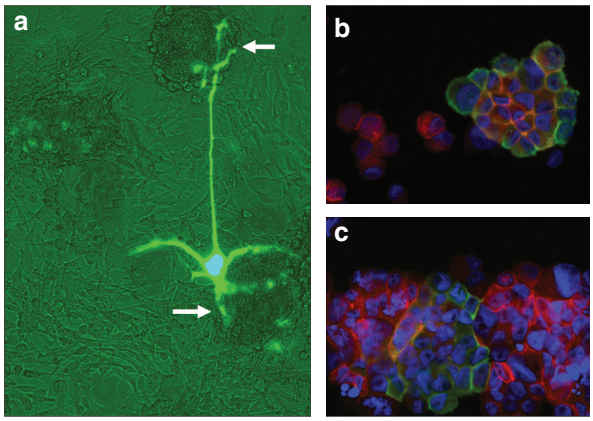

Figure 6. Melanoma cells displaying developmental pathways. Melanoma cells are dynamic and the heterogeneous features of the cell population are best detected in long-term dense cultures, presumably due to local microenvironments created by the tumor. Neuronal cells can be identified as shown here in a GFP-positive melanoma cell after 4 months in culture (**a**). This cell is extending dendrites into two tumor spheroids (white arrows). In the DM2N melanoma line, both KIT and the pluripotent neural stem cell marker nestin are expressed. In this system, waves of nestin (FITC-stained, green) and KIT (Cy3-stained, red) are expressed in developing spheroids (**b**) and sheets (**c**) of cells appearing similar to those which would be anticipated during embryonic development.

(Figure 6) as well as the presence of neuronal appearing melanoma cells with dendrites reaching into tumor spheroids (Figure 6). We hypothesize that this is due to enhanced cell-to-cell and cell-to-matrix microenvironments as would be expected during normal development. Matrix microenvironment components have been shown to promote endothelial differentiation of melanoma cells (Hendrix *et al.*, 2003). Local environmental factors have also been proposed to control melanoma growth (Hendrix *et al.*, 2007; Lee and Herlyn, 2007).

Thus, melanomas are heterogeneous and behave in a manner consistent with a developmental system. Within this mass of cells is a subpopulation of cells with stem cell-like features. The data support a tumor derivation from a stem cell. The data from the CD133 experiments clearly reinforce arguments for a stem cell derivation given the lack of tumorgenicity of CD133-negative cells.

Genomic instability and concept of meiomitosis. Melanomas, unlike benign nevi, have markedly unstable genomes. Large fragments of chromosomes are often gained or lost (Bastian *et al.*, 2000; Stark and Hayward, 2007). DNA repair pathways clearly play a role in the development of melanoma and other skin cancers as markedly demonstrated in patients with xeroderma pigmentosa (Kraemer *et al.*, 1994). It is quite possible that these DNA repair mechanisms lead to significant genomic instability (Charames and Bapat, 2003). Another process that has been speculated to cause genomic instability is telomere crisis in which critically shortened telomeres result in strand breaks and chromosomal fusion (Gilley *et al.*, 2005). However, given the stem cell biology and plasticity of melanoma, there is another area that needs to be seriously considered: the collision of two cellular division pathways, meiosis and mitosis, resulting in marked genomic instability (meiomitosis).

Meiosis is a cellular division pathway in germ cells that promotes the recombination of large expanses of chromosomal DNA, potentially similar in size to those lost or gained in cancers. In meiosis, homologous chromosomes are held together by cohesions, and after resolution of chiasma (the recombination crossover points with the other chromosome pair), the homologous chromosomes are segregated together (Figure 7). In normal mitosis, there are no chiasma and sister chromatids are directly separated. In meiomitosis, the mitotic cancer cells would also have partially expressed meiotic machinery. The presence of chiasma, cohesins, and an aberrantly assembled mitotic spindle would be expected to result in significant shearing and/or mis-segregation of DNA sequences. Cells surviving this event would likely be aneuploid. Melanoma may be particularly prone to meiomitosis given the high frequency of testis antigen expression in the tumor cells, 77% for at least 1 of 6 antigens in melanoma, 0% in nevi (Luftl *et al.*, 2004). Two of the testis antigens expressed in melanoma are known meiosis proteins (Tureci *et al.*, 1998; Chen *et al.*, 2005), supporting the possibility that meiomitosis plays a role in genomic instability.

Why would germ cell differentiation be important to a tumor? The answer may lie in self-renewal. No cell system is better equipped than the germ cell system to produce cells with an infinite life span. The highest level of telomerase in the adult is in the testis, and its expression is highest in the primary spermatocyte just prior to meiosis I, as it gives rise to the secondary spermatocyte (Bekaert *et al.*, 2004). Thus, tumor cells differentiating along the germ cell pathway may become immortal via telomerase expression but at the cost of meiosis protein expression and genomic instability. It is also tempting to speculate that the reason the germ cell compartment is ''privileged'' is so that if testis antigens are expressed in cells outside the germ cell compartment, they will be destroyed before the cell has a significant chance to express self-renewal and meiotic pathways driving further tumor progression.

Genomic instability has been studied in metastatic melanoma lesions in a patient who survived with the disease for an extended period of time (Wang *et al.*, 2006). The melanoma was found to have a β-catenin mutation and that specific mutation was present in all subsequent metastases. However, karyotyping and genetic analysis revealed marked genomic instability in each of the metastasis and the pattern was sufficiently different from metastasis to metastasis (and from cell to cell in some cultures), as to not be due to a linear progression. These findings suggested that the metastatically competent cell was more genomically intact than the

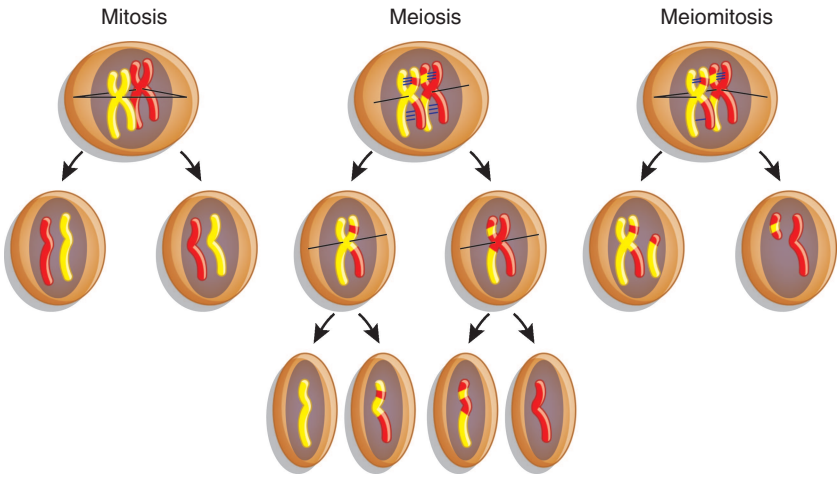

Figure 7. Meiomitosis. During normal mitosis sister chromatids are separated into different cells. During meiosis, homologous chromosomes are held together by cohesions (purple lines) and are segregated together after resolution of chiasma (recombination crossover points) with the other chromosome pair. A second cycle separates the sister chromatids. The term ''meiomitosis'' is used for mitotic cells, in which meiotic machinery is partially expressed. In these cells, chiasma occur and sister chromatids are partially linked together with cohesions. During cell division, the attempt to separate the sister chromatids results in shearing and/or mis-segregation of DNA sequences and creation of an aneuploid state.

rest of the locally produced tumor bulk (Grichnik, 2006a). Thus, ongoing marked genomic instability might hinder the capacity of daughter cells to successfully metastasize.

The stem cell characteristics of melanoma and the expression of testis antigens should lead us to consider the potential role of germ cell differentiation in enhancing self-renewal while creating genomic instability through meiomitosis. Given the destruction wreaked upon the cell with genomic instability, only a subpopulation of cells may remain metastatically competent.

Melanoma stem cell progression model. Melanoma progression and metastasis is traditionally modeled as a stepwise process with the initial mutagenic event occurring in a melanocyte in the epidermis, with further mutation resulting in the proliferation passing through nevus and dysplastic nevus phases. The malignant cells eventually acquire the ability to digest their way through the basement membrane. Then with additional mutations, the cells acquire a migratory form and eventually physically penetrate the lymphatic vessel walls and travel to the local lymph node where they remain trapped until they expand sufficiently to populate the next node in the chain.

There are a number of questions the traditional model cannot readily explain. These include: (1) If dermal invasion is a second malignant step in melanoma development, how do we write off the fact that benign nevi readily ''invade'' the dermis? (2) If precursor nevus and dysplastic nevus stages are critical, then why do 75% of melanomas arise in normal skin? (3) How can we explain the fact that benign nevus cells can also be found in lymph nodes? (4) If the lymph node traps the tumor cells, why does sentinel lymph biopsy and regional lymph node dissection fail to improve survival? (5) Why despite an exuberant immunologic response to the primary melanoma or to the vaccine therapy does the immune system fail to eliminate metastatic disease? And finally (6) How can an aggressive tumor remain dormant for decades and then reoccur?

The stem cell model (Figure 8) more readily addresses many of these issues. In contrast to the traditional theory of melanoma derivation from a differentiated melanocyte, the stem cell model suggests that the initial mutations accumulate in a quiescent stem cell. Eventually, environmental signaling would activate the stem cell, but due to the mutations, proliferation would not be appropriately controlled. Benign tumors would be growth restricted whereas malignant tumors would not. Secondary mutations could occur in the expanding benign cells creating the development of a melanoma within a nevus, but the nevus step would not be required. The neoplastic cells produced would attempt to follow normal melanocytic differentiation pathways including migration into the epidermis. In the epidermis, local growth factors would likely drive further proliferation, and the excess tumor cells would be discharged through the epidermis into the stratum corneum. The stem cell component in the dermis would continue to expand, and some TSCs would inappropriately express germ cell pathways not only allowing for increased self-renewal but also driving genomic instability due to meiomitosis. In the malignant tumors, this would result in different subclones of cells and would display the potentially diagnostic non-uniform surface features (Lucas *et al.*, 2003). Although lymph node metastasis is often modeled as an active process, it is important to recall that carbon and tattoo ink can readily pass from the skin to the lymph node. Some of the dermal cells from both the benign and the malignant tumors could be washed into the lymph system. Thus, although more adherent melanoma cells would bind in the lymph node and possibly migrate toward the capsule, the weakly adherent TSCs may pass straight through the nodal chain and enter the circulatory system. The small quiescent TSCs would be overlooked by the immune system and could continue to circulate long term, but eventually would reinsert in tissues in accordance with normal stem cell regenerative pathways. These TSCs would again activate when the environmental signals where favorable and the process would continue.

Treatment

Regardless of the model applied, it is clear that melanomas are heterogeneous and include a distinct subpopulation of TSCs. These TSCs may be difficult to target with conventional treatments, as they may lack significant proliferation decreasing the effectiveness of anti-proliferative agents, lack significant antigen expression inhibiting vaccine efforts, and may not be

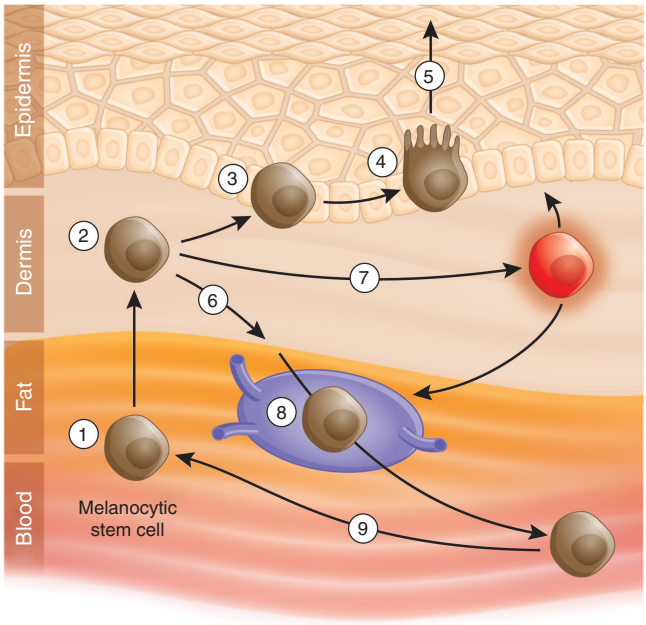

Figure 8. Stem cell-based model of melanocytic neoplasia. In this model, stem cell lineage cells (1) implant into the dermis (2). When necessary to replenish melanocytes in the epidermal compartment, these cells (2) migrate and implant in the basal layer of the epidermis (3). The cells in the epidermis proliferate and differentiate giving rise to mature melanocytes (4). Eventually, the mature melanocytes are shed through the stratum corneum (5). Mutations could occur at any level, but for the tumors with a dermal component, mutations would occur in the dermal cells (2). For tumors confined to the epidermis, mutations could occur in the epidermal cells (3) or in a dermal cell (2) that completely migrated into the epidermis. The mutated cells would attempt to follow normal differentiation pathways, including the shedding of excess cells through the stratum corneum. Some mutations could also result in aberrant dermal migration, including along nerves and blood vessels (6). Cells in the dermis could accumulate additional mutations (7). If the initial stem cell included only mutation for a benign nevus, the additional mutation could then result in a focus of melanoma within the nevus. If the original stem cell included a complement of mutations to create a melanoma, then these additional mutations would create new tumor subpopulations. Loose non-adherent cells (from benign or malignant process) could ''wash'' into the lymph system (8). Some of these cells would stick in the lymph node and cells retaining migratory pathways may migrate into the capsule. The non-adherent stem-like cells may pass through the lymph node and circulate systemically (9) as a mutated stem-like cell (1). This cell could then under the appropriate conditions re-enter the dermis (2) (or some other tissue) and reinitiate the process.

dependent on a targetable mutated pathway while in a quiescent state. In addition, even if the TSCs were susceptible to a particular agent, they may have an enhanced ability to pump the agent out of the cell minimizing its effect (as noted with ATP binding cassette transporters—Frank *et al.*, 2005). Furthermore, even if it was possible to fully eradicate the TSCs, it is also possible that some of the TA cells could revert to TSCs, similar to that noted in the murine melanocytic stem cell experiments (Nishimura *et al.*, 2002). Thus, treatment of this heterogeneous population of melanoma cells will be quite a challenge, requiring eradication of the TSC subpopulation as well as any cells capable of giving rise to more TSCs. For the purpose of discussion, we will consider the TA cells, the only population with the potential capacity to revert to a TSC state, the term TSC/TAs will be used to describe the tumorigenic cell population within the entire heterogeneous population of melanoma cells.

There are a number of treatment approaches that may be considered for the TSC/TAs. One approach would be to activate the TSCs and differentiate the entire TSC/TA population to a chemotherapy sensitive or non-proliferative cell type. A second approach would be a targeted multiantigenic approach against the TSC/TA population either by activating the immune system or by utilizing targeted reagent antibodies. It is possible that targeting the CD133+ melanoma cells in a specific manner will destroy all TSC/TAs, but it is likely that additional approaches will be required. A third approach would be to target a specific mutation or aberrantly activated pathway within the TSC/TA population. However, this therapy might only kill the TAs in which it was active, and this approach might need to be coupled with a reagent activating the quiescent TSCs to make them susceptible. A fourth approach would be to force all the TSC/TA cells into a quiescent state, preventing further development of downstream tumor. These patients would require long-term suppressive therapies.

Thus, TSCs have inherited a number of protective properties that will make them difficult to destroy. It is clear that any potentially successful therapy will need to find creative methods to eradicate the TSC subpopulation as well as eliminating any cells capable of making TSCs (TAs).

CONCLUSION

Stem cells function to replenish terminally differentiated cell populations in adult tissues. It is these stem cells that are the most likely source for cancers. The ultimate stem cell, the fertilized egg, can give rise to over a trillion cells comprising numerous different cell populations. A cancer stem cell potentially has access to this same cellular machinery. The pigmentary system serves as an ideal model for the study of developmental biology and the role of stem cells in tumorigenesis. A discrete series of developmental exposures, *in vivo* or *in vitro*, can differentiate stem cells toward the melanocytic lineage. Certain mutations in stem cells, such as B-Raf activation, may drive melanocytic differentiation without the need for external signaling pathways. Both benign and malignant melanocytic tumors develop directly on the skin surface allowing for the evaluation of early events. Through the study of melanoma and nevus development, it is likely we will be able to better understand the early events and the role of stem cell biology in tumorigenesis.

Numerous studies have demonstrated the existence of TSCs within human melanomas. Given the pre-

sence of TSC in melanomas and the presence of stem cell populations in human skin, it is reasonable to assume that the skin stem cells serve as the cells of origin for melanoma development. However, the specific stem cell type involved in the transformation process and its location in the skin remains undefined. Although many of the stem cell population studies have focused on follicular stem cells, most melanomas do not appear to have a follicular origin. Given the normal developmental direction of migration, it seems likely that invasive melanomas are based on a stem cell within the dermis. Nevi would also be based on this dermal stem cell, and existence of blue nevi (heavily pigmented melanocytic neoplasm that originates in the dermis without any epidermal or follicular involvement) also supports a derivation from a melanocytic stem cell present in the dermis.

A promising marker for dermal stem cells is CD133. CD133+ cells have been shown to give rise to endothelial cells (Gehling *et al.*, 2000), mast cells (Dahl *et al.*, 2002), and neurons and glial cells (Uchida *et al.*, 2000). Adipocytes, osteocytes, and chondrocytes have also been generated (Tondreau *et al.*, 2005). Although melanocytes have not yet been reported to differentiate from this population, the presence of CD133+ melanoma TSC is highly suggestive. Therefore, it is possible that different mutations in a CD133+ stem cell could result in different tumor types, including malignant melanoma, benign nevi, hemangiomas, lipomas, neurofibromas, and mastocytomas. CD133+ cells have been noted to be increased in circulation after trauma (Liu *et al.*, 2007). It is also feasible that mutant (possibly B-Raf) CD133+ stem cells could be mobilized from a patient's bone marrow due to their immunosuppressed state, and present in the skin as eruptive nevi. The fact that only the CD133+ melanoma cells are tumorigenic (Monzani *et al.*, 2007) supports the role of a stem cell-driven process.

Although melanoma derivation from a stem cell seems likely, the potential role for dedifferentiation from a melanocyte cannot be ruled out. Support for a dedifferentiation process includes that fact that melanized quail cells can dedifferentiate into multipotent stem cells (Real *et al.*, 2006), oncogenes added to cultured melanocytes can create tumors (Chudnovsky *et al.*, 2005), and genes driven from the tyrosinase promoter can give rise to melanomas in transgenic mice (Kelsall and Mintz, 1998; Wong and Chin, 2000). Certainly, developmental models include plasticity, allowing neighboring cells to assume the function of cells lost during development. Thus, normal developmental biology does allow for both ''stem cell'' and ''dedifferentiation/plasticity'' mechanisms. At this point, it is reasonable to assume that both mechanisms may be at play to varying degrees in melanoma.

Melanocytic tumors are probably best viewed as a pliable developmental system with a TSC component. These tumors may be best defined by four biologic parameters. The first parameter is the nature of the critical pathway(s) that have been mutated in the tumor cells. These pathway(s) are likely to define the direction of cellular differentiation as well as the regulation over the proliferative and apoptotic process. The second parameter is the range of cell's differentiation/plasticity capacity. The extent of this capacity may be, in part, defined by the mutated pathway(s), but it may also be defined by the state of differentiation (organization of the nuclear matrix) in the cell of origin. Theoretically, tumor origin in a stem cell would allow for a far greater range of differentiation/plasticity capacity. This capacity would allow cells to better survive under different environmental conditions and allow for greater diversity in expression programs, such as allowing germ cell pathway differentiation, which may be advantageous for the expression of telomerase but hazardous due to the potential for meiomitosis. The third parameter is the intactness of the genome. Increasing genomic instability will result in the loss of primary DNA sequence data. Surviving cells may benefit initially from acceleration of certain pathways but at the same time will loose other potentially useful capabilities. The fourth and final parameter is telomere length. As telomeres shorten, the cells ability to continue to replicate is diminished. Thus, when viewed as a pliable, but mutated, developmental system, it may be necessary to consider these four different facets in defining the neoplasm's ultimate behavior.

In summary, melanoma tumors, similar to other solid cancers, clearly include a subpopulation of TSCs. These cells appear to have the unique capacity to perpetuate the tumor as well as the capacity to differentiate along different cellular developmental lines. The melanocytic system is a model system for normal and abnormal adult tissue stem cell biology. The development of these tumors on the skin surface and the advance of skin surface imaging technologies will help us to study the complexities of the oncologic process. The lessons learned will be able to be applied across many solid tumor types and will have ramifications for tumor development, diagnosis, prognosis, and treatment.

CONFLICT OF INTEREST

Founder and major shareholder—Digital Derm Inc.—MoleMapCD (total body photography). Consulting and Grants—Electro-Optical Sciences Inc.—Melafind (melanoma detection device) and Spectral Image Inc.—Dermatologic Diagnostics.

ACKNOWLEDGMENTS

Division of Dermatology and the Benefactors to the Duke Comprehensive Cancer center, especially Fred and Sharon Matt (in honor of James Matt), Carolyn Holding, Carmen Ames, and Rebecca Hundley for their generous financial support, Harold Rabinovitz for his image of the melanoma with blue features, and KP Grichnik, KK Hipp, and JA Burch for their editorial review.

REFERENCES

Alanko T, Rosenberg M, Saksela O (1999) FGF expression allows nevus cells to survive in three-dimensional collagen gel under conditions that induce apoptosis in normal human melanocytes. *J Invest Dermatol* 113:111–6

Alexeev V, Yoon K (2006) Distinctive role of the cKit receptor tyrosine kinase signaling in mammalian melanocytes. *J Invest Dermatol* 126:1102–10

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 100:3983–8

Amoh Y, Li L, Katsuoka K, Penman S, Hoffman RM (2005) Multipotent nestin-positive,

keratin-negative hair-follicle bulge stem cells can form neurons. *Proc Natl Acad Sci USA* 102:5530–4

Arbiser JL (2003) Activation of B-raf in non-malignant nevi predicts a novel tumor suppressor gene in melanoma (MAP kinase phosphatase). *J Invest Dermatol* 121:xiv

Banky JP, Kelly JW, English DR, Yeatman JM, Dowling JP (2005) Incidence of new and changed nevi and melanomas detected using baseline images and dermoscopy in patients at high risk for melanoma. *Arch Dermatol* 141:998–1006

Bastian BC, Kashani-Sabet M, Hamm H, Godfrey T, Moore DH II, Brocker EB *et al.* (2000) Gene amplifications characterize acral melanoma and permit the detection of occult tumor cells in the surrounding skin. *Cancer Res* 60:1968–73

Bataille V, Kato BS, Falchi M, Gardner J, Kimura M, Lens M *et al.* (2007) Nevus size and number are associated with telomere length and represent potential markers of a decreased senescence *in vivo*. *Cancer Epidemiol Biomarkers Prev* 16:1499–502

Bauer J, Curtin JA, Pinkel D, Bastian BC (2007) Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. *J Invest Dermatol* 127:179–82

Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE *et al.* (1994) Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* 79:1277–85

Bekaert S, Derradji H, Baatout S (2004) Telomere biology in mammalian germ cells and during development. *Dev Biol* 274:15–30

Belicchi M, Pisati F, Lopa R, Porretti L, Fortunato F, Sironi M *et al.* (2004) Human skin-derived stem cells migrate throughout forebrain and differentiate into astrocytes after injection into adult mouse brain. *J Neurosci Res* 77: 475–86

Bennett DC, Medrano EE (2002) Molecular regulation of melanocyte senescence. *Pigment Cell Res* 15:242–50

Bevona C, Goggins W, Quinn T, Fullerton J, Tsao H (2003) Cutaneous melanomas associated with nevi. *Arch Dermatol* 139:1620–4

Bickenbach JR, Grinnell KL (2004) Epidermal stem cells: interactions in developmental environments. *Differentiation* 72:371–80

Blanpain C, Fuchs E (2006) Epidermal stem cells of the skin. *Annu Rev Cell Dev Biol* 22:339–73

Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3:730–7

Botchkareva NV, Khlgatian M, Longley BJ, Botchkarev VA, Gilchrest BA (2001) SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. *FASEB J* 15:645–58

Burkhart CG, Burkhart CN (2005) The mole theory: primary function of melanocytes and melanin may be antimicrobial defense and immunomodulation (not solar protection). *Int J Dermatol* 44:340–2

Charames GS, Bapat B (2003) Genomic instability and cancer. *Curr Mol Med* 3:589–96

Chen YT, Venditti CA, Theiler G, Stevenson BJ, Iseli C, Gure AO *et al.* (2005) Identification of CT46/HORMAD1, an immunogenic cancer/testis antigen encoding a putative meiosis-related protein. *Cancer Immun* 5:9

Chudnovsky Y, Adams AE, Robbins PB, Lin Q, Khavari PA (2005) Use of human tissue to assess the oncogenic activity of melanoma-associated mutations. *Nat Genet* 37:745–9

Cotsarelis G (2006) Epithelial stem cells: a folliculocentric view. *J Invest Dermatol* 126: 1459–68

Cramer SF (1984) The histogenesis of acquired melanocytic nevi. Based on a new concept of melanocytic differentiation. *Am J Dermatopathol* 6:289–98

Cramer SF (1991) The origin of epidermal melanocytes. Implications for the histogenesis of nevi and melanomas. *Arch Pathol Lab Med* 115:115–9

Curtin JA, Busam K, Pinkel D, Bastian BC (2006) Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 24:4340–6

Dahl C, Saito H, Nielsen HV, Schiotz PO (2002) The establishment of a combined serum-free and serum-supplemented culture method of obtaining functional cord blood-derived human mast cells. *J Immunol Methods* 262: 137–43

Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW *et al.* (2007) Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 104:10158–63

de Cholnoky T (1941) Malignant Melanoma: A clinical study of one hundred seventeen cases. *Ann Surg* 113:392–410

Dupin E, Calloni G, Real C, Goncalves-Trentin A, Le Douarin NM (2007) Neural crest progenitors and stem cells. *C R Biol* 330:521–9

Etienne G, Cony-Makhoul P, Mahon FX (2002) Imatinib mesylate and gray hair. *N Engl J Med* 347:446

Ewing J (1930) The Problems of Melanoma. *B J Med* 11:852–6

Fang D, Leishear K, Nguyen TK, Finko R, Cai K, Fukunaga M *et al.* (2006) Defining the conditions for the generation of melanocytes from human embryonic stem cells. *Stem Cells* 24:1668–77

Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S *et al.* (2005) A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 65:9328–37

Farmer PJ, Gidanian S, Shahandeh B, Di Bilio AJ, Tohidian N, Meyskens FL Jr (2003) Melanin as a target for melanoma chemotherapy: pro-oxidant effect of oxygen and metals on melanoma viability. *Pigment Cell Res* 16: 273–9

Fernandes KJ, McKenzie IA, Mill P, Smith KM, Akhavan M, Barnabe-Heider F *et al.* (2004) A dermal niche for multipotent adult skin-derived precursor cells. *Nat Cell Biol* 6:1082–93

Frank NY, Margaryan A, Huang Y, Schatton T, Waaga-Gasser AM, Gasser M *et al.* (2005) ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. *Cancer Res* 65:4320–33

Frank NY, Pendse SS, Lapchak PH, Margaryan A, Shlain D, Doeing C *et al.* (2003) Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter. *J Biol Chem* 278: 47156–65

Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M *et al.* (2000) *In vitro* differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 95:3106–12

Gilley D, Tanaka H, Herbert BS (2005) Telomere dysfunction in aging and cancer. *Int J Biochem Cell Biol* 37:1000–13

Grichnik JM (2006a) Genomic instability and tumor stem cells. *J Invest Dermatol* 126: 1214–6

Grichnik JM (2006b) Kit and melanocyte migration. *J Invest Dermatol* 126:945–7

Grichnik JM, Ali WN, Burch JA, Byers JD, Garcia CA, Clark RE *et al.* (1996) KIT expression reveals a population of precursor melanocytes in human skin. *J Invest Dermatol* 106:967–71

Grichnik JM, Burch JA, Burchette J, Shea CR (1998) The SCF/KIT pathway plays a critical role in the control of normal human melanocyte homeostasis. *J Invest Dermatol* 111:233–8

Grichnik JM, Burch JA, Schulteis RD, Shan S, Liu J, Darrow TL *et al.* (2006) Melanoma, a tumor based on a mutant stem cell? *J Invest Dermatol* 126:142–53

Grichnik JM, Crawford J, Jimenez F, Kurtzberg J, Buchanan M, Blackwell S *et al.* (1995) Human recombinant stem-cell factor induces melanocytic hyperplasia in susceptible patients. *J Am Acad Dermatol* 33:577–83

Halaban R (2000) The regulation of normal melanocyte proliferation. *Pigment Cell Res* 13:4–14

Halaban R, Ghosh S, Duray P, Kirkwood JM, Lerner AB (1986) Human melanocytes cultured from nevi and melanomas. *J Invest Dermatol* 87:95–101

Hardy K, Martin KL, Leese HJ, Winston RM, Handyside AH (1990) Human preimplantation development *in vitro* is not adversely affected by biopsy at the 8-cell stage. *Hum Reprod* 5:708–14

Hendrix MJ, Seftor EA, Hess AR, Seftor RE (2003) Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. *Nat Rev Cancer* 3:411–21

Hendrix MJ, Seftor EA, Seftor RE, Kasemeier-Kulesa J, Kulesa PM, Postovit LM (2007) Reprogramming metastatic tumour cells with embryonic microenvironments. *Nat Rev Cancer* 7:246–55

Hirobe T (2005) Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian

epidermal melanocytes. *Pigment Cell Res* 18:2–12

Ho MM, Ng AV, Lam S, Hung JY (2007) Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer Res* 67:4827–33

Hong L, Simon JD (2007) Current understanding of the binding sites, capacity, affinity, and biological significance of metals in melanin. *J Phys Chem B* 111:7938–47

Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A *et al.* (1994) Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 79:1267–76

Hu YF, Zhang ZJ, Sieber-Blum M (2006) An epidermal neural crest stem cell (EPI-NCSC) molecular signature. *Stem Cells* 24:2692–702

Hui P, Perkins A, Glusac E (2001) Assessment of clonality in melanocytic nevi. *J Cutan Pathol* 28:140–4

Ichii-Nakato N, Takata M, Takayanagi S, Takashima S, Lin J, Murata H *et al.* (2006) High frequency of BRAFV600E mutation in acquired nevi and small congenital nevi, but low frequency of mutation in medium-sized congenital nevi. *J Invest Dermatol* 126:2111–8

Jiao Z, Zhang ZG, Hornyak TJ, Hozeska A, Zhang RL, Wang Y *et al.* (2006) Dopachrome tautomerase (Dct) regulates neural progenitor cell proliferation. *Dev Biol* 296:396–408

Kantor GR, Wheeland RG (1987) Transepidermal elimination of nevus cells. A possible mechanism of nevus involution. *Arch Dermatol* 123:1371–4

Kavak A, Akcan Y, Korkmaz U (2005) Hair repigmentation in a hepatitis C patient treated with interferon and ribavirin. *Dermatology* 211:171–2

Kelsall SR, Mintz B (1998) Metastatic cutaneous melanoma promoted by ultraviolet radiation in mice with transgene-initiated low melanoma susceptibility. *Cancer Res* 58: 4061–5

Klein WM, Wu BP, Zhao S, Wu H, Klein-Szanto AJ, Tahan SR (2007) Increased expression of stem cell markers in malignant melanoma. *Mod Pathol* 20:102–7

Kondo T, Setoguchi T, Taga T (2004) Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. *Proc Natl Acad Sci USA* 101:781–6

Kraemer KH, Lee MM, Andrews AD, Lambert WC (1994) The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. *Arch Dermatol* 130:1018–21

Krengel S, Hauschild A, Schafer T (2006) Melanoma risk in congenital melanocytic naevi: a systematic review. *Br J Dermatol* 155:1–8

Kumamoto T, Shalhevet D, Matsue H, Mummert ME, Ward BR, Jester JV *et al.* (2003) Hair follicles serve as local reservoirs of skin mast cell precursors. *Blood* 102:1654–60

Kunisada T, Lu SZ, Yoshida H, Nishikawa S, Nishikawa S, Mizoguchi M *et al.* (1998) Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. *J Exp Med* 187:1565–73

Lahav R, Heffner G, Patterson PH (1999) An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 96:11496–500

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J *et al.* (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 367:645–8

Lee JT, Herlyn M (2007) Microenvironmental influences in melanoma progression. *J Cell Biochem* 101:862–72

Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V *et al.* (2007) Identification of pancreatic cancer stem cells. *Cancer Res* 67:1030–7

Liang L, Bickenbach JR (2002) Somatic epidermal stem cells can produce multiple cell lineages during development. *Stem Cells* 20:21–31

Lin F, Burch JA, Singer S, Grichnik JM (2000) SCF induced proliferation of human melanocytes is masked *in vitro* by bFGF. *J Invest Dermatol* 114:859 (abstract)

Liu L, Liu H, Jao J, Liu H, Bergeron A, Dong JF *et al.* (2007) Changes in circulating human endothelial progenitor cells after brain injury. *J Neurotrauma* 24:936–43

Lucas CR, Sanders LL, Murray JC, Myers SA, Hall RP, Grichnik JM (2003) Early melanoma detection: nonuniform dermoscopic features and growth. *J Am Acad Dermatol* 48:663–71

Luftl M, Schuler G, Jungbluth AA (2004) Melanoma or not? Cancer testis antigens may help. *Br J Dermatol* 151:1213–8

Mackenzie MA, Jordan SA, Budd PS, Jackson IJ (1997) Activation of the receptor tyrosine kinase Kit is required for the proliferation of melanoblasts in the mouse embryo. *Dev Biol* 192:99–107

Mancianti ML, Gyorfi T, Shih IM, Valyi-Nagy I, Levengood G, Menssen HD *et al.* (1993) Growth regulation of cultured human nevus cells. *J Invest Dermatol* 100:281S–7S

Megahed M, Schon M, Selimovic D, Schon MP (2002) Reliability of diagnosis of melanoma *in situ*. *Lancet* 359:1921–2

Meyskens Jr FL, Farmer PJ, Anton-Culver H (2004) Etiologic pathogenesis of melanoma: a unifying hypothesis for the missing attributable risk. *Clin Cancer Res* 10:2581–3

Molino A, Lucas C, Benitez-Graham A, Burch JA, Herman C, Selim A *et al.* (2003) Immature dermal melanocytic cells are present in growing melanocytic neoplasms. *J Invest Dermatol* 121:1174 (abstract)

Monzani E, Facchetti F, Galmozzi E, Corsini E, Benetti A, Cavazzin C *et al.* (2007) Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur J Cancer* 43:935–46

Motohashi T, Aoki H, Chiba K, Yoshimura N, Kunisada T (2007) Multipotent cell fate of neural crest-like cells derived from embryonic stem cells. *Stem Cells* 25:402–10

Na GY, Paek SH, Park BC, Kim DW, Lee WJ, Lee SJ *et al.* (2006) Isolation and characterization of outer root sheath melanocytes of human hair follicles. *Br J Dermatol* 155:902–9

Nishikawa S, Kusakabe M, Yoshinaga K, Ogawa M, Hayashi S, Kunisada T *et al.* (1991) *In utero* manipulation of coat color formation by a monoclonal anti-c-kit antibody: two distinct waves of c-kit-dependency during melanocyte development. *EMBO J* 10:2111–8

Nishikawa S, Osawa M (2007) Generating quiescent stem cells. *Pigment Cell Res* 20:263–70

Nishimura EK, Granter SR, Fisher DE (2005) Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* 307:720–4

Nishimura EK, Jordan SA, Oshima H, Yoshida H, Osawa M, Moriyama M *et al.* (2002) Dominant role of the niche in melanocyte stem-cell fate determination. *Nature* 416: 854–60

Oback B, Wells DN (2007) Donor cell differentiation, reprogramming, and cloning efficiency: elusive or illusive correlation? *Mol Reprod Dev* 74:646–54

O'Brien CA, Pollett A, Gallinger S, Dick JE (2007) A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 445:106–10

Okura M, Maeda H, Nishikawa S, Mizoguchi M (1995) Effects of monoclonal anti-c-kit antibody (ACK2) on melanocytes in newborn mice. *J Invest Dermatol* 105:322–8

Peters EM, Tobin DJ, Botchkareva N, Maurer M, Paus R (2002) Migration of melanoblasts into the developing murine hair follicle is accompanied by transient c-Kit expression. *J Histochem Cytochem* 50:751–66

Petronic-Rosic V, Shea CR, Krausz T (2004) Pagetoid melanocytosis: when is it significant? *Pathology (Phila)* 36:435–44

Pharis DB, Zitelli JA (2001) Sunburn, trauma, and the timing of biopsies of melanocytic nevi. *Dermatol Surg* 27:835–6

Pierce KE, Michalopoulos J, Kiessling AA, Seibel MM, Zilberstein M (1997) Preimplantation development of mouse and human embryos biopsied at cleavage stages using a modified displacement technique. *Hum Reprod* 12:351–6

Pizzichetta MA, Massone C, Grandi G, Pelizzo G, Soyer HP (2006) Morphologic changes of acquired melanocytic nevi with eccentric foci of hyperpigmentation (''Bolognia sign'') assessed by dermoscopy. *Arch Dermatol* 142:479–83

Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P *et al.* (2007) Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 104:973–8

Real C, Glavieux-Pardanaud C, Le Douarin NM, Dupin E (2006) Clonally cultured differentiated pigment cells can dedifferentiate and generate multipotent progenitors with self-renewing potential. *Dev Biol* 300:656–69

Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414:105–11

Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C *et al.* (2007) Identification and expansion of human colon-cancer-initiating cells. *Nature* 445:111–5

Robinson WA, Lemon M, Elefanty A, Harrison-Smith M, Markham N, Norris D (1998) Human acquired naevi are clonal. *Melanoma Res* 8:499–503

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J *et al.* (2003) Identification of a cancer stem cell in human brain tumors. *Cancer Res* 63:5821–8

Slominski A, Wortsman J, Tuckey RC, Paus R (2007) Differential expression of HPA axis homolog in the skin. *Mol Cell Endocrinol* 265–266:143–9

Stark M, Hayward N (2007) Genome-wide loss of heterozygosity and copy number analysis in melanoma using high-density single-nucleotide polymorphism arrays. *Cancer Res* 67:2632–42

Takeda K, Takahashi NH, Shibahara S (2007) Neuroendocrine functions of melanocytes: beyond the skin-deep melanin maker. *Tohoku J Exp Med* 211:201–21

Tobin DJ, Hagen E, Botchkarev VA, Paus R (1998) Do hair bulb melanocytes undergo apoptosis during hair follicle regression (catagen)? *J Invest Dermatol* 111:941–7

Toma JG, Akhavan M, Fernandes KJ, Barnabe-Heider F, Sadikot A, Kaplan DR *et al.* (2001) Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 3:778–84

Tondreau T, Meuleman N, Delforge A, Dejeneffe M, Leroy R, Massy M *et al.* (2005) Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, Oct4 expression, and plasticity. *Stem Cells* 23:1105–12

Tsao H, Goel V, Wu H, Yang G, Haluska FG (2004) Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. *J Invest Dermatol* 122:337–41

Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M *et al.* (2004) Defining the epithelial stem cell niche in skin. *Science* 303:359–63

Tureci O, Sahin U, Zwick C, Koslowski M, Seitz G, Pfreundschuh M (1998) Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens. *Proc Natl Acad Sci USA* 95:5211–6

Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV *et al.* (2000) Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci USA* 97:14720–5

Wang E, Voiculescu S, Le Poole IC, El-Gamil M, Li X, Robbins PF *et al.* (2006) Clonal persistence and evolution during a decade of recurrent melanoma. *J Invest Dermatol* 126: 1372–7

Wilkie AL, Jordan SA, Jackson IJ (2002) Neural crest progenitors of the melanocyte lineage: coat colour patterns revisited. *Development* 129:3349–57

Wong AK, Chin L (2000) An inducible melanoma model implicates a role for RAS in tumor maintenance and angiogenesis. *Cancer Metastasis Rev* 19:121–9

Wong CE, Paratore C, Dours-Zimmermann MT, Rochat A, Pietri T, Suter U *et al.* (2006) Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. *J Cell Biol* 175: 1005–15

Worret WI, Burgdorf WH (1998) Which direction do nevus cells move? Abtropfung reexamined. *Am J Dermatopathol* 20:135–9

Yamane T, Hayashi S, Mizoguchi M, Yamazaki H, Kunisada T (1999) Derivation of melanocytes from embryonic stem cells in culture. *Dev Dyn* 216:450–8

Yang CT, Johnson SL (2006) Small molecule-induced ablation and subsequent regeneration of larval zebrafish melanocytes. *Development* 133:3563–73

Yoshida H, Kunisada T, Kusakabe M, Nishikawa S, Nishikawa SI (1996) Distinct stages of melanocyte differentiation revealed by analysis of nonuniform pigmentation patterns. *Development* 122:1207–14

Yu H, Fang D, Kumar SM, Li L, Nguyen TK, Acs G *et al.* (2006) Isolation of a novel population of multipotent adult stem cells from human hair follicles. *Am J Pathol* 168:1879–88

Yu Y, Flint A, Dvorin EL, Bischoff J (2002) AC133-2, a novel isoform of human AC133 stem cell antigen. *J Biol Chem* 277:20711–6

Zalaudek I, Ferrara G, Argenziano G (2007) Dermoscopy insights into nevogenesis: ''Abtropfung'' vs ''Hochsteigerung''. *Arch Dermatol* 143:284

Zalaudek I, Grinschgl S, Argenziano G, Marghoob AA, Blum A, Richtig E *et al.* (2006) Age-related prevalence of dermoscopy patterns in acquired melanocytic naevi. *Br J Dermatol* 154:299–304