

## MINI REVIEW

# Integrating tumor-initiating cells into the paradigm for melanoma targeted therapy

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There is growing evidence to suggest that not all cancer cells have similar levels of malignant potential and that tumor progression may be driven by specialized sub-sets of “tumor initiating” cells. It is likely that as tumor initiating cells have lower proliferation rates and enhanced survival mechanisms they may also drive drug resistance. Melanoma is known to be an exceptionally therapy resistant tumor, with no treatment yet identified to alter the natural progression of the disseminated disease. In the current review, we discuss evidence for the existence of melanoma initiating cells and described possible therapeutic strategies to eradicate this population via the targeting of specific cell-surface markers or through the disruption of the interaction of the melanoma initiating cells with their local microenvironment. It is hoped that the targeting of melanoma initiating cells may be one approach to overcome the incredible therapy resistance of this tumor.

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The clinical management of melanoma remains difficult, with no therapeutic intervention tried thus far able to halt the progression of disseminated disease. Recent work has suggested that the presence of malignantly transformed populations of stem cells may underlie the initiation and drug resistance of many tumor types. In the current review, we discuss the evidence for melanomas arising from transformed stem cells, and how this may impact upon future approaches to melanoma targeted therapy.

### Stem cell hierarchies in normal tissues and the melanocytic system

Tumors typically arise in tissues with rapid cell turnover, such as the gastrointestinal tract, the hematopoietic system and the skin. In these organs, there is a continuous ordered cycle of cell proliferation that replaces the short-lived differentiated cells. This cell proliferation is a highly controlled process, whereby a small pool of self-renewing stem cells gives rise to a population of proliferating progenitor cells that undergo limited rounds of cell division before reaching a state of terminal differentiation. In this system only the stem cells are long-lived, and they have the unique property of being able to undergo self-renewing cell division where at least one of the progeny “daughter cells” remains as a stem cell—a process termed asymmetric cell division. The daughter cells then either remain as stem cells or undergo a further process of differentiation to become either a multi-potent progenitor or a transient amplifying cell. It is through the generation of many transient amplifying cells that stem cells can generate large cell numbers to repopulate entire tissues. As transient amplifying cells undergo multiple rounds of cell division their progeny become progressively more differentiated and start losing their potential for further cell proliferation. This delicate balance between self-renewal and differentiation is critical in retaining the size of stem cell pool. Stem cells exist within a specialized microenvironment termed as stem cell niche. The niche plays a critical role in maintaining the undifferentiated state of stem cell pool through the provision of paracrine and extracellular matrix signals. In human and

Murine skin, melanocyte stem cells reside in the hair follicle bulge of the lower permanent portion of the hair follicle.<sup>1,2</sup> During the growth phase of the hair follicle or wound healing, signals from the hair bulge niche provide the melanocyte stem cells with the necessary signals for growth and differentiation. Although the exact nature of these signals is not known, recent work has shown that WNT-3a may play a role in this process.<sup>3</sup>

### Do cancers arise from oncogenically transformed stem cells?

The past few years have seen rising support for the idea that tumor initiation and progression is driven by the oncogenic transformation of a stem cell population. The cancer stem cell theory postulates that tumor formation, growth and metastatic spread are driven by a minority population of tumor initiating or cancer stem cells. The hypothesis explains a number of key observations that have perplexed the cancer research community for many years; such as why the majority of tumor cells are actually nontumorigenic and readily transplantable into new hosts. In a similar vein, the genetic model of cancer predicts that a serial acquisition of oncogenes and inactivation of tumor suppressors is required for oncogenic transformation. This model, which would require a 10–30 year time frame, has always been difficult to reconcile with relatively short lifespan of most somatic cells.

Most of the known tumor-initiating cells come from a series of pioneering studies from the hematology field. Early transplantation experiments on lethally irradiated mice, where the native bone marrow was completely eradicated, showed that a minority population of cells (equal to about 0.05% of total bone marrow) was able to completely reconstitute the mouse hematopoietic system.<sup>4</sup> Further investigation revealed that these “regenerating cells” were from a very primitive lineage that lacked every known hematopoietic cell surface marker. Subsequent work has shown the hematopoietic system to possess a well-structured cellular hierarchy, in which only the stem cells have the ability to self-renew and generate the entire range of differentiated cells. Transplantation of the more differentiated transient-amplifying cell population was unable to reconstitute the hematopoietic system. As these early studies on hematopoiesis have been applied to the study of leukemia and show that only a specialized population of acute myeloid leukemic (AML) cells, with the cell surface markers CD34<sup>+</sup>/CD38<sup>-</sup>, were able to transplant the disease into a recipient animal.<sup>4</sup> Infusion of more differentiated leukemic cells into similar mice were unable to transplant the AML.<sup>4</sup> The leukemia-initiating cells were further shown to have all of the important key features of stem cells, in that they had the potential for self-renewal, asymmetric cell division and could differentiate into multiple lineages. This work led to the proposal that leukemia was the

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result of aberrant hematopoiesis and that the leukemia-initiating cells were in fact oncogenically transformed stem cells. Thus, a central aspect of the tumor-initiating cell model is the observation that not all cancer cells have equal tumorigenic potential, and that tumors are made up of heterogeneous cell populations with varying states of differentiation and malignancy.

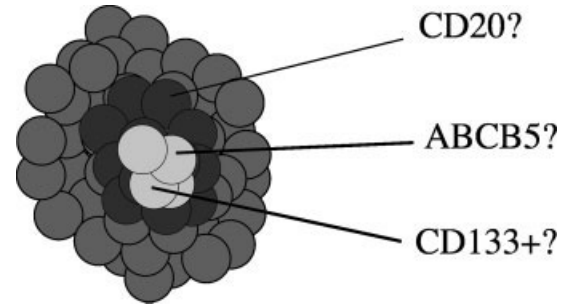
### Characterizing the tumor initiating cells

There are many similarities between stem cells and tumor-initiating cells. Both populations are known to be relatively quiescent and have multiple mechanisms to overcome exogenous genotoxic stimuli, through the high expression levels of ATP-binding cassette (ABC)-family drug transporters and an increased capacity for DNA repair. Similarly, both tumor cells and stem cells are able to suppress the activity of p53, following genotoxic insult, leading to reduced levels of apoptosis.<sup>5</sup> In addition, both normal stem cells and over 90% of human tumors express the enzyme telomerase that maintains chromosomal integrity at the chromosome ends following prolonged rounds of cell proliferation.<sup>6</sup>

In addition to leukemia, putative tumor-initiating populations have now also been identified in many solid tumors.<sup>7</sup> Tumor-initiating cells share key characteristics of normal stem cells in that they can self-renew and differentiate into more benign differentiated cells that cannot initiate new tumors. They differ from normal stem cells in that they have escaped the physiological growth controls imposed by the niche and proliferate in an uncontrolled manner. Although the tumor-initiating population and the nontumorigenic population of cancer cells may contain similar oncogenic mutations, the nontumorigenic population lacks the capacity to self-renew. Whilst compelling, these arguments are not direct proof of the existence of cancer stem cells, as it is well known that malignant potential can be regulated through the local microenvironment and via epigenetic means. Evidence for the epigenetic regulation of transformed cells comes from embryology studies showing that the injection of embryonal carcinoma cells into a blastocyst gives rise to a normal chimeric mouse.<sup>8</sup> It has also been shown that nuclear transfer from a mouse melanoma cell into an oocyte can give rise to a normal (albeit more cancer-prone) mouse,<sup>9</sup> suggesting that even a normal cellular milieu can regulate oncogenic behavior. It is therefore clear that even highly malignant cells can give rise to differentiated, benign progeny, and that a "normal" cellular environment can hold even the most malignant genetic changes in check.

The first evidence of tumor-initiating cells in a solid tumor came from studies on breast cancer, where it was shown that only a minor sub-population of tumor cells isolated from patient lesions were able to establish new tumors in mice.<sup>10</sup> Again, the tumorigenic cells were distinguished from the nontumorigenic population on the basis of cell surface marker selection and were defined as being CD24<sup>-low</sup>/CD44<sup>+</sup>.<sup>10</sup> Limiting dilution xenograft experiments showed that tumors could be initiated from as little as 100 of the CD24<sup>-low</sup>/CD44<sup>+</sup> cells. In contrast, injections of tens of thousands of the non-CD24<sup>-low</sup>/CD44<sup>+</sup> cells were unable to initiate tumor growth in mice. Histological evaluation of the resulting tumors from the CD24<sup>-low</sup>/CD44<sup>+</sup> mice showed that the full phenotypic heterogeneity of the original breast tumor could be recapitulated by these few tumor-initiating cells. Since this initial work, similar tumor-initiating cell populations have been identified in colon,<sup>11</sup> brain<sup>12</sup> and prostate cancer,<sup>13</sup> as well as melanoma.<sup>14</sup>

The evidence for the presence of cancer stem cells within solid tumors is not as compelling as that for hematological malignancies. Part of the difficulty in confirming the leukemia data in solid tumors has been the lack of good differentiation markers and defined hierarchal lineages. As a result, it is not clear whether the correct cell surface markers have been selected, this making it difficult to obtain highly purified populations of stem cells. As a consequence, the sub-grouping of putative solid tumor cancer stem cells on the basis of cell surface markers (such as CD133<sup>+</sup>), has



**FIGURE 1** – Melanomas are made up of heterogeneous populations of cells, some with enhanced tumorigenic potential. Studies have shown that melanoma sub-populations with increased CD20 and ABCB5 expression identify a melanoma-initiating population. Other ongoing studies point to a possible role of CD133 in melanoma.

resulted in relatively large sub-groups, often accounting for up to 20% of the total cell number. This lack of proper cell fractionation has raised the possibility that CD133<sup>+</sup> stromal/supporting cells are being transferred along with the tumor cell population. The presence of contaminating cells may be an important issue, as it is known that CD133<sup>+</sup> endothelial cell precursors can also enhance the growth of transplanted human cancer cells *in vivo*.<sup>15</sup>

Our group has recently identified a slow growing melanoma cell population with enhanced tumor forming ability in SCID mice.<sup>14</sup> Initial isolation of these cells was based upon their ability to grow in embryonic stem cell media. These cells were shown to be CD20<sup>+</sup> and to differentiate into multiple cellular lineages, such as adipocytes, chondrocytes and osteocytes. There is some suggestion that this CD20<sup>+</sup> population of melanoma cells may harbor the tumor initiating population. Although well characterized, these CD20<sup>+</sup> cells often have an unstable phenotype in culture, and we have begun the search for more robust stem cell population markers.

It is highly likely that melanomas may contain multiple populations of potential tumor-initiating cells, each with a different set of cell surface markers (Fig. 1). A recent study from the laboratory of Markus Frank at Harvard identified another putative population of melanoma initiating cells, characterized by the expression of a novel member of the ABC drug transporter family (ABCB5).<sup>16</sup> Sorting of the cells into ABCB5<sup>+</sup> and ABCB5<sup>-</sup> fractions showed that the ABCB5<sup>+</sup> cell population had an enhanced tumor forming ability *in vivo* and that these tumors could be serially transplanted into new animals.<sup>16</sup> It was further shown that administration of a monoclonal antibody against ABCB5 significantly reduced melanoma growth, demonstrating the requirement of an ABCB5<sup>+</sup> population to propagate melanoma growth. Another recent study identified a minor population of melanoma cells with enhanced tumorigenic potential that stained positively for CD133 and the ABC transporter protein ABCG2.<sup>17</sup> As the ABC transporters are multi-drug resistance pumps involved in drug resistance, these studies raise the intriguing possibility that the presence of melanoma-initiating cells is linked to the intrinsic chemoresistance of melanoma. It further suggests that the melanoma-initiating cells may be targeted pharmacologically, as a number of small molecule inhibitors exist that target the ABC-family of drug transporters.<sup>18</sup>

### Controversies in the "stem cell" theory of cancer

One of the central tenets of the cancer stem cell model is that tumor growth is driven by the presence of rare cells with unlimited self-renewal potential. Much of this work is based on observations from the hematology field, where normal hematopoietic stem cells can restore normal hematopoiesis to mice that have been sublethally irradiated. The hematological model presents a cellular hierarchy where only very restricted groups of cells have renewal

potential, the majority of the cells is terminally differentiated and, thus, lacks the potential to self-renew. As more data becomes available, it is becoming clear that this simplified model may not hold true for solid tumors. One problem in recapitulating the hematological model of cancer stem cells has been the difficulty in defining a detailed hierarchy of cell differentiation for solid tumors with clearly defined cell surface markers. Many of the markers thus far defined for solid tumor cancer stem cells, such as CD133<sup>+</sup> for colon carcinoma, have not held up to close scrutiny. A recent study reported that both the CD133<sup>+</sup> and CD133<sup>-</sup> populations of colon carcinoma cells were able to induce tumor formation *in vivo*.<sup>19</sup> In an interesting twist on previous findings, this study showed that the CD133<sup>+</sup> carcinoma cell subpopulation gave rise to a CD133<sup>-</sup> sub-population with an even more aggressive phenotype than the original CD133<sup>+</sup> cells. This illustrates that there is still much to be learnt about how these defined subpopulations of cancer cells behave, particularly as CD133<sup>+</sup> is not even a marker of normal human colon stem cells.<sup>20,21</sup>

As a reflection of these uncertainties, the cancer stem cell field is seeing a shift in the terminology used, so there is now less discussion of “cancer stem cells” and a move toward the more neutral terms such as “tumor-initiating” and “tumor-propagating” cells. There is now an alternate model of tumorigenesis that takes an account of what we now know about tumor-initiating cells and fuses it with the more established “genetic” theories of cancer. In essence, this hybrid hypothesis posits that a tumor is made up of a number of heterogeneous clones of cancerous cells, which may well have differing mutational profiles, and that these subsets of cells may represent different stages of the oncogenic process. This refined model has the advantage of fully taking the microenvironment into account, as it is likely that each of the clones may well have a different growth advantage depending upon the growth conditions they find themselves in. Evidence from the melanoma field certainly supports this idea, and it has been shown in clinical melanoma samples that multiple cell clones with different mutational profiles coexist within the same tumor.<sup>22</sup> What is beyond doubt is that not all tumor cells are created equal, making it critically important to develop strategies to therapeutically target all of the clones—particularly those with enhanced malignant potential.

### The response of tumor initiating cells to therapeutic intervention

Although the past 40 years have seen many breakthroughs in the treatment of childhood leukemia and testicular cancer, there has been little tangible increase in survival of patients with most solid tumors. The classical approaches to cancer therapy have relied upon killing the rapidly proliferating population of tumor cells through the generation of genotoxic stress, such as chemotherapy/radiation, or by using targeted agents that block the signaling pathways that drive tumor growth.<sup>23</sup> The stem cell model suggests that as the tumor initiating cells cycle very slowly, and express multiple drug transporter proteins, they are also almost totally resistant to conventional anticancer therapies.<sup>24</sup> Although these ideas are widely accepted in the field, data to confirm this hypothesis has only recently become available. It has been recently shown that growth of breast cancer cells under mammosphere culture conditions, which are thought to enrich for the stem cell population, leads to increased radiation resistance and less DNA damage, compared to similar cells grown as adherent cell cultures.<sup>25</sup> Intriguingly, radiation treatment actually led to an increase in the percentage of the nonadherent CD24<sup>-low</sup>/CD44<sup>+</sup> cell population, suggesting that radiation treatment increases the size of the tumor initiating cell pool.<sup>25</sup> Similar results were also found in glioma, where the tumor initiating population is defined through the cell surface expression of CD133<sup>+</sup>.<sup>26</sup> In these studies, the CD133<sup>+</sup> glioma cell population were more resistant to radiation treatment than the CD133<sup>-</sup> population. Again like in the breast cancer studies, it was shown that the CD133<sup>+</sup> cell fraction

expanded following radiation treatment and that this relatively small increase in the CD133<sup>+</sup> population had very profound effects on the tumor growth rate.<sup>26</sup> Comparison of DNA damage marker induction following radiation treatment showed that the CD133<sup>+</sup> glioma cell population were able to activate the DNA damage checkpoints more efficiently than the CD133<sup>-</sup>, leading to a more radio resistant phenotype. Hints of a possible strategy, to overcome this resistance came when it shown that inhibition of the DNA damage responsive kinases CHK1 and CHK2 using pharmacological inhibitors sensitized the CD133<sup>+</sup> cells to radiation.<sup>26</sup> Similar findings were reported in colon carcinoma, where the cancer stem cell population was found to be more drug resistant than the differentiated “daughter” tumor cells from the same patient.<sup>27</sup>

The marked resistance of tumor initiating cells to therapeutic intervention also applies to the newer breed of targeted cancer therapies. This is best illustrated by the treatment of CML with the targeted therapy agent imatinib (Gleevec<sup>®</sup>, STI-571). The progression of CML is driven by a mutation resulting in the splice of the genes encoding for Bcr and the Abl kinase.<sup>28</sup> Imatinib works by inhibiting the activity of Bcr-Abl and typically induces a total remission in CML patients. The remission is ultimately followed by disease relapse associated with imatinib resistance, through the acquisition of imatinib insensitive Bcr-Abl mutations. Recent work has statistically modeled this process based on a cellular hierarchy consisting of terminally differentiated cells, differentiated cells, progenitor cells and stem cells.<sup>29</sup> It showed that during the imatinib treatment there was an initial rapid decline of Bcr-Abl transcript number that correlated with the reduction in the number of differentiated leukemia cells observed. After this, there was a prolonged, but slower, reduction in Bcr-Abl transcripts consistent with a depletion of the leukemia progenitor cells. Following treatment cessation or the acquisition of a Bcr-Abl mutation, transcript numbers again began to increase in a manner that was consistent with the failure of imatinib to eradicate the stem cell population.<sup>29</sup> Taken together, our current understanding of the response of tumor initiating cells to therapy indicates that most regimens exclusively target the daughter cell or nontumorigenic cell population. Some regimens, such as radiotherapy, may even lead to expansion of the tumor-initiating cell pool, causing rapid tumor regrowth and resistance.

### The therapeutic targeting of melanoma-initiating cells

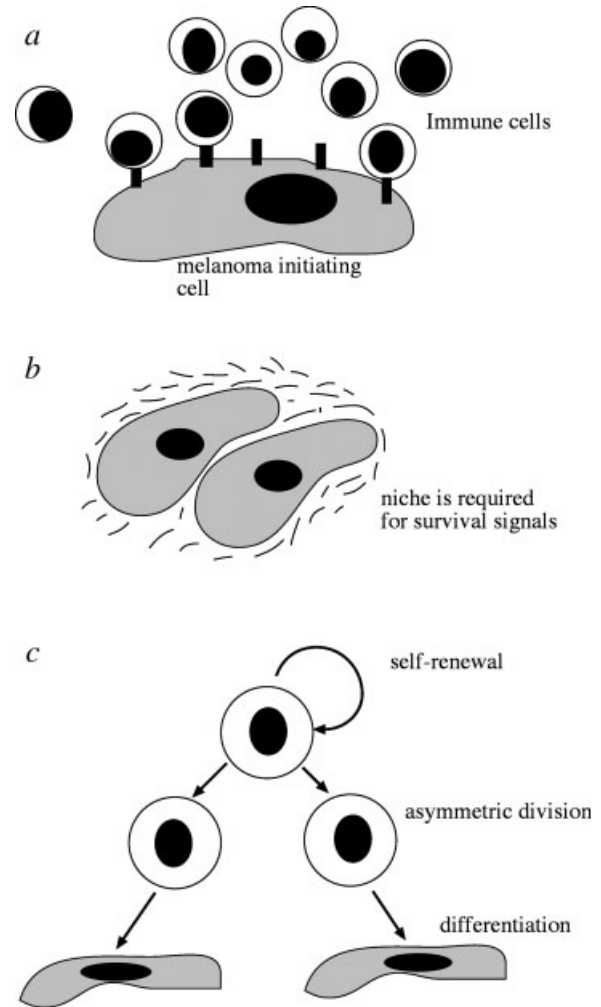
At present, we know very little about the mutational profiles and spectrum of cell signaling activity between the melanoma initiating cells and the bulk of the melanoma “daughter cells”. It is therefore unclear, whether any of the novel therapies currently undergoing clinical evaluation in melanoma will eradicate the melanoma-initiating population. To date, most progress in therapeutically targeting tumor-initiating populations has focused upon inhibiting the interaction of these cells with their microenvironment. Most progress in this area has been made in studies on leukemia. Recent work has shown that the bone marrow niche provides a protective microenvironment for acute lymphoblastic leukemia stem cells (ALL), through the stromal cell-derived secretion of asparagine synthetase, an enzyme that is critical for the biosynthesis of the amino acid asparagine.<sup>30</sup> Asparaginase, which depletes cells of asparagine, has long been used as an ALL treatment, even though the mechanism of action was unknown. There is a direct correlation between treatment outcome and the intensity of the asparaginase treatment regimen.<sup>31</sup> Thus, it was shown that the bone marrow stromal cells produced significantly more asparaginase than that of the leukemia initiating cells, and that the resistance of the leukemia cells to asparaginase was directly correlated with the expression levels of asparaginase from the bone marrow stromal cells.<sup>30</sup>

Another series of leukemia studies demonstrated that the direct targeting of the interaction between the tumor initiating cells and their niche is a promising future therapeutic strategy. CD44 is a

ubiquitously expressed cell-surface protein with multiple roles in cell-cell adhesion and cell-matrix adhesion. In the hematopoietic system, CD44 is important for leukocyte recruitment to sites of inflammation, and it also seems to play a key role in the adhesion of stem cells to the bone marrow stroma. The possible importance of CD44 in leukemia development was indicated by two recent studies showing that anti-CD44 antibodies prevent leukemia engraftment into immunodeficient mice by preventing migration of the leukemia initiating cells to the bone marrow.<sup>32,35</sup>

Solid tumor initiating cells do not reside in the bone marrow and rather less is known about their interactions with the niche. One possible site of the glioma tumor initiating cell niche is in the vasculature.<sup>34</sup> Soluble factors from vascular endothelial cells have been shown to promote self-renewal and inhibit differentiation of neural stem cells, suggesting that this may also be the site of these tumor initiating cells.<sup>35</sup> Evidence for this comes from a recent study showing that the simultaneous targeting of the vascular niche using antiangiogenic strategies (antivascular endothelial growth factor receptor 2, anti-VEGFR2 antibodies) in combination with chemotherapy leads to depletion of the tumor initiating cell compartment.<sup>36</sup> Relatively little is known about the niche for melanoma initiating cells. Previous studies have shown that melanoma cells interact preferentially with both endothelial cells and stromal fibroblasts, and it is possible that these cells may define the melanoma niche<sup>37,38</sup> (Fig. 2). The CD20<sup>+</sup> population of melanoma initiating cells retains the expression of classic melanoma markers such as MCAM (MUC-18) and  $\beta 3$  integrin, suggesting that these adhesion proteins may be involved in the tumor-initiating cell-microenvironmental niche interaction.<sup>14</sup> Studies are ongoing to determine the nature of the melanoma initiating cell niche and whether there are any specific molecules that are amenable to therapeutic targeting.

Alternative therapeutic strategies to deplete the tumor-initiating cell population involve either inhibiting the intracellular signaling pathways responsible for regulating stem cell self-renewal and survival or by directly targeting cell-surface antigens found on the tumor initiating cell population. Some melanomas are known to respond to immunotherapy approaches, raising the possibility of developing specific melanoma initiating cell vaccines (Fig. 2). In some cases, there are specialized proteins that regulate the survival of the cancer stem cells. A recent study showed that the targeting of the promyelocytic leukemia protein tumor suppressor exhausts the quiescent CML stem cell pool,<sup>39</sup> leading to disease regression. In addition, it has been shown that tumor-initiating cells rely more on pathways involved in development such as Notch, Wnt and Hedgehog, than normal somatic daughter cells. In pancreatic cancer cells, blockade of the hedgehog signaling pathway using cyclopamine, reduces metastatic spread and is associated with a reduced fraction of aldehyde dehydrogenase positive tumor initiating cells.<sup>40</sup> Similarly, studies in glioblastoma show that cyclopamine treatment decreases the self-renewal potential of the tumor initiating cell population<sup>41</sup> (Fig. 2). Subsequent intracranial injection of the remaining cyclopamine-treated glioblastoma cells was associated with a complete inhibition of tumor formation, indicating a total depletion of the tumor initiating cell fraction.<sup>41</sup> In melanoma, there is evidence that the Wnt, Notch and Hedgehog pathways are all involved in cell proliferation and survival<sup>42-44</sup> and it is likely these same pathways are also critical for self-renewal and differentiation. Notch in particular looks to be important in the metastatic behavior of melanoma, with the overexpression of the Notch converting early-stage non-tumorigenic melanomas into those with enhanced potential to form lung metastases.<sup>43,44</sup> Under these circumstances, Notch activation involves the activity of  $\beta$ -catenin, suggesting some cross-talk between the Notch and WNT signaling pathways. A number of pharmacological inhibitors of Notch, the  $\gamma$ -secretase inhibitors are now available for preclinical testing and have been found to reduce melanoma tumorigenicity.<sup>45</sup> It remains to be seen whether treatment with  $\gamma$ -secretase inhibitors depletes the melanoma initiating cell population. There is also evidence that the pathways well known



**FIGURE 2** – Possible sites of therapeutic intervention in the melanoma initiating population. (a) As melanoma initiating cells express unique surface antigens, it may be possible to use immunotherapy approaches to directly target and destroy them. (b) The niche provides important paracrine survival signals and maintains the melanoma initiating cells in a more primitive undifferentiated state. Inhibition of these key survival factors and the physical interaction between the initiating cells and the surrounding matrix and stromal cells is likely to lead to depletion of the initiating cell pool. (c) The self-renewal and differentiation of the melanoma initiating cell population is likely to rely upon developmental pathways such as Notch, Wnt and Hedgehog (HH). The pharmacological targeting of these pathways is likely to block the process of asymmetric division and expansion of the daughter cell population.

to be involved in melanoma survival and progression, such as the PI3K/AKT pathway, can play a role in stem cell maintenance in certain models. Inhibition of the PI3K pathway using a small molecule inhibitor blocks the expansion of the bronchioalveolar stem cell population in a mouse model of KRAS-induced lung cancer.<sup>46</sup> Whether BRAF or MAPK pathways plays any role in the maintenance of the tumor initiating population is unclear.

There is a growing realization that any future anticancer treatment will need to target the tumor initiating population. Ultimately, most solid tumor therapy is associated with eventual relapse and metastatic spread. The successful targeting of the tumor-initiating population is an absolute must to prevent relapse, and it is hoped that the eradication of the cells can reduce most cancers to the level of non-life threatening chronic diseases. The challenge for the melanoma field is great. At present, we know very little about the melanoma-initiating cells in terms of their

mutational profiles, and which signaling pathways are activated. Little is also known about the hierarchy of the melanoma-initiating cells, and how the CD20, CD133+ and ABCB5+ cells relate to each other in terms of differentiation status and tumorigenic potential (Fig. 1). It is highly likely that other putative melanoma-initiating population exist with different patterns of cell-surface marker expression. It is beyond doubt that the melanoma-initiating

cells represent an excellent therapeutic target, and we need to now focus our efforts on learning about how these cell populations differ from the “daughter” melanoma population that we have all worked with for so many years. We are confident that a concerted effort from the melanoma community can demystify these elusive melanoma-initiating cells, leading to improved therapies and renewed hope for melanoma patients the world over.

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