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Cancer Stem Cells: Lessons From Melanoma

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Abstract The model of cancer stem cells in tumor development states that tumors contain a subset of cells that both self renew and give rise to differentiated progeny. Like normal adult tissue stem cells, cancer stem cells are a minority of the whole tumor and are the only cells that are able to maintain tumor growth indefinitely. In the present review is critically discussed the actually existence of a cancer stem cell subpopulation in melanoma. The self-renewal signaling pathways as well as specific markers like as CD133, ABCB5 and ABCG2 recently identified in putative melanoma cancer stem cells are also discussed.

Keywords Cancer stem cells · CD133 · ABCG2 · ABCB5 · Self-renewal

Cancer Stem Cells "State of the Art"

The model of cancer stem cells (CSC) in tumor development states that tumors contain a subset of cells that both self renew and give rise to differentiated progeny. Like normal adult tissue stem cells, cancer stem cells are a minority of the whole tumor and are the only cells that are able to maintain tumor growth indefinitely. According to the cancer stem cell model, although the majority of the cells differentiate and die, the driving force of the tumor is the self-renewal properties of cancer stem cells. In this connection, it is quite clear that the identification of biomarkers that allow the prospective isolation of such a subpopulation from whole tumors is important not only from a diagnostic point of view but also in order to understand the biological properties of these cells.

The first problem is to validate a putative cancer stem cell subpopulation. The most widely accepted assay to validate a candidate cancer stem cell subpopulation is tumor initiation and serial transplantation in immunocompromised mice where the cells can recapitulate the heterogeneity of the primary tumor. However, xenotransplantation in spite of syngeneic transplant, does not require the intricate network of interactions with diverse support such as fibroblasts, endothelial cells, macrophages, mesenchymal stem cells and many of the cytokines and receptors involved in these interactions [1]. Recently, Pearce and colleagues, showed that the engraftment of hematopoietic cells in NOD-SCID mice is dependent by self-renewal, proliferation and differentiation potential [2]. Thereby, a high number of cells should be introduced if their self renewal is low. In this connection, several studies in syngeneic transfer suggested that the tumor is not driven by a minor subpopulation of tumor stem cells [3–6]. It might be that certain oncogenes can activate extensively self-renewal in more differentiated cells, perhaps enhancing expression of genes that can impose stem cell character such as those of the Wnt-beta-catenin pathway or bmi1 and certain hox genes [7]. Recently, Adams and Strasser proposed that tumors are sustained by a substantial proportion of cells perhaps all the cells that can form colonies *in vitro* under optimal conditions rather than by a minor subpopulation as expected by cancer stem cell model [1]. As far as their hypothesis is intriguing, there are tumors grown in syngeneic animals (i.e.mouse leukemia, breast cancer

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and B16F10 melanoma) which display some features expected from cancer stem cell model [8–12]. On the other hand, in all human biopsies studied and reported in the literature a low percentage of putative cancer-initiating cells was shown [13–20]. It is, of course, possible that the environmental condition both *in vitro* or *in vivo* (using syngeneic or xenotransplant models) might help the growth of putative CSCs. Our group has recently shown that after injecting CD133+ melanoma cells in NOD-SCID mice, most of the tumor cells became CD133- [17]. Furthermore, growing these cells *in vitro* after few passages they re-expressed CD133 [17]. In this connection, in human glioma, CD133—cells were able to form a CD133+ cells in nude rats [21]. On the other hand, CD133 might not be a good bio-marker to define correctly CSCs. As far as previous evidences regarding the presence of a CD133+ subpopulation in colon cancer supporting the tumor growth [22–24], a recent paper showed that even CD133—cells are able to initiate tumors and that CD133 is expressed on a wide range of differentiated epithelial cells in adult tissues and on spontaneous primary colon tumor in mice [25]. Therefore, the use of multiple markers to define such a CSC subpopulation is, in my view, strongly necessary. Hematopoietic stem cells are defined by a panel of markers as well. For instance, in melanoma our group have recently identified in the CD133+ cells a subpopulation expressing ABCG2 and, more interestingly, such a population decreased in tumor xenograft but it was re-expressed in cells obtained by tumor xenograft and grown for few passages *in vitro* [17]. We are right now studying the role of ABCG2 subpopulation and the possible relationship with others ATP-binding cassette (ABC) members like as ABCB5.

Another interesting aspect is that regulatory factors might modulate self-renewal affecting therefore the capability to undergo an asymmetric or symmetric division. In haematopoietic stem cells the cells grow asymmetrically and the probability of asymmetric division depends by the presence of certain cytokines [26, 27]. Interestingly, hematopoietic stem cells as well as oligodendrocytes precursor cells show an intrisic property to grow asymmetrically and appear to be regulated only in a permissive way by extrinsic factors [26, 28]. Furthermore, some proteins have been shown to be asymmetrically distributed during haematopoietic stem cell division [29]. In a recent review, the developmental signaling pathways involved in hematopoietic stem cells self-renewal like the Notch pathway, WNT—and TGFbeta-signaling as well as chemical regulators like as retinoic acid have been summarized [30]. It is important to highlight that is not a valid concept that stemness involved a set of genes common to all the stem cells. In fact, when comparing the gene-expression patterns of stem cells in distinct tissues, a specific pattern appears for each [31]. Therefore, factors produced, for instance, by CSCs, might modulate CSCs self-renewal.

Melanoma Cancer Stem Cells: a Critical Discussion

Seven papers came out in the last 2 years showing that a CSC subpopulation occurs in melanoma [12, 17–19, 31–33]. The markers used have been: CD133 [17–19, 31, 32]; CD20 [19]; side population using the flow cytofluorimetric technique based on heightened capacity of stem cells to efflux fluorescent dyes [32]; and ABC transporter family members like as MDR1, ABCG2 and ABCB5 [17, 18, 33]. It is important to highlight that all these papers used multiple bio-markers to define CSCs.

In the present review, I underscore the possible relationship between CD133 and ABC transporter family and melanoma CSC.

CD133

CD133 (prominin-1) was the first identified member of the prominin family of pentaspan membrane protein. It is localized in membranous protusions of the plasma membrane such as microvilli of epithelial cells. Thereby, a functional role of CD133 is as organizer of plasma membrane topology [34]. Interestingly, interactions between CD133 and cholesterol within such novel membrane micro-domains suggested that CD133 might also be important in maintaining a correct lipid composition within the plasma membrane [34].

In human melanoma biopsy, CD133 was shown to be expressed in less than 1% of the cells[17] as well as in several melanoma cell lines with different degrees of expression [17–19]. CD133 was also found expressed in primary human cultured melanocytes [19]. In this connection, Klei et al have observed an increased expression of CD133, CD166 and nestin in primary and metastaic melanoma compared to banal nevi [31]. In mouse melanoma (B16F10) a subpopulation CD133/CD44/CD24+ was demonstrated to have similar properties of cancer stem cells [12].

Several signaling pathways have been shown to regulate the CD133 epitope-positive cells. In human cord blood-derived CD133+ cells, Wnt3a preserved an undifferentiated phenotype; whereas Wnt5a, Wnt11, and Wnt4 induced expression of endothelial markers von Willebrand factor and CD31 [35]. The Notch, Hedgehog, and bone morphogenic protein (BMP) signaling pathways were demonstrated to be dependent on the tissue-specific expression of splice variants of prominin family molecules [36–39].

All together these data strongly show that the identification of the biocellular function of CD133 could be important in order to clarify its role as a biomarker of CSCs.

ATP-binding Cassette (ABC) Transportes

First described in the 1970s, ABC transporters are conserved proteins that typically translocate solutes across the cellular membranes [40]. The human genome contains 48 genes that encode ABC transporters which have been divided into seven subfamilies labelled A–G [41].

Recently, a self-renewing melanoma cell population marked by MDR1 was demonstrated [33]. Interestingly, these cells appeared enriched in precursor promoting conditions and possess higher clonogenic and self-renewal capacity, in addition to anchorage independence. In fact, they expressed higher levels of hTERT, nanog and ABCB5 as compared to MDR1negative cells [33].

ABCB5 is the third member of the human P-gp family next to its structural analogues ABCB1 [42–46] and ABCB4 [43], described as a pivotal factor of membrane potential and a regulator of cell fusion [44]. Furthermore, during melanoma progression, elevated levels of ABCB5 were demonstrated to occur in primary and metastatic melanoma with respect to benign nevi [44]. Recently, two novel isoforms, ABCB5alpha and ABCB5beta, were identified both in melanoma cells, melanocytes and retinal pigment epithelial cells, suggesting that they might be involved in melanogenesis [46]. In this connection, more recently, Schatton *et al*. described a tumor-initiating cells capable of self-renewal and differentiation in human melanoma expressing ABCB5 [18]. As suggested by the same authors, ABCB5 is reasonably a marker of melanoma progression [29, 39]. It is not a surprise, since ABCB5 is coded on human chromosome 7, one of the most frequent with a copy number gain in melanomas [47]. Accordingly, in human melanoma WM115 CSCs, our group have found ABCB5 with a copy number gain (unpublished data). Furthermore, Shatton *et al*. showed by isolating single cells from clinical melanoma that a subpopulation ABCB5+ occurred in tumor cells with a high frequency ranging from 2 to 20% [18]. They also demonstrated that ABCB5+ cells were able to recapitulate the tumor when injected in immunodeficient mice; and they are capable of re-establishing the original parent tumor heterogeneity [18]. However, despite serial passage in mice, ABCB5 percentages remained similar to the original parent tumor [19]. More interestingly, ABCB5—cells could not regenerate the ABCB5+ cells, suggesting that these cells lack a stem cell phenotype [18]. Finally using a monoclonal anti-ABCB5 antibody in nude mice, initial tumor growth as well as established tumors were inhibited by antibody-dependent cell-mediated cytotoxicity [18]. However, the ABCB5+ subpopulation was not completed eradicated by the treatment in established tumors [18].

ABCG2 is the second member of the G family of ABC transporters which is identical to the placental ABC protein (ABCP1) [48], mitoxantrone-resistance protein (MXR) and the breast cancer-resistance protein (BCRP1) [49]. In melanoma, ABCG2 was demonstrated to be expressed in a subpopulation (4%) of melanoma cells expressing high percentage of CD133 (80–90%) [17]. Since ABCG2 appeared to be expressed only in a subpopulation of CD133 positive cells, our group is now studying the possible role of ABCG2 with respect to self-renewal. Furthermore, since ABCG2 positive cells represent only a small fraction of CD133+ cells, it is not a surprise that melanoma specimens are negative for ABCG2 using conventional immunohistochemical analysis [50]. Interestingly, preliminary cytofluorimetric analysis in collaboration with the group of Markus Frank (Children Hospital, Boston, MA), showed that in spite of a high number of cells expressing ABCB5, there is a subpopulation of cells expressing both ABCB5+/ABCG2+ (unpublished data). Further studies are needed to clarify the possible relationship between the two ABC drug resistance markers.

Conclusion

It is not easy to define a cancer stem cell. However, the questions whether CSCs do exist as a distinct subpopulation inside the tumor and what are their origin are valid ones based on existing data. For melanoma, there are the following evidences:

1- CD133 is expressed in discrete cell populations with proportionately higher tumor propagating activity.
2- ABCB5 is expressed, and it is a marker of progression. The gene encoding this protein maps to a typical gain region in melanoma; but we do not know if it contributes alone to the stemness or self renewal capacity of putative melanoma cancer stem cells.
3- ABCG2 is expressed by a smaller subpopulation, but we do not have yet a clear demonstration of its involvement in self-renewal capacity in melanoma cancer stem cells.

In the future, it will be essential to investigate all these points. The outcome of these and other investigations like them, in laboratories exploring other tumor types, will bring greater definition and clarity to the CSC hypothesis.

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