It is currently thought that adult tissue homeostasis originates from primitive cells that differentiate into several lineages. The most primitive are thought to be adult stem cells (SCs), which are normally quiescent. These are characterized by their specific properties of long-term self-renewal, clonogenicity, pluripotentiality and most importantly, the ability to undergo asymmetrical division. This maintains a base SC whilst simultaneously producing a daughter cell [1]. This latter cell proliferates, producing a transit-amplifying cell population (TAP). These cells are committed down a specific lineage and are characterized by their ability to undergo several symmetrical divisions, producing many terminally differentiated cells.

The presence of stem-like cells within the prostate was postulated after studies of the effect of androgen withdrawal and replacement. These experiments showed regression of the prostatic epithelium to the basal layer on castration, and subsequent re-formation of all the epithelial layers when the normal androgen milieu was restored [2]. This process can be repeated many times, suggesting a process of long-term self-renewal by a progenitor cell. This cellular attribute has led to the hypothesis that a cancer SC (CSC) is the root cause of malignant prostatic proliferation, with CSCs arising from direct mutation of normal SCs. TAP cells might also gain mutations and, despite having only limited self-renewal capability, they might contribute significantly to increased malignant cell numbers via their enhanced replicative potential [3,4]. Alternatively, the TAP might undergo de-differentiation, regaining the SC property of self-renewal [5,6] leading to the disruption of cell growth regulation. Study of the SCs and TAPs, and their role in BPH and carcinoma of the prostate, has been hampered by the lack of specific biomarkers required for the isolation and characterization of these cellular populations, both *in vitro* and *in vivo*.

CD133: A MARKER OF TRANSIT AMPLIFICATION RATHER THAN STEM CELL PHENOTYPE IN THE PROSTATE?

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Accepted for publication 10 September 2008

Over the last decade several specific prostate SC markers have been proposed, including PSCA, $\alpha_2\beta_1$ -integrin and CD44⁺/CD24⁻. However, it is the haematological marker CD133 that has gained favour. CD133 (prominin-1) is a five-domain transmembrane glycoprotein within the outer cell membrane, and it has been shown to be a marker for primitive haemopoietic cells. Enhanced long-term re-population potential was shown in cells harvested from primary sheep donors, which were successfully engrafted in secondary recipients.

Isolating cellular subpopulations using a separation protocol based on expression of CD133, CD44⁺ and the $\alpha_2\beta_1$ -integrin in normal/BPH tissue resulted in the isolation of an epithelial subpopulation representing 0.75% of the basal cell population. This population was enriched for cells with the SC properties of high colony-forming ability, prolonged clonal growth and, importantly, the capacity to differentiate and form glandular acini *in vivo* [7]. This population has also been reported in prostatic malignancy, suggesting that CD133 might be a marker expressed on CSCs and that expression could be used to differentiate between the CSC ($\alpha_2\beta_1$ -integrin^{hi}/CD44⁺/CD133⁺) and the TAP ($\alpha_2\beta_1$ -integrin^{hi}/CD44⁺/CD133⁻). CD133⁺ cells were shown to be maintained in culture at a constant level and they could differentiate into a population with an androgen receptor (AR)-positive phenotype, highly suggestive of the presence of a SC-like population [8].

However, there is uncertainty as to whether the CD133⁺ phenotype represents a true SC population. The proposed SC population in CD133-based studies in the prostate [7,8] was expanded from the outset, with potential for non-SC population outgrowth and for 'genetic drift'. Furthermore, the CD133⁻ population in that study also contained clonogenic cells, albeit in a lower proportion. Whilst the CD133⁺ cells did not express the TAP marker c-MET [9] or markers of terminal differentiation (PSA, AR), colony-forming efficiency is only relative to the populations being compared and is not exclusive to the SC, as the early TAP will also form colonies. Furthermore, the study by Collins *et al.* [8] failed to show true asymmetric division, characterized by retention of the original genome by the SC. There is therefore a lack of definitive evidence for the proposition that CD133 positivity is synonymous with the SC phenotype.

The role of CD133 as a CSC marker has also been questioned in other tumours. In a study of CSCs in glioblastoma, four of the 15 tumours examined were composed of cells with a CD133⁻ phenotype; these cells also had SC characteristics [10]. Although the CD133⁻ cells had a lower proliferative index than the CD133⁺ phenotype, the findings suggest that in neural CSCs, CD133 status is not the determinant of the SC compartment.

The question that remains is whether CD133 is a SC/CSC marker or a marker of early transient amplification. Wang *et al.* [11] suggested that CD133 expression is not

required for brain tumour initiation, but that it might be involved in its progression. Brain tumour biopsy material expressed little or no CD133 until passaged. During passage, expression of CD133 appeared and increased, coinciding with a shorter survival; a finding more characteristic of an amplifying/differentiating population than a SC one. Subsequent implantation of CD133^{-ve} cells into the brains of six nude rats led to the confirmation of tumorigenesis in three cases. Interestingly, CD133^{+ve} cells were ultimately harvested from these tumour populations. It is therefore arguable that a more likely hierarchy in relation to CD133 is that the CD133^{-ve} population represents either the SC or terminally differentiated populations, and that the TAP has a CD133^{+ve} phenotype (Fig. 1).

However, it might be the specific AC133 epitope of CD133 and not the actual CD133/prominin-1 mRNA expression that is important for detecting SCs. Florek *et al.* [12] found that although the antigen AC133 decreased, CD133/prominin-1 expression did not alter during the differentiation of the colonic adenocarcinoma cell line Caco-2. This was also confirmed by the detection of CD133/prominin-1 in a range of renal cells, as well as cells within the lactiferous ducts of the breast [12]. To attempt to resolve any uncertainties about CD133 expression, Shmelkov *et al.* [13] developed a transgenic mouse model using CD133 endogenous promoters driving the expression of *lacZ*. This study showed that both undifferentiated and differentiated colonic epithelial cells expressed CD133. Interestingly, of the tumours formed in this model, it was the CD133^{-ve} cells that were the most aggressive and they expressed the typical cancer-initiating phenotype (CD44^{+ve}CD24^{-ve}), whereas the CD133^{+ve} fraction was composed of CD44^{low}CD24^{+ve} cells [13].

Recent work in renal tissue showed that although isolated CD133^{+ve} cells had a greater proliferative capacity than comparable CD133^{-ve} cells, there was no other enhancement for SC behaviour or expression of putative SC markers. However, this work identified a primitive population of CD133^{-ve} cells that possessed enhanced SC-like behaviour and marker expression, whilst also having the ability to acquire CD133 expression after passage. This suggested that CD133 is a marker of the TAP in renal cells [14].

FIG. 1. A flow diagram illustrating the proposed hierarchy for the CD133 phenotype within the prostate epithelial population.

FIG. 2. Dual-parameter flow cytometry density plots illustrating the identification of both the side population (SP) or tail and the non-SP (NSP) in a live prostate epithelial cell population. The SP is accurately defined by the use of a verapamil control (right).

It is therefore still unclear as to where the CD133^{+ve} phenotype fits in to the prostate cellular hierarchy. To address the issues raised about the CD133 status of prostate SCs studies of unexpanded SC populations must be conducted and verified by different isolation techniques based on SC functionality. The Hoechst 33342 dye-efflux assay, defined by Goodell *et al.* [15] in the haemopoietic system, has been shown to isolate a 'side population' (Fig. 2) enriched for cells with SC characteristics. This technique has been adapted to many solid tissues and malignancies, including the prostate [16,17].

Genetic comparison of the unexpanded CD133^{+ve} phenotype with the side population might provide the final insight into the true nature of the CD133^{+ve} prostate cell.

ACKNOWLEDGEMENTS

Ben Grey was supported by Prostate UK and the Peel Medical Research Trust.

CONFLICT OF INTEREST

None declared.

REFERENCES

- 1 Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 2000; **100**: 157–68
- 2 Isaacs JT. Antagonistic effect of androgen on prostatic cell death. *Prostate* 1984; **5**: 545–57
- 3 Huntly BJ, Shigematsu H, Deguchi K *et al*. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell* 2004; **6**: 587–96
- 4 Krivtsov AV, Twomey D, Feng Z *et al*. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* 2006; **442**: 818–22
- 5 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105–11
- 6 Jordan CT, Guzman ML, Noble M. Cancer stem cells. *N Engl J Med* 2006; **355**: 1253–61
- 7 Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT. CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci* 2004; **117**: 3539–45
- 8 Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65**: 10946–51
- 9 van Leenders GJ, Schalken JA. Stem cell differentiation within the human prostate epithelium: implications for prostate carcinogenesis. *BJU Int* 2001; **88** (Suppl. 2): 35–42
- 10 Beier D, Hau P, Proescholdt M *et al*. CD133+ and CD133- Glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res* 2007; **67**: 4010–5
- 11 Wang J, Sakariassen PO, Tsinkalovsky O *et al*. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int J Cancer* 2008; **122**: 761–8
- 12 Florek M, Haase M, Marzesco AM *et al*. Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. *Cell Tissue Res* 2005; **319**: 15–26
- 13 Shmelkov SV, Butler JM, Hooper AT *et al*. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. *J Clin Invest* 2008; **118**: 2111–20
- 14 Oates JE, Grey BR, Swann R, Hart C, Brown MD, Clarke NW. CD133 is a marker for early proliferative cells but not cancer stem-cells in renal cell carcinoma. *BJU Int* 2008; **101** (Suppl. 5): 43
- 15 Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med* 1996; **183**: 1797–806
- 16 Bhatt RI, Brown MD, Hart CA *et al*. Novel method for the isolation and characterisation of the putative prostatic stem cell. *Cytometry A* 2003; **54A**: 89–99
- 17 Brown MD, Gilmore PE, Hart CA *et al*. Characterization of benign and malignant prostate epithelial Hoechst 33342 side populations. *Prostate* 2007; **67**: 1384–96

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Abbreviations: (C)SC, (cancer) stem cell; TAP, transit-amplifying cell population; AR, androgen receptor.