

Focus on Stem Cells in Human Reproduction

A cancer stem cell origin for human endometrial carcinoma?

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Abstract

Endometrial cancer (EC) is the most common gynaecological malignancy affecting women in the western world. Cancer stem cells (CSCs) are defined as a subset of tumour cells with the capacity to self-renew and give rise to the differentiated cells that comprise the bulk of the tumour. Given that a rare population of epithelial stem/progenitor cells has been identified in human endometrium, it is possible that these cells or their progeny may be the source of the putative CSCs that may initiate and maintain EC. Studies have shown that some cells within EC have the capacity to initiate clones that undergo self-renewing cell division and form tumours *in vivo* that can be serially passaged, demonstrating self-renewal, proliferation and differentiation abilities of the potential EC stem cells (ECSCs). These potential ECSCs may be located within the tumour cell population expressing CD133 and/or within the side population. With the discovery of markers for ECSCs, it is hoped that ECSCs can be isolated and characterised, and that their role in the development of human EC will be further investigated. This knowledge opens the way for the development of new treatment modalities that target the CSCs, but spares normal endometrial stem/progenitor cells and other cells. Such treatments will be particularly useful for early-stage and pre-menopausal EC candidates where the uterus may be conserved, and for late-stage cases where hysterectomy is not curative and current treatments target the bulk tumour cells rather than CSCs.

Reproduction (2010) **140** 23–32

The cancer stem cell concept

Many human cancers are composed of cell populations with heterogeneous immunological and genetic profiles, proliferation potentials and differentiation capacities (Heppner 1984, Visvader & Lindeman 2008). To accommodate this heterogeneity, the cancer stem cell (CSC) hypothesis has been proposed. CSCs are defined as a subset of tumour cells with the capacity to self-renew and give rise to the differentiated cells that comprise the bulk of the tumour (Reya *et al.* 2001, Visvader & Lindeman 2008, Rosen & Jordan 2009). It also suggests that tumours are similar to an organ where there is a hierarchy of cells with varying differentiation and proliferation capacities.

Differences in proliferation potential of the component cell types have been observed in numerous cancers, where it has been demonstrated that only a few cells from the cancer form clones *in vitro* (Hamburger & Salmon 1977, Reya *et al.* 2001, Bapat *et al.* 2005, Tokar *et al.* 2005, Zhang *et al.* 2008), and autotransplantation

of malignant cells in humans did not always establish a tumour (Southam & Brunschwig 1961, Visvader & Lindeman 2008). Furthermore, several studies have demonstrated that not all human cancer cells xenografted into immunocompromised animal models were able to initiate tumours (Lapidot *et al.* 1994, Patrawala *et al.* 2006, 2007, Vermeulen *et al.* 2008, Zhang *et al.* 2008). This indicates that only certain cells within the tumour retain the capacity to initiate, maintain and promote the development of the tumours, supporting the CSC hypothesis.

Evidence for the CSC theory has been demonstrated in leukaemic cells, where the CSCs represented <1% of the total cell population (Lapidot *et al.* 1994, Bonnet & Dick 1997, Rosen & Jordan 2009). It was hypothesised that CSCs expressed cell surface markers or a combination of specific markers that distinguish them from the bulk of the tumour populations, and these can be used to purify populations of CSCs in the same way that normal adult stem cells (ASCs) can be isolated from the remainder of the organ in which they reside. The CSC theory, although controversial, has important implications for our understanding of the biology and development of cancers and offers an alternative target for anti-tumour therapies.

This paper is one of four papers that form part of a special Focus Issue section on Stem Cells in Human Reproduction. The Guest Editor for this section was C E Gargett, Clayton, Australia; she was not involved in the handling of this paper, on which she is listed as an author.

Properties of cancer stem cells

By definition, CSCs have similar properties to ASCs. CSCs may not necessarily acquire the first genetic mutation that initiates tumourigenesis, but they are the cells that maintain the tumour over time (Visvader & Lindeman 2008). Subsequent mutations within a cancer may create new CSCs that overtake or coexist with the older CSCs (Visvader & Lindeman 2008). Whatever the mutation, the CSCs must retain the capacity to self-renew. To date, the best *in vivo* indicator of CSC self-renewal is the serial transplantation of CSC-enriched populations into immunocompromised mice, where the tumour is re-established, with a similar phenotype to the original tumour, with each serial xenografting (Visvader & Lindeman 2008). This technique also demonstrates that CSCs are able to give rise to large numbers of differentiated progeny. CSCs may also be relatively quiescent, allowing them to escape the cytotoxic effects of chemotherapeutic agents targeting rapidly cycling cells. Chemotherapeutic failure can also be acquired by upregulating drug transporters (Visvader & Lindeman 2008, Rosen & Jordan 2009), which rapidly transport harmful agents from the cell before they exert their effects. Emerging evidence also indicates that CSCs may be less radiosensitive than the majority of cancer cells (Diehn *et al.* 2009). It is hypothesised that CSCs possess DNA protective mechanisms that prevent the effects of radiation (Diehn *et al.* 2009).

Recent evidence indicates that CSCs may not be as rare as originally thought (Visvader & Lindeman 2008, Rosen & Jordan 2009). In normal organs, homeostatic mechanisms tightly regulate the proliferation of the normal ASCs, maintaining their numbers at relatively low levels. In cancer, these mechanisms no longer apply and CSCs may self-replicate to expand the mutated stem cell pool (Rosen & Jordan 2009). However, numbers of CSCs are often inferred from the percentage of cells expressing a particular surface marker phenotype, which may also be present on more differentiated cells. CSCs and ASCs have many similar properties which may operate through similar molecular pathways, albeit aberrantly in CSCs.

Source of cancer stem cells

There appear to be several sources from which CSCs can arise. They may arise from normal ASCs, from more restricted progenitor cells or even from differentiated cells (Reya *et al.* 2001, Visvader & Lindeman 2008). Normal stem cells are the likely targets of mutagenesis leading to the formation of CSCs as they already possess active self-renewal pathways, whereas induction of self-renewal genes is required to transform differentiated cells. Furthermore, normal stem cells are the only cells with a lifespan long enough to accumulate the genetic mutations that lead to tumourigenesis (Reya *et al.* 2001).

To date, CSCs and their ASC counterparts have similar surface marker phenotypes (Lapidot *et al.* 1994), lacking differentiation markers, but it is unclear whether these markers are related to stem cell functions. Finally, some CSCs have been located in the same region as their normal ASC counterparts, and it is possible that niche signals attract the CSCs to the niche rather than the CSCs arising from the normal ASC (Rosen & Jordan 2009). The ASC niche is composed of one or more cells that protect and maintain the ASCs in a quiescent, undifferentiated state. Niche cells sense the environment and signal ASCs to divide when new tissue cells are required (Fuchs *et al.* 2004). It is possible for progenitors and other differentiated cells to give rise to CSCs, although they would have to acquire more genetic mutations, particularly in self-renewal genes (Visvader & Lindeman 2008, Rosen & Jordan 2009). Similarly, it has not yet been determined whether a mutant niche can cause a tumour. It is possible that niche cells receive the first genetic mutations for tumourigenesis, sending aberrant signals to the resident ASC, conferring CSC properties. It has been hypothesised that CSCs arising from normal stem cells result in more aggressive cancer phenotypes, whereas those arising from progenitor cells are less aggressive, though this remains to be proven (Visvader & Lindeman 2008).

Cancer stem cell markers

Cells express a variety of markers on their cell surface. The expression or absence of these markers has been used to isolate subpopulations of cancer cells for the examination of CSC properties. Some cell surface markers, including CD133 (PROM1), CD44, CD24 and THY1 (Table 1), are common to several cancers (Visvader & Lindeman 2008). Investigations into solid

Table 1 Common human cell surface cancer stem cell markers.

| Marker | Additional markers | Tumour type | References |
|--|--|---|---|
| CD44 ⁺ | CD24 ^{-/low} EpCAM ^{hi} CD24 ⁺ ESA ⁺ | Head and neck Breast Colon Pancreas | Prince <i>et al.</i> (2007) Al-Hajj <i>et al.</i> (2003) Dalerba <i>et al.</i> (2007) Li <i>et al.</i> (2007) |
| CD49 ^{hi} CD133 ⁺ | EpCAM ⁺ | Breast Glioblastoma Medulloblastoma Brain Colon Pancreas Lung Ovarian Endometrial | Lim <i>et al.</i> (2009) Singh <i>et al.</i> (2004) Singh <i>et al.</i> (2004) Bao <i>et al.</i> (2006) O'Brien <i>et al.</i> (2007) Hermann <i>et al.</i> (2007) Tirino <i>et al.</i> (2009) Kusumbe <i>et al.</i> (2009) Rutella <i>et al.</i> (2009) |
| ALDH1 ⁺ | | Breast Colon Lung | Ginestier <i>et al.</i> 2007 Huang <i>et al.</i> (2009) Jiang <i>et al.</i> (2009) |
| CD90 ⁺ ABC5 ⁺ SP | | Liver Melanoma Mesenchymal Endometrial Ovarian | Yang <i>et al.</i> (2008) Schatten <i>et al.</i> (2008) Wu <i>et al.</i> (2007) Friel <i>et al.</i> (2008) Szotek <i>et al.</i> (2006) |

tumour CSC markers require further investigation as markers in some tumours have only been investigated by a single laboratory, different laboratories have found different markers for the same tumour types, and a potential CSC marker is not always expressed in every tumour (Fillmore & Kuperwasser 2007, Visvader & Lindeman 2008). The exact function of these markers is yet to be determined, and they may be involved in essential stem cell functions such as self-renewal, although this is unlikely given that they are also present on more differentiated cells (Visvader & Lindeman 2008). Other markers to intracellular targets have been useful in confirming CSC activity in a subpopulation of cancer cells. An example is musashi-1, which appears to mark ASCs in the intestinal and neural systems and may be useful as a confirmatory marker in subpopulations of cells obtained using specific cell surface markers (Pilkington 2005).

CD133 as a cancer stem cell marker

A commonly investigated potential CSC marker is CD133 or prominin-1, a 120 kDa five transmembrane domain cell surface glycoprotein that may be involved in cell–cell interactions and mature organ homeostasis (Ricci-Vitiani *et al.* 2007, Klonisch *et al.* 2008, Ferrandina *et al.* 2009). CD133 has been identified on 0–42.1% of human colon, ovarian and prostate cancer cells, specifically localising to the luminal surface of colon cancer epithelial cells (Collins *et al.* 2005, Dalerba *et al.* 2007, O'Brien *et al.* 2007, Ricci-Vitiani *et al.* 2007, Ferrandina *et al.* 2008, Horst *et al.* 2008, Choi *et al.* 2009, Chu *et al.* 2009). Non-adherent colon cancer cells cultured as spheres express CD133 and generate tumours when transplanted into non-obese diabetic (NOD)/severe combined immunodeficient (SCID) mice, even after 1 year of sphere culture (Ricci-Vitiani *et al.* 2007, Vermeulen *et al.* 2008). It is not clear whether these spheres arose from CSCs or progenitor cells; however, when the spheres differentiated, they stopped expressing CD133 and lost their ability to form tumours *in vivo* (Ricci-Vitiani *et al.* 2007, Vermeulen *et al.* 2008). Furthermore, CD133⁺ ovarian cancer cells were more efficient at forming clones and proliferated more extensively than the CD133[−] population (Ferrandina *et al.* 2008), and in the ovarian cancer cell lines, A2780 and PEO1, the CD133⁺ cells were more resistant to chemotherapeutic drugs, upregulated anti-apoptotic and development genes, and downregulated death cascade genes, in comparison with the CD133[−] population (Baba *et al.* 2008). When transplanted into mice, as few as 100 CD133⁺ cells isolated from primary and metastatic colon and brain cancers, and ovarian cancer cell lines, initiated serially transplantable tumours with similar histoarchitecture and differentiation into CD133⁺ and CD133[−] cells in a similar ratio to the parent tumour (Singh *et al.* 2004, O'Brien *et al.* 2007,

Ricci-Vitiani *et al.* 2007, Baba *et al.* 2008). In contrast, many more CD133[−] cells were required to initiate tumours that formed at a much slower rate and were smaller than those initiated by CD133⁺ cells (Singh *et al.* 2004, O'Brien *et al.* 2007, Ricci-Vitiani *et al.* 2007, Baba *et al.* 2008). Importantly, not all CD133⁺ cells were able to initiate tumourigenesis (O'Brien *et al.* 2007), and not all cancers expressed CD133, indicating that it may not mark the CSCs in all types of cancer (Dalerba *et al.* 2007, Choi *et al.* 2009, Chu *et al.* 2009, Ferrandina *et al.* 2009). In some cases of colon cancer, CD133 was correlated with more advanced stage tumours, suggesting that it may be a marker for metastasis (Choi *et al.* 2009); however, in ovarian cancer, the expression of CD133 decreased with metastasis or was unrelated to tumour stage (Ferrandina *et al.* 2008, 2009).

CD44 as a cancer stem cell marker

The hyaluronan receptor, CD44, is a surface glycoprotein with many signalling functions (Patrawala *et al.* 2006, Klonisch *et al.* 2008) that appears to be expressed on populations enriched for CSCs in human breast, prostate, ovarian and colon cancers. Similar to CD133, spheres generated from unsorted ovarian and colon cancer cells, and those from prostate cancer cell lines expressed CD44 (Patrawala *et al.* 2006, Vermeulen *et al.* 2008, Zhang *et al.* 2008). These spheres were capable of undergoing self-renewing divisions *in vitro* and expressed other stem cell pluripotency markers including *POU5F1* (*OCT-4*), *NANOG* and *BMI1* (Patrawala *et al.* 2006, Vermeulen *et al.* 2008, Zhang *et al.* 2008). With differentiation, the spheres lost their expression of CD44 (Vermeulen *et al.* 2008), indicating that these cells might represent CSCs. *In vitro* evidence for CD44 as a marker for CSCs has been found in colon cancer and prostate cancer cell lines, where the CD44⁺ population is more efficient at forming large clones (Patrawala *et al.* 2006, Chu *et al.* 2009). *In vivo*, the CD44⁺ population in breast, prostate and colon cancers is more efficient at producing larger, more aggressive, serially transplantable tumours in NOD/SCID mice that recapitulated the original tumour phenotype in a shorter time frame, compared to the CD44[−] population (Al-Hajj *et al.* 2003, Dalerba *et al.* 2007, Chu *et al.* 2009). However, studies have found a positive correlation with CD44 expression and colon tumour size, and increased CD44 expression with increased malignancy in a number of cancer cell lines, which may explain this association in the transplanted mice (Patrawala *et al.* 2006, Choi *et al.* 2009).

Some CSCs have been identified in different and sometimes non-overlapping subpopulations. Human breast CSCs have been identified in the CD44⁺CD24^{−/low} population and in the ALDH⁺ fraction (Al-Hajj *et al.* 2003, Ginestier *et al.* 2007). Both sets of cells produced tumours *in vivo* that could be serially transplanted and recapitulated the original tumour phenotype

(Al-Hajj *et al.* 2003, Ginestier *et al.* 2007). Interestingly, only 0.1–1.2% of the cells have overlapping marker expression and these cells are highly tumorigenic (Ginestier *et al.* 2007). The reason for this distinction may be because they examined different tumour subtypes and the Al-Hajj investigation focused on metastatic rather than primary breast cancers. Alternatively, the combined $\text{ALDH}^+\text{CD44}^+\text{CD24}^{-/\text{low}}$ population may represent the true CSC, with the $\text{ALDH}^-\text{CD44}^+\text{CD24}^{-/\text{low}}$ and $\text{ALDH}^+\text{non}(\text{CD44}^+\text{CD24}^{-/\text{low}})$ populations representing more differentiated progenitors that retain some capacity to proliferate *in vivo* (Ginestier *et al.* 2007). Similarly, the CD133^+ and LGR5^+ fractions appear to locate to different subpopulations in mouse colon cancer (Becker *et al.* 2008). Differences in CSC subpopulations both within the same tissue and between tissues may be related to culture effects, the types of cancers examined (metastatic versus primary), the ethnicity of the patients, sorting technologies (magnetic activated cell sorting (MACS) versus fluorescent activated cell sorting (FACS)) and location of the xenograft.

Cancer stem cells and metastasis

Cancer cells that have not developed into new growths have been noted at sites distant from the original tumour. These cancer cells are often identified by pathologists based on their abnormal karyotype, or other cancer cell morphological features. These cells may not have had time to establish a tumour due to the efficiency in their removal by the immune system, lack of blood supply or their intrinsic lack of tumour initiation capability (Southam & Brunschwig 1961, Fidler & Kripke 1977, Clark *et al.* 2000, Reya *et al.* 2001). There are two theories regarding metastasis and the CSC. The first is that the CSC is the only cell with the traits required to establish a tumour at another site, and thus needs to escape the original tumour to establish metastasis elsewhere. Evidence for the role of CSC in establishing metastasis comes from breast cancer studies, where metastatic cells expressing the CSC phenotype, $\text{CD44}^+\text{CD24}^{-/\text{low}}$, formed self-renewing mammospheres, gland-like structures in soft agar, and gave rise to differentiated progeny including $\text{CD44}^{\text{low}}\text{CD24}^{\text{high}}$ cells at a higher efficiency than non-metastatic cells (Al-Hajj *et al.* 2003, Mani *et al.* 2008). The second theory postulates that CSCs and metastatic cells are different, that the metastatic cell travels to a distant site to establish a niche and then signals to the CSC to travel and take residence at the new site and initiate a metastatic tumour. This is evidenced by the increased number of CD24^+ cells in distant metastases of human breast cancer (Visvader & Lindeman 2008). It has also been suggested that the $\text{CD44}^+\text{CD24}^{-/\text{low}}$ cells metastasize and then alter their phenotype in response to the new environment.

Cancer stem cells as prognostic indicators

While the CSC model is still controversial (Kelly *et al.* 2007), it is important to note that other cells may also play key roles in the development and progression of cancer (Visvader & Lindeman 2008, Rosen & Jordan 2009). If CSC surface markers relate to functional characteristics, it seems likely that the same cancer subtypes with similar molecular characteristics between patients will be initiated by similar populations of CSCs. A correlation between putative stem cell markers and poor prognosis has been observed in some cancers (Prawala *et al.* 2006, Ginestier *et al.* 2007, Horst *et al.* 2008, Visvader & Lindeman 2008, Zeppernick *et al.* 2008); however, no statistically significant correlation between progression-free survival and CSC number has been observed in breast cancer (Abraham *et al.* 2005, Kern & Shibata 2007).

Cancer stem cells as targets for novel therapeutics

In cancers with strong evidence for CSCs, a new cellular target is available for drug development that focuses on eradicating the CSCs to prevent recurrence. Combining these drugs with traditional cytotoxic agents targeting the remaining cancer cells (Kern & Shibata 2007) will prevent them mutating, acquiring self-renewal properties and becoming CSCs themselves. Similarly, it will be necessary to ensure terminal differentiation of all CSCs when using differentiation induction therapies, such as ATRA for acute promyelocytic leukaemia (Petrie *et al.* 2009). Targeting the CSCs may be especially important in higher grade and advanced tumours, where evidence indicates that the frequency of CSCs is greater than in lower grade and lower staged tumours (Visvader & Lindeman 2008, Rosen & Jordan 2009), and also in tumours where the majority of tumour cells have already been eradicated (Kern & Shibata 2007). It will be important that new treatment options spare normal stem cells or are delivered in a targeted manner that avoids normal stem cell exposure.

Endometrial carcinoma

Incidence

Endometrial cancer (EC) is the most common gynaecological malignancy affecting women in the western world (National Institutes of Health (NIH) NCI, <http://www.cancer.gov/cancertopics/types/endometrial>, 2/9/2009). In 2009, it was expected that there would be 42 160 new cases diagnosed and 7780 deaths from the disease in the USA (NIH NCI, <http://www.cancer.gov/cancertopics/types/endometrial>, 2/9/2009). It has an incidence rate of 23.3 per 100 000 women, and the average age at diagnosis is 62 years (Horner *et al.* 2009). The 5-year survival rate for EC is 82.9%, mainly

because cases are diagnosed at an early stage due to the readily detectable symptom of abnormal uterine bleeding in post- and peri-menopausal women (Horner *et al.* 2009).

Histological classification of endometrial carcinomas

There are a number of histological types of EC; endometrioid carcinoma, mucinous adenocarcinoma, papillary serous adenocarcinoma, clear cell adenocarcinoma, undifferentiated carcinoma and mixed carcinoma (Creasman *et al.* 2006). Around 10% of ECs are non-sporadic and mostly associated with hereditary non-polyposis colorectal cancer (Ryan *et al.* 2005, Bansal *et al.* 2009). In this condition, there are abnormalities in DNA mismatch repair genes that eventually results in microsatellite instability (MSI; Ryan *et al.* 2005). Women with this genetic susceptibility have a tenfold higher lifetime risk of developing EC in comparison to the general population (Ryan *et al.* 2005). The remaining 90% of EC cases are considered sporadic (Ryan *et al.* 2005, Bansal *et al.* 2009), with various genetic mutations being identified.

The International Federation of Gynecology and Obstetrics (FIGO) has classified EC into three grades on the basis of morphology (FIGO 1989). Grade 1 tumours are neoplasms with $\leq 5\%$ non-squamous component or those with a non-morular solid growth pattern (FIGO 1989, Creasman *et al.* 2006, Zaino 2009). In grade 2 tumours, 6–50% of the neoplasm is non-squamous and non-morular, and in grade 3 tumours, $>50\%$ of the tumour is a solid mass (FIGO 1989, Zaino 2009). In general, grade 1 tumours are well-differentiated, grade 2 moderately differentiated and grade 3 poorly differentiated (Creasman *et al.* 2006). FIGO grading increases by 1 when nuclear atypia appears inappropriate for the architecture of the grade (e.g. grade 1 becomes grade 2), and when adenocarcinomas contain squamous differentiation, the cells are graded according to the nuclear grade of the glandular component (Creasman *et al.* 2006). Serous, clear cell and pure squamous cancers are designated grade 3 by convention due to their poor prognosis (Zaino 2009).

Types of endometrial carcinoma

In 1983, Bokhman performed a large study over 20 years and classified sporadic ECs into two types based on their aetiology and clinical behaviour (Bansal *et al.* 2009). This classification has been confirmed on the basis of genetic mutation differences between the two types. Type I ECs have an endometrioid histology and account for 70–85% of sporadic cases (Creasman *et al.* 2006, Di Cristofano & Ellenson 2007, Bansal *et al.* 2009). They have a favourable prognosis as they are often diagnosed at an early stage and are of a low grade (Bokhman 1983,

Creasman *et al.* 2006, Di Cristofano & Ellenson 2007, Bansal *et al.* 2009). These tumours often arise from endometrial hyperplasia in a setting of unopposed oestrogen in peri- and post-menopausal women and commonly express oestrogen and progesterone receptors (Bokhman 1983, Di Cristofano & Ellenson 2007, Bansal *et al.* 2009). Mutations in *PTEN*, β -catenin (*CTNNB1*), *KRAS* and *MSI* are molecular lesions associated with type I EC (Di Cristofano & Ellenson 2007).

Type II tumours account for 10–20% of sporadic ECs and often have a serous papillary or clear cell histology (Bokhman 1983, Prat *et al.* 2007, Bansal *et al.* 2009). They tend to be composed of markedly atypical cells that grow in papillary, glandular or solid patterns (Di Cristofano & Ellenson 2007). Type II tumours arise in a background of atrophic post-menopausal endometrium independent of oestrogen and may be preceded by endometrial intraepithelial carcinoma (EIC; Bokhman 1983, Di Cristofano & Ellenson 2007, Prat *et al.* 2007, Bansal *et al.* 2009). EIC is composed of cells that are indistinguishable from carcinoma cells, but confined to endometrial epithelium, without invasion into the underlying stroma (Di Cristofano & Ellenson 2007, Prat *et al.* 2007). Type II tumours have a poor prognosis as they tend to spread from the site of origin early in the development of the disease (Bokhman 1983, Prat *et al.* 2007, Bansal *et al.* 2009). Mutations in *TP53* and *ERBB2* (*HER-2/neu*) genes and aneuploidy are commonly found in type II cancers, whereas mutations in *KRAS*, *PTEN* or *MSI* are relatively uncommon, suggesting different molecular pathways for tumourigenesis in the two types of EC (Di Cristofano & Ellenson 2007).

Cancer stem cells in endometrial carcinoma

Given that a rare population of epithelial stem/progenitor cells has been identified in human endometrium (Chan *et al.* 2004, Gargett *et al.* 2009), it is possible that these cells or their progeny may be the source of the putative CSCs that may initiate and maintain EC (Fig. 1; Gargett *et al.* 2008). The first evidence for a stem cell origin of EC was suggested in 1997 in a study on a uterine carcinosarcoma-derived cell line (Gorai *et al.* 1997). In this study, colony-initiating cells grew for over 50 serial passages and were composed of cells with columnar, small epithelial, moderately sized or large epithelial-like, malignant tumour giant and spindle-shaped morphologies, similar to those found in the original cell line (Gorai *et al.* 1997). These highly proliferative clonal cells expressed immunohistochemical and molecular markers consistent with their parental tissue, re-capitulated the tumour phenotype *in vitro* and were considered stem cells responsible for propagating the cell line (Gorai *et al.* 1997). While these results were promising, they were not performed on freshly isolated EC cells, or even a pure EC cell line.

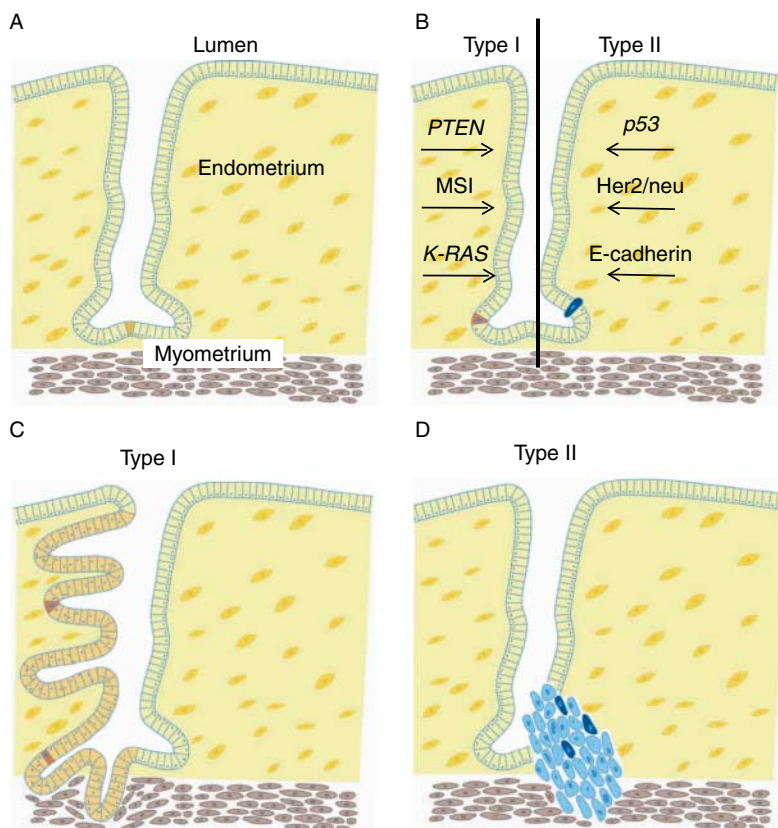


Figure 1 Hypothesised role of cancer stem cells in the development of endometrial carcinoma. (A) Normal endometrial gland with a potential epithelial stem/progenitor cell located at the base of the gland. (B) Epithelial progenitor cell acquires genetic mutations resulting in the development of the CSCs in either type I (left, orange) or type II (right, dark blue) EC. Expansion of the CSC clone producing heterogeneous neoplastic epithelial cells in type I (C) and type II (D) ECs, comprising a small number of CSCs (C, orange; D, dark blue) and numerous differentiated tumour cells (C, light orange; D, light blue).

Identification of CSC activity in human EC samples

Recently, freshly isolated cells from EC tissues have been investigated for CSC properties (Hubbard *et al.* 2009). A small population of freshly isolated EC cells has the capacity to initiate clones that undergo self-renewing cell divisions and initiate tumours *in vivo* that can be serially passaged, demonstrating self-renewal, proliferation and differentiation abilities of the potential CSCs.

Less than 1% of single EC cells from all grades and both types of EC initiated clones *in vitro* (Hubbard *et al.* 2009). As a range of clone sizes were observed, more differentiated progenitor cells may have also contributed to the observed cloning efficiency (Hubbard *et al.* 2009). This demonstrated that only rare cells within EC are able to proliferate and initiate large colonies, conforming to the CSC hypothesis.

Heterogeneous proliferative potential of EC cells was further confirmed in tumourigenicity studies. EC cells were subrenally transplanted in NOD/SCID mice in limiting dilution, but not all dilutions initiated tumours, a phenomenon observed in all grades and both types of EC (Fig. 2; Hubbard *et al.* 2009). The tumours re-capitulated the original tumour histoarchitecture and differentiation marker expression, including ESR1(ER α), PGR (PR), vimentin and cytokeratin (Hubbard *et al.* 2009). Type I EC cells also formed tumours when injected subcutaneously; however, larger numbers of cells were

required to initiate tumour formation (Friel *et al.* 2008, Hubbard *et al.* 2009). This is probably related to the superior blood supply from kidney tissue into the xenograft as has been observed in other tumours (Visvader & Lindeman 2008). The ability of some EC cells to initiate tumours supports a CSC origin of EC, and these cells may be similar to the original cells that gave rise to the tumour in the patient.

Colony-forming units/cells from all grades and both types of EC underwent several rounds of serial subcloning *in vitro*, indicating substantial self-renewal, an essential CSC property (Hubbard *et al.* 2009). A trend of increasing self-renewal ability was observed with increasing tumour aggressiveness in this study (Hubbard *et al.* 2009). Secondary clones also expressed several self-renewal genes, including *BM11*, *CTNNB1*, *SOX2* and *NANOG* (Hubbard *et al.* 2009). Also, primary subcutaneous tumours established from freshly isolated xenografted EC cells were serially passaged up to five times in immunocompromised mice, maintaining the original tumour morphology and phenotype when examined by immunohistochemistry, demonstrating the CSC traits of self-renewal and differentiation *in vivo* (Friel *et al.* 2008, Hubbard *et al.* 2009). While type II tumours appear to initiate tumours more efficiently (Hubbard *et al.* 2009), no trend of increasing self-renewal with increasing tumour

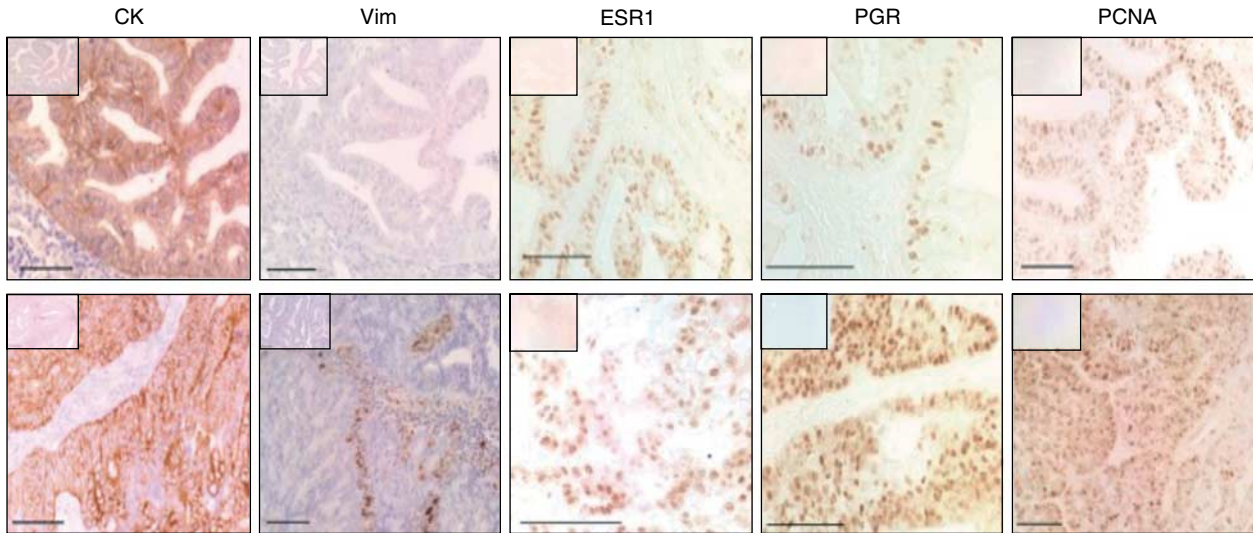


Figure 2 Differentiation marker analysis of transplanted and parent EC tumours. Immunohistochemistry of representative tumours derived from transplanted cells (top panels) and corresponding parent tumours (lower panels). Insets, isotype controls. Transplants and parent tumours are classified as type I, grade 3. CK, cytokeratin; Vim, vimentin; ESR1, oestrogen receptor- α ; PGR, progesterone receptor (brown staining; blue, haematoxylin counter-stained with CK and Vim). Transplants were highly proliferative as indicated by PCNA staining. Scale bars 100 μ M. Modified and reprinted, with permission, from Hubbard SA, Friel AM, Kumar B, Zhang L, Rueda BR & Gargett CE 2009 Evidence for cancer stem cells in human endometrial carcinoma. *Cancer Research* 69 8241–8248. ©2009 American Association for Cancer Research.

aggressiveness was observed *in vivo*, perhaps due to low sample sizes of the investigations.

Together, these data from *in vitro* and *in vivo* studies on EC and EC cell lines provide substantial evidence for a CSC origin for EC. However, these studies are generally retrospective and a cell surface marker or combination of markers for the isolation of EC CSCs is required, so that the CSCs can be isolated, characterised and their role in the development of the disease was studied.

Side population cells as EC stem cells

Side population (SP) cells are cells capable of expelling the Hoechst 33342 dye, often due to the presence of plasma membrane ABCG2 transporters. A recent study investigating CSCs in EC has examined several EC cell lines and four high grade ECs for the presence of SP cells (Friel *et al.* 2008). In the AN3CA and Ishikawa, but not the SKUT-2, HEC-1-A and HEC-1-B EC cell lines, and in four EC cell suspensions, rare (<3.4% of cells sorted by FACS) SP cells were detected (Friel *et al.* 2008; Fig. 3). SP cells from the cell lines demonstrated several CSC traits, including slow growth rate *in vitro*, demonstrated by a higher percentage of cells in the G1 phase of the cell cycle than the main population (MP), suggesting that SP cells cycled less frequently than the majority of the tumour cells (Friel *et al.* 2008). Another trait was the resistance of SP cells to paclitaxel, suggestive of the presence of drug transporters, such as ABCG2. *In vitro* culture of the SP cells from the cell lines recapitulated the original SP:MP cellular ratio, suggesting self-renewal

and differentiation potential of cells in the SP fraction (Friel *et al.* 2008). Finally, SP cells, but not MP cells, from the cell lines and the four EC tissues initiated tumours in NOD/SCID mice when injected subcutaneously (Friel *et al.* 2008). However, it is not known whether the resultant tumours expressed typical EC markers (e.g. ESR1, PGR, cytokeratin, vimentin), nor was it determined whether the SP-initiated tumours contained both SP and MP cells, indicating that differentiation and self-renewal of the SP cells had occurred *in vivo*. However, this study does provide evidence for a CSC origin of EC.

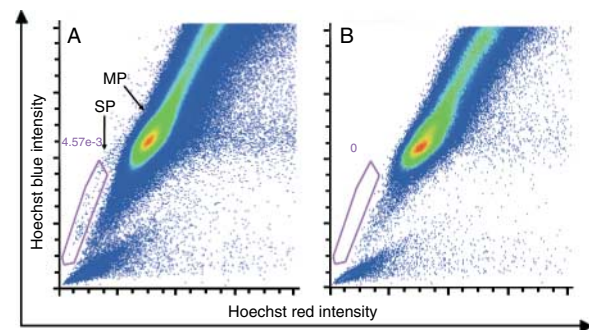


Figure 3 Identification of SP cells in the human endometrial cancer cell line AN3CA. Cells were labelled with Hoechst 33342 and analysed by flow cytometry (A) before and (B) after treatment with verapamil. Reprinted, with permission, from Friel AM, Sergent PA, Patnaude C, Szotek PP, Oliva E, Scadden DT, Seiden MV, Foster R & Rueda BR 2008 Functional analyses of the cancer stem cell-like properties of human endometrial tumor initiating cells. *Cell Cycle* 7 242–249. © 2008 Landes Bioscience.

Markers identifying EC stem cells

Molecular and cell surface markers of CSCs have recently been identified in EC tissues. Musashi-1, a RNA-binding protein expressed by epithelial progenitors of the gastric mucosa, intestine, mammary glands, epidermis and hair follicles, is associated with self-renewal functioning of ASCs (Götte *et al.* 2008, Sureban *et al.* 2008). In xenografted colon adenocarcinomas, si-RNA-mediated knockdown of musashi-1 reduced cellular proliferation and increased apoptosis (Sureban *et al.* 2008), indicating that the potential CSCs maintaining the cancer were unable to function in the absence of musashi-1. Musashi-1-expressing single cells were found in sections of EC samples. It was hypothesised that these were EC progenitor or CSCs. However, functional assays of CSC activity (Gargett 2007) are required to confirm that musashi-1 identifies CSCs in human EC.

CD133 as a marker of EC stem cells

CD133/1 (referred to as CD133) is the first marker used to identify and prospectively isolate EC stem cells (ECSCs; Rutella *et al.* 2009). Both the CD133⁺ and CD133⁻ sorted fractions of EC single cell suspensions grew in culture, giving rise to CD133⁺ and CD133⁻ cells, although the CD133⁺ fraction had a higher proliferation rate (Rutella *et al.* 2009). The CD133⁺ fraction produced single cell-derived colonies at greater efficiency than the CD133⁻ fraction (Rutella *et al.* 2009), and the CD133⁺ cells were more resistant to the cytotoxic effects of cisplatin and paclitaxel than the CD133⁻ fraction (Rutella *et al.* 2009). Interestingly, both populations were equally sensitive to doxorubicin, indicating that this drug may be effective in treating EC. Furthermore, as EC is an oestrogen-dependent disease, the proliferation potential between the populations was compared in the presence of oestradiol (Rutella *et al.* 2009), with the CD133⁺ population showing increased proliferation, whereas the CD133⁻ population did not respond. The CD133⁺ population also had a higher expression of oestrogen receptors than the CD133⁻ population (Rutella *et al.* 2009). However, it is not known whether the CD133⁺ population includes niche cells which may also express oestrogen receptor and relay proliferative signals to the putative ECSCs. In normal mouse endometrium, the potential epithelial progenitor cell (label retaining cell) did not express ESR1, but the neighbouring niche cells did (Chan & Gargett 2006), indicating that normal progenitor cells responded indirectly to oestradiol, via signals provided by niche cells (Chan & Gargett 2006). However, it is not known whether ECSCs will have similar properties to mouse endometrial epithelial label retaining cells. The case may be different in human cancer as it is not known from which cells the CSC arises. Also, the growth kinetics and changes in CD133 expression of the unsorted

populations were not compared, and thus it is not known whether there is any interaction between the two populations. Together, these data indicate that ECSCs may be located within the CD133⁺ population; however, definitive evidence is still lacking.

Unsorted single EC cells subcutaneously transplanted into NOD/SCID mice formed tumours which contained an increased proportion of cells expressing CD133 (20 to 80–90%; Rutella *et al.* 2009), suggesting that CD133⁺ cells may have a greater survival potential within the mouse host. However, freshly isolated CD133⁺ or CD133⁻ cells were each incapable of forming tumours *in vivo* (Rutella *et al.* 2009), indicating that the two fractions may interact during tumourigenesis. It is important to note that in the samples where CD133⁺ or CD133⁻ cells were transplanted, tumours also failed to develop when the unsorted cells from these same samples were transplanted, potentially indicating that these tumours were devoid of TICs. It will be important to undertake additional investigations to confirm this possibility. Furthermore, both CD133⁺ and CD133⁻ fractions isolated from primary xenografts derived from unsorted cells formed tumours of comparable sizes when re-transplanted into NOD/SCID mice. This apparent contradiction to the differential *in vitro* abilities of the CD133⁺ and CD133⁻ cells indicates that TICs may be present in both the CD133⁺ and CD133⁻ fractions, especially after the cells have had the opportunity to adapt to the mouse kidney microenvironment (Rutella *et al.* 2009). It is also possible that CD133 may be expressed on non-tumour-initiating progenitor or transit-amplifying cells unable to proliferate *in vitro*. This study highlights the importance of undertaking a range of CSC assays to confirm the utility of potential markers to enrich for CSC.

Interestingly, CD133 expression was not correlated with tumour grade or CA-125 serum levels, but with endometrioid subtype, as the tumours were mainly early stage, and accordingly CD133 expression was not found in patients with lymph node metastases (Rutella *et al.* 2009). Further work is required to confirm whether CD133 is a marker present on ECSCs and also needs to address the apparent differences between the *in vitro* and *in vivo* experiments.

With the continued investigation of CD133 as a marker of ECSC and the future discovery of other markers for ECSCs, it is hoped that ECSCs can be isolated and characterised, and their role in the development of human EC further investigated. This knowledge opens the way for the development of new treatment modalities that target the CSCs, but spare the remaining normal endometrial stem/progenitor cells and other cells. Such treatments will be particularly useful for early-stage and pre-menopausal EC candidates where the uterus may be conserved, and for late-stage cases where hysterectomy is not curative and current treatments target the bulk tumour cells rather than CSCs.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this work.

Funding

This study is supported by grants from the Cancer Council Victoria (491079), and NHMRC RD Wright Career Development Award (465121) to C E Gargett, Australian Postgraduate Award to S A Hubbard.

Acknowledgements

The authors wish to acknowledge that the endometrial cancer specimens used in the work referred to in their laboratory were provided by the Victorian Cancer Biobank, which is supported by the Victorian Government.

References

- Abraham BK, Fritz P, McClellan M, Hauptvogel P, Athelou M & Brauch H 2005 Prevalence of CD44⁺/CD24⁻/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. *Clinical Cancer Research* **11** 1154–1159.
- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ & Clarke MF 2003 Prospective identification of tumorigenic breast cancer cells. *PNAS* **100** 3983–3988.
- Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, Huang Z, Bentley RC, Mori S, Fujii S *et al.* 2008 Epigenetic regulation of CD133 and tumorigenicity of CD133⁺ ovarian cancer cells. *Oncogene* **28** 209–218.
- Bansal N, Yendluri V & Wenham RM 2009 The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. *Cancer Control* **16** 8–13.
- Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD & Rich JN 2006 Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Research* **66** 7843–7848.
- Bapat SA, Mali AM, Koppikar CB & Kurrey NK 2005 Stem and progenitor-like cells contribute to the aggressive behaviour of human epithelial ovarian cancer. *Cancer Research* **65** 3025–3029.
- Becker L, Huang Q & Mashimo H 2008 Immunostaining of Lgr5, an intestinal stem cell marker, in normal and premalignant human gastrointestinal tissue. *Scientific World Journal* **8** 1168–1176.
- Bokhman JV 1983 Two pathogenetic types of endometrial carcinoma. *Gynecologic Oncology* **15** 10–17.
- Bonnet D & Dick JE 1997 Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine* **3** 730–737.
- Chan RWS & Gargett CE 2006 Identification of label-retaining cells in mouse endometrium. *Stem Cells* **24** 1529–1538.
- Chan R, Schwab K & Gargett C 2004 Clonogenicity of human endometrial epithelial and stromal cells. *Biology of Reproduction* **70** 1738–1750.
- Choi D, Lee HW, Hur KY, Kim JJ, Park G-S, Jang S-H, Song YS, Jang K-S & Paik SS 2009 Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. *World Journal of Gastroenterology* **15** 2258–2264.
- Chu P, Clanton DJ, Snipas TS, Lee J, Mitchell E, Nguyen M-L, Hare E & Peach RJ 2009 Characterization of a subpopulation of colon cancer cells with stem cell-like properties. *International Journal of Cancer* **124** 1312–1321.
- Clark EA, Golub TR, Lander ES & Hynes RO 2000 Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* **406** 532–535.
- Collins AT, Berry PA, Hyde C, Stower MJ & Maitland NJ 2005 Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Research* **65** 10946–10951.
- Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, Heintz APM, Ngan HYS & Pecorelli S 2006 Carcinoma of the corpus uteri. *International Journal of Gynecology and Obstetrics* **95** S105–S143.
- Dalerba P, Dylla SJ, Park I-K, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM *et al.* 2007 Phenotypic characterization of human colorectal cancer stem cells. *PNAS* **104** 10158–10163.
- Di Cristofano A & Ellenson LH 2007 Endometrial carcinoma. *Annual Review of Pathology. Mechanisms of Disease* **2** 57–85.
- Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M *et al.* 2009 Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* **458** 780–783.
- Ferrandina G, Bonanno G, Pierelli L, Perillo A, Procoli A, Mariotti A, Corallo M, Martinelli E, Rutella S, Paglia A *et al.* 2008 Expression of CD133-1 and CD133-2 in ovarian cancer. *International Journal of Gynecological Cancer* **18** 506–514.
- Ferrandina G, Martinelli E, Petrillo M, Prisco M, Zannoni G, Sioletic S & Scambia G 2009 CD133 antigen expression in ovarian cancer. *BMC Cancer* **9** 221.
- Fidler IJ & Kripke ML 1977 Metastasis results from preexisting variant cells within a malignant tumor. *Science* **197** 893–895.
- FIGO, The International Federation of Gynecology and Obstetrics 1989 Announcements. *Gynecologic Oncology* **35** 125–127.
- Fillmore C & Kuperwasser C 2007 Human breast cancer stem cell markers CD44 and CD24: enriching for cells with functional properties in mice or in man? *Breast Cancer Research* **9** 303.
- Friel AM, Sargent PA, Patnaude C, Szotek PP, Oliva E, Scadden DT, Seiden MV, Foster R & Rueda BR 2008 Functional analyses of the cancer stem cell-like properties of human endometrial tumor initiating cells. *Cell Cycle* **7** 242–249.
- Fuchs E, Tumber T & Guasch G 2004 Socializing with the neighbours: stem cells and their niche. *Cell* **116** 769–778.
- Gargett CE 2007 Uterine stem cells: what is the evidence? *Human Reproduction Update* **13** 87–101.
- Gargett CE, Chan RWS & Schwab KE 2008 Hormone and growth factor signaling in endometrial renewal: role of stem/progenitor cells. *Molecular and Cellular Endocrinology* **288** 22–29.
- Gargett CE, Schwab KE, Zilwood RM, Nguyen HPT & Wu D 2009 Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. *Biology of Reproduction* **80** 1136–1145.
- Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S *et al.* 2007 ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* **1** 555–567.
- Gorai I, Yanagibashi T, Taki A, Udagawa K, Miyagi E, Nakazawa T, Hirahara F, Nagashima Y & Minaguchi H 1997 Uterine carcinosarcoma is derived from a single stem cell: an *in vitro* study. *International Journal of Cancer* **72** 821–827.
- Götte M, Wolf M, Staebler A, Buchweitz O, Kelsch R, Schüring AN & Kiesel L 2008 Increased expression of the adult stem cell marker Musashi-1 in endometriosis and endometrial carcinoma. *Journal of Pathology* **215** 317–329.
- Hamburger AW & Salmon SE 1977 Primary bioassay of human tumor stem cells. *Science* **197** 461–463.
- Heppner GH 1984 Tumor heterogeneity. *Cancer Research* **44** 2259–2265.
- Herrmann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ & Heeschen C 2007 Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* **1** 313–323.
- Horner MJ, Ries LAG, Krapcho M, Neyman N, Aminou R, Howlader N, Altekruse SF, Feuer EJ, Huang L, Mariotto A *et al.* (Eds) 2009 *SEER Cancer Statistics Review, 1975–2006*. Bethesda, MD: National Cancer Institute. http://seer.cancer.gov/csr/1975_2006/
- Horst D, Kriegl L, Engel J, Kirchner T & Jung A 2008 CD133 expression is an independent prognostic marker for low survival in colorectal cancer. *British Journal of Cancer* **99** 1285–1289.
- Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, Fields JZ, Wicha MS & Boman BM 2009 Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Research* **69** 3382–3389.

- Hubbard SA, Friel AM, Kumar B, Zhang L, Rueda BR & Gargett CE 2009 Evidence for cancer stem cells in human endometrial carcinoma. *Cancer Research* **69** 8241–8248.
- Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, Wang H, Liu Z, Su Y, Stass SA *et al.* 2009 Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Molecular Cancer Research* **7** 330–338.
- Kelly PN, Dakic A, Adams JM, Nutt SL & Strasser A 2007 Tumor growth need not be driven by rare cancer stem cells. *Science* **317** 337.
- Kern SE & Shibata D 2007 The fuzzy math of solid tumor stem cells: a perspective. *Cancer Research* **67** 8985–8988.
- Klonisch T, Wiehche E, Hombach-Klonisch S, Ande SR, Wesselborg S, Schulze-Osthoff K & Los M 2008 Cancer stem cell markers in common cancers – therapeutic implications. *Trends in Molecular Medicine* **14** 450–460.
- Kusumbe AP, Mali AM & Bapat SA 2009 CD133-expressing stem cells associated with ovarian metastases establish an endothelial hierarchy and contribute to tumor vasculature. *Stem Cells* **27** 498–508.
- Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA & Dick JE 1994 A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367** 645–648.
- Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF & Simeone DM 2007 Identification of pancreatic cancer stem cells. *Cancer Research* **67** 1030–1037.
- Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat M-L, Gyorki DE, Ward T, Partanen A *et al.* 2009 Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nature Medicine* **15** 907–913.
- Mani SA, Guo W, Liao M-J, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M *et al.* 2008 The epithelial–mesenchymal transition generates cells with properties of stem cells. *Cell* **133** 704–715.
- 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** 106–110.
- Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, Reilly JG, Chandra D, Zhou J, Claypool K *et al.* 2006 Highly purified CD44⁺ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* **25** 1696–1708.
- Patrawala L, Calhoun-Davis T, Schneider-Broussard R & Tang DG 2007 Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44⁺α2–β1⁺ cell population is enriched in tumor-initiating cells. *Cancer Research* **67** 6796–6805.
- Petrie K, Zelent A & Waxman S 2009 Differentiation therapy of acute myeloid leukemia: past, present and future. *Current Opinion in Hematology* **16** 84–91.
- Pilkington GJ 2005 Cancer stem cells in the mammalian central nervous system. *Cell Proliferation* **38** 423–433.
- Prat J, Gallardo A, Cuatrecasas M & Catusas L 2007 Endometrial carcinoma: pathology and genetics. *Pathology* **39** 72–87.
- Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF & Ailles LE 2007 Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *PNAS* **104** 973–978.
- Reya T, Morrison SJ, Clarke MF & Weissman IL 2001 Stem cells, cancer, and cancer stem cells. *Nature* **414** 105–111.
- Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C & De Maria R 2007 Identification and expansion of human colon-cancer-initiating cells. *Nature* **445** 111–115.
- Rosen JM & Jordan CT 2009 The increasing complexity of the cancer stem cell paradigm. *Science* **324** 1670–1673.
- Rutella S, Bonanno G, Procoli A, Mariotti A, Corallo M, Prisco MG, Eramo A, Napoletano C, Gallo D, Perillo A *et al.* 2009 Cells with characteristics of cancer stem/progenitor cells express the CD133 antigen in human endometrial tumors. *Clinical Cancer Research* **15** 4299–4311.
- Ryan A, Susil B, Jobling T & Oehler M 2005 Endometrial cancer. *Cell Tissue Research* **322** 53–61.
- Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C *et al.* 2008 Identification of cells initiating human melanomas. *Nature* **451** 345–349.
- Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD & Dirks PB 2004 Identification of human brain tumour initiating cells. *Nature* **432** 396–401.
- Southam CM & Brunschwig A 1961 Quantitative studies of autotransplantation of human cancer. Preliminary report. *Cancer* **14** 971–978.
- Sureban SM, May R, George RJ, Dieckgraefe BK, McLeod HL, Ramalingam S, Bishnupuri KS, Natarajan G, Anant S & Houchen CW 2008 Knockdown of RNA binding protein Musashi-1 leads to tumor regression *in vivo*. *Gastroenterology* **134** 1448–1458.
- Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, MacLaughlin DT & Donahoe PK 2006 Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. *PNAS* **103** 11154–11159.
- Tirino V, Camerlingo R, Franco R, Malanga D, La Rocca A, Viglietto G, Rocco G & Pirozzi G 2009 The role of CD133 in the identification and characterisation of tumour-initiating cells in non-small-cell lung cancer. *European Journal of Cardio-Thoracic Surgery* **36** 446–453.
- Tokar EJ, Ancrile BB, Cunha GR & Webber MM 2005 Stem/progenitor and intermediate cell types and the origin of human prostate cancer. *Differentiation* **73** 463–473.
- Vermeulen L, Todaro M, de Sousa Mello F, Sprick MR, Kemper K, Alea MP, Richel DJ, Stassi G & Medema JP 2008 Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *PNAS* **105** 13427–13432.
- Visvader JE & Lindeman GJ 2008 Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nature Reviews. Cancer* **8** 755–768.
- Wu C, Wei Q, Utomo V, Nadesan P, Whetstone H, Kandel R, Wunder JS & Alman BA 2007 Side population cells isolated from mesenchymal neoplasms have tumor initiating potential. *Cancer Research* **67** 8216–8222.
- Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PWK, Lam CT, Poon RTP & Fan ST 2008 Significance of CD90⁺ cancer stem cells in human liver cancer. *Cancer Cell* **13** 153–166.
- Zaino RJ 2009 FIGO staging of endometrial adenocarcinoma: a critical review and proposal. *International Journal of Gynecological Pathology* **28** 1–9.
- Zeppernick F, Ahmadi R, Campos B, Dictus C, Helmke BM, Becker N, Lichter P, Unterberg A, Radlwimmer B & Herold-Mende CC 2008 Stem cell marker CD133 affects clinical outcome in glioma patients. *Clinical Cancer Research* **14** 123–129.
- Zhang S, Balch C, Chan MW, Lai H-C, Matei D, Schilder JM, Yan PS, Huang THM & Nephew KP 2008 Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Research* **68** 4311–4320.

Received 21 September 2009

First decision 27 October 2009

Revised manuscript received 16 December 2009

Accepted 20 January 2010