Breast cancer chemoresistance: Emerging importance of cancer stem cells

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Abstract
Cancer stem cells (CSCs) have recently been documented in solid tumours. Evidence has suggested that CSCs are involved in carcinogenesis, tumour invasion and metastases, and resistance to various forms of therapies, including chemotherapy. Breast CSCs are characterised by the expression of CD44 but lack of CD24 (CD44+/CD24−/CD0− cells). The mechanisms involved in chemoresistance of breast CSCs are complex and not clearly defined. Overexpression of ABC transporters, detoxification enzymes (aldehyde dehydrogenase), low cell turnover rate and the ability to activate the DNA check point response are possibly all involved. Innovative therapies, based on a better understanding of CSCs, should lead to enhanced and long-term cure rates in breast cancer.

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KEYWORDS
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Abbreviations: CSCs, cancer stem cells; ABC, ATP binding cassette; SP, side population; ALDH1, aldehyde dehydrogenase-1; VEGF, vascular endothelial growth factor.

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Introduction: stem cells in modern medicine

Stem cells are defined as a small and distinct population of cells that have the capacity to divide, and produce progeny of differentiated cells with specific functions. Stem cells, therefore, have two major key characteristics: a) self-renewal and b) the production of “progenitor” cells, which can differentiate into more mature and differentiated cells.

Self-renewal is a characteristic feature of stem cells, thereby, ensuring that the stem cells survive a long time. All stem cells must regulate the balance between self-renewal and differentiation. The self-renewal and differentiation of stem cells is regulated by many signalling pathways, and some pathways are associated with carcinogenesis including the Notch, Shh, BMI-1 and Wnt signalling pathways [1]. In addition, recent evidence suggests that adult stem cells show plasticity, and that these adult tissue-restricted stem cells may develop into cells resembling pluripotent stem cells [2–4]. This property of adult stem cells has raised the possibility of their use in the treatment of a range of degenerative diseases. For example, human CD34+ adult bone marrow stem cells have been recently used for the treatment of chronic liver disease with preliminary impressive outcomes [5,6]. These haematopoietic stem cells have also been used in a number of phase I clinical trails in the treatment of ischaemic heart disease, diabetes and neurodegenerative diseases with impressive preliminary outcomes [7–9]. However, long-term clinical outcome data has still to be documented and carefully evaluated.

Cancer stem cells: minimal key population in tumours

Stem cell patho-biology in cancer was originally described in haematologic malignancies and rapidly expanded into solid tumours. The concepts of cancer stem cells (CSCs) arose from the observations of the capacity to and comparability of self-renewal between stem cells and cancer cells. CSCs are believed to be involved in carcinogenesis, local invasion and metastasis formation, as well as playing a key role in chemo- and radiotherapy resistance [10–12].

Clonogenic studies in vitro and in vivo have demonstrated that only a very small percentage of cells (~0.1%) from various solid cancers produced colonies and gave rise to differentiated mature cancer cells [13]. This very small population of cells is believed to harbour CSCs. In the seminal experiment by Southam and Brunschwig in 1960 (not able to be repeated today because of ethical considerations), patients had their own tumour cells injected into their own body subcutaneously. A low success rate of tumour growth occurred at the injection site (14.3%) [14]. Also, a large number of cancer cells (at least 1 x 10^6 cells) were required in order to induce tumour growth at the autotransplantation site. A possible explanation for the results of Southam’s experiment is likely to be the small percentage of CSCs in the tumour inoculations. Out of the 1 x 10^6 cells that Southam implanted into his patients, possibly less than 0.5% consisted of CSCs and would be able to induce a new tumour at the injection site.

Cancer stem cells and carcinogenesis

Recently, the possible roles of CSCs in carcinogenesis have become more obvious. The CSC hypothesis was firstly proposed by Bonnet and Dick (1997) who documented that a minority of leukaemic cells were able to self-renew and differentiate to generate a new tumour in human acute myeloid leukaemia [15]. Subsequently, experimental and clinical evidence has demonstrated that tumours are derived from individual cancer-initiating cells, rather than from the population as a whole [11]. Normally, the ability of stem cells to undergo self-renewal and to generate differentiated cells is tightly regulated in order to maintain normal homeostasis in the body. However, if this tight regulation is lost, normal stem cells can mutate and the mutated stem cell is known as the “CSC”. This latter cell can differentiate, as do normal stem cells, but gives rise to a malignant growth [16]. The model carcinogenesis induced by CSCs is summarized in Fig. 1. This model is comparable with the model of multi-stage carcinogenesis, where at least two mutations are required, at the stem cell and progenitor cell level, as outlined in Fig. 1 panel B, C.

Breast cancer stem cells

Breast epithelium is composed of two main cell types — luminal epithelial and myoepithelial cells. The mammary stem cell populations reside in the luminal, but not in the myoepithelial compartment [17]. Mammary stem cells are found in the cap of the terminal end bud of mouse mammary glands, and could be identified by in vivo labeling of proliferating cells [18]. The exact derivation of breast CSCs has not been clearly identified. However, it is suggested that, as a result of accumulated oncogenic insults
over time normal breast stem/progenitor cells may transform into breast CSCs/progenitor cells [12]. Of all the CSCs identified in solid tumours, breast CSCs are one of the most commonly studied [19,20].

Previously, phenotypic identification and isolation of stem cells in solid tumour was not possible due to lack of accurate and reliable cell surface markers. However, with the rapid development in biomolecular techniques, various new cell surface markers specific to different types of cancers have been discovered. Consequently, identification and isolation of CSCs from the other cell populations have become realistic goals. Stem cells identified in solid tumours express organ-specific cell surface markers. For example, EpCAM$^{\text{high}}$/CD44$^+$/CD166$^+$ and CD133 are specific markers for the human colonic CSCs [20,21]. CD133 is the specific stem cell marker for human central nervous system cancers [19,21]. CD44$^+$ was the surface phenotypic profile of breast CSCs [22]. A small number of CD44$^+$/CD24$^-$ cells (as few as 200 cells) were able to give rise to new tumours after injection into the mammary fat pad of non-obese diabetic/severe combined immunodeficient mice [22,23]. This biological behaviour (differentiation into mature differentiated cancer cells) of CD44$^+$/CD24$^-$ cells is the important hallmark of breast CSCs.

CD44 is a 37 kDa cell adhesion molecule expressed in most cell types, including putative breast CSCs [24]. CD24 was originally found during the early stage of B cell development and is highly expressed in neutrophils, but not in normal T cells or monocytes [25]. Genomic studies of CD44$^+$ cells, extracted from human breast tissues, have shown the expression of genes associated with self-renewal, including hedgehog signalling pathway-related genes: Gli1 and Gli2 [26]. As well, the TGF-signalling pathway, known to be important in human embryonic stem cells and promoting invasion and angiogenesis, was found to be activated in CD44$^+$ breast cancer cells [26].

**Role of cancer stem cells in breast cancer chemoresistance**

**Breast cancer stem cells and ATP binding cassette (ABC) transporters**

One characteristic of CSCs that differentiates them from other normal cells in the tumour is that they have high levels of ABC transporter proteins, in particular ABCG2 [27]. The ABC transporter molecules are responsible for protecting cells from drug damage via efflux pumping mechanisms. Thus, CSCs, as a result of these biological properties, are rendered resistant to drug treatment, including chemotherapeutic drugs [12].

![Figure 1](image_url) A schematic diagram which demonstrates CSC mutation and tumour progression. Normal stem cells give rise to multipotent progenitors, committed progenitors and mature cells (panel A). However, if there is a mutation in the stem cells, the result is the premalignant lesion (panel B). An additional mutation in the progenitor cells result in malignant tumour transformation (panel C).
In clinical practice, optimal chemotherapy treatment can kill most cells within solid tumours. However, a small fraction of cells (postulated to be CSCs) are drug resistant, possibly because of enrichment of ABC transporter proteins and resultant rapid and effective efflux of drugs out of the cells. This small fraction of CSCs remains quiescent in the G-null phase. Over a period of time and due to stimuli (e.g. release of cytokines, heat-shock proteins) associated with tumour cell death, these quiescent stem cells are induced to divide and produce progenitor cells. Subsequently, some of these progenitor cells differentiate into new mature tumour cells with a chemoresistant phenotype [12]. This is the postulated model of acquired chemoresistance in breast cancer observed in the clinic (Fig. 2). Patients at this stage will develop recurrent tumours and fail to be responsive to further chemotherapy treatment.

The high expression of ABC transporter proteins in tumour stem cells results in exclusion of the fluorescent dye Hoechst 33342 and Rhodamine 123, and can be detected by flow-cytometry. The cells that are able to efflux Hoechst 33342, as detected on flow-cytometry, are known as the “side population” (SP) cells [28,29]. Use of Hoechst efflux assay was originally documented for isolation of murine tumour cells with a chemoresistant phenotype [12]. This is confirmed that chemoresistant breast cancer cells contained a higher population of SP cells, compared with the chemosensitive cells [30]. We also documented a significantly higher population of CD44+ /CD24− cells in adriamycin and paclitaxel resistant breast cancer cells, compared with the chemosensitive cells [30]. Similar findings have also been reported in a recent study where SP cells were more tumourigenic and more resistant to chemotherapy [31]. Furthermore, in a proteomic analysis of SP cells of breast cancer cell lines, thymosin β4, a small actin-binding protein involved in cell motility, has been linked with chemoresistance [31]. Suppression of thymosin β4 in a validated functional assay resulted in activating apoptosis during paclitaxel and doxorubicin treatment, thus enhancing the chemosensitivity [31].

Breast cancer stem cells and resistance to other therapies

As well as a phenotypic marker of breast CSCs and chemoresistance, CD44+/CD24− cells have been linked to resistance to other forms of treatment, including radiation therapy. CD44+/CD24− cells isolated from MCF-7 and MDA-MB-231 breast cancer cells were more radio-resistant than non CD44+/CD24− cells [32]. The mechanisms involved in radio-resistance are not well understood, possibly due to decreased induction of reactive oxygen species in the CD44+/CD24− cells, and activation of the DNA damage check point response [32,33]. Moreover, an in vitro study has documented that during a fractioned course of radiation, the number of CSCs has increased with the activation of Jagged-1 and Notch-1, and has suggested the possible induction of radiotherapy resistance via the Notch signalling pathway [32].

Aldehyde dehydrogenase-1 (ALDH1) is a detoxification enzyme, involved in catalyzing the oxidation of acetaldehydes produced from ethanol, and was first described in hepatic cells; higher than normal levels of this enzyme have been documented in liver cancer cells [34]. High ALDH1 activity is another characteristic of human breast and colonic cancer stem/progenitor cells [35,36]. As few as 500 ALDH1-positive cells (as documented by the ALDEFLOUR assay) can give rise to a new tumour in NOD/SCID mice [35]. High ALDH1 activity cells were also documented in our adriamycin and paclitaxel resistant breast cancer cell lines (unpublished data). Other studies have found over-expression of ALDH1 in cyclophosphamide resistant leukaemic and colonic cancer cells [36,37]. Due to the known function of ALDH1 as a detoxification enzyme, and as this enzyme is overexpressed in CSCs, this is possibly another reason why CSCs are resistant to various treatments of cancer, including chemotherapy and radiation therapy resistance.

Breast cancer stem cells and cancer invasiveness

CSCs are postulated to be involved not only in carcinogenesis but also in tumour invasion and formation of metastases [10,11]. In a recent study of 13 different types of
breast cancer cell lines, with different levels of invasiveness, CD44+/CD24− cells were confirmed to have higher levels of expression of genes associated with invasion [38]. As a result, CD44+/CD24− cells have been shown to have a significantly higher relative invasion index than CD44+/CD24+ cells [38]. The presence of CD44+/CD24− cells was also linked with the increase of distant metastases in human breast cancers. In a study of 136 breast cancer tissues, the presence of a high number of CD44+/CD24− cells (~10%) in the breast cancer tissue samples was correlated with a high incidence of distant metastases [39]. Another study showed that all the disseminated cancer cells in the bone marrow of breast cancer patients were CD44+/CD24− cells [25]. These findings have highlighted the possible roles of putative breast cancer stem/progenitor cells in invasion and dissemination.

Strategies to overcome chemoresistant breast cancer by targeting breast cancer stem cells

If the chemoresistant cells are CSCs, targeting treatment at these cells would be the logical way forward to overcome the chemoresistance and could improve the outcome of breast cancer treatment. The traditional approach of changing chemotherapeutic regimens, after tumours develop resistance to one chemotherapeutic regimen, may not be useful in terms of achieving long-term survival advantage. Most current chemotherapeutic drugs are targeted on rapidly dividing cells within the tumour, but probably spare the slowly dividing and inherently resistant CSCs and, thus, may not lead to long-term cures [40].

CSCs may be eliminated by selectively targeted therapies against various self-renewal signalling pathways including the Notch, Shh, BMI-1 and Wnt signalling pathways [41]. However, if normal stem cells and CSCs share the same pathways to maintain their self-renewal, it would be more complex to selectively target at self-renewal pathways of CSCs without inducing harmful damage to normal stem cells. Fortunately, it appears that CSCs are more likely to be more dependent on certain putative pathways [42].

CSCs may be protected from external toxic agents via the overexpression of ABC transporter proteins. Therefore, targeting at these proteins may be an alternative strategy and, thus, a way to overcome chemoresistance. Recently, an in vitro study has shown the benefit of gefitinib, a tyrosine kinase inhibitor, in reversing chemotherapy resistance in multidrug resistant breast cancer cells expressing ATP transporters [43]. Also, gefitinib has been recently reported to successfully overcome SN-38-resistance in small-cell lung cancer cells in vitro [44]. Therefore, gefitinib, in combination with current chemotherapeutic agents, may be an alternative approach in eradicating breast CSCs.

Moreover, instead of killing tumour cells with chemotherapy, biological therapy with monoclonal antibodies targeted against specific cellular surface molecules or receptors should be considered. Targeting at the apoptotic pathway could be an attractive and therapeutically beneficial option. Chemotherapeutic-induced cell death is generally programmed by apoptosis [45]. The elimination of CSCs may be feasible by increasing the ratio of pro-apoptotic to anti-apoptotic proteins and signal pathways, perhaps targeting at pro-apoptotic members of the Bcl-2 family [46]. Alternatively, targeting CSCs at the niche endothelium is a possible therapeutic strategy. CSCs niches are likely to be well endowed with a blood supply as a result of enhanced angiogenesis [45]. Therefore, blockade of action of vascular endothelial growth factor (VEGF) signalling with the anti-VEGF agent, bevacizumab, could be an alternative approach.

However, all the strategies proposed above are speculative. Published data, so far, has not yet confirmed the benefit of these approaches in chemoresistant patients where CSCs are believed to be the predominant factor. If CSCs are key factors responsible for chemoresistance, there is an urgent need to enhance both experimental and clinical studies to support the use of these biological therapies in chemoresistant breast cancers. It is likely that additional agents, following chemotherapy, may be needed to eradicate CSCs, if a good long-term outcome is to be achieved.

Conclusions

New concepts of chemoresistance in solid tumours have been proposed and involve the resistance and persistence of a minority of cells (CSCs) that result in progressive cancer cell growth and the development of new tumours. The mechanisms responsible for chemotherapy resistance by CSCs have not been clearly identified, but overexpression of ABC transporters and detoxification enzymes, and their slow turn over rate and ability to activate the DNA damage check point response, are all possible pathobiological mechanisms. Current published data on CSCs and chemoresistance is limited. It is essential, therefore, to characterise more fully these findings in in vivo human breast cancers. If the CSCs are key cells in induction of chemoresistance, as hypothesised, current chemotherapy may not be the best approach to fully eradicate solid tumours. Other strategies aimed at killing breast CSCs would be a more reliable approach, if we are to achieve permanent cures in breast cancer.

Conflict of interest statement

All authors declare no conflict of interest.

References


