Review

Breast cancer stem cells: implications for therapy of breast cancer

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Abstract

The concept of cancer stem cells responsible for tumour origin, maintenance, and resistance to treatment has gained prominence in the field of breast cancer research. The therapeutic targeting of these cells has the potential to eliminate residual disease and may become an important component of a multimodality treatment. Recent improvements in immunotherapy targeting of tumour-associated antigens have advanced the prospect of targeting breast cancer stem cells, an approach that might lead to more meaningful clinical remissions. Here, we review the role of stem cells in the healthy breast, the role of breast cancer stem cells in disease, and the potential to target these cells.

Introduction

The past decades have seen advances in the diagnosis and treatment of breast cancer. Despite this progress, breast cancer is still a leading cause of cancer-related deaths among women, with as many as 40% relapsing with metastatic disease [1]. Breast cancer survival rates have been shown to plateau after 7 to 10 years, whereas most cancer survival curves take between 2 and 5 years to plateau [2]. The length of time for the survival rate to plateau in breast cancer might indicate the involvement of a cell type capable of disease recurrence which is able to withstand primary treatment and reside in the body, often undetected, for prolonged periods. Interestingly, it has been shown that, of the 40% of patients with lymph node involvement who did not undergo surgical removal, only 15% had recurrence of disease [3]. This raises the point that immune system surveillance of tumours or other protective mechanisms of the body might be capable of controlling breast cancer relapses.

Prominent in the breast cancer field has been the notion of the existence of a transformed population of cells with many of the properties of stem cells that may be responsible for the origin and maintenance of tumours. These stem cell-like cells, designated as cancer stem cells, represent a minor subset of cells in the tumour and are distinct from the more differentiated tumour cells. It is thought that these cancer stem cells may play an important role in cancer establishment, progression, and resistance to current treatments. Traditional cancer therapies are effective at debulking some tumours but often fail to produce long-term clinical remissions, possibly due to their inability to eradicate the cancer stem cell population. Therefore, novel treatments aimed at targeting the cancer stem cell population could find use in treating both primary and metastatic tumours.

Therapies aimed at targeting cancer stem cells may prove clinically relevant in inducing long-term clinical remission of cancer. A variety of methods, including inducing differentiation of the cancer stem cells or targeting of cancer stem cells for elimination, are being studied to disrupt the cancer stem cell pool. Cancer stem cell antigens may also provide a new target for cancer immunotherapy. The targeting of breast cancer stem cells (BCSCs) through immunotherapy, such as dendritic cell (DC)-based therapies or adoptive T-cell

ABC = ATP-binding cassette; ALDH1 = aldehyde dehydrogenase-1; BCSC = breast cancer stem cell; BMP = bone morphogenetic protein; CK = cytokeratin; CT = cancer/testis; CTL = cytolytic T-cell response; DC = dendritic cell; EGFR = epidermal growth factor receptor; ER = oestrogen receptor; ESA = epithelial surface antigen; Hh = Hedgehog; Lin$^-$ = lineage-negative; MDR = multidrug resistance; MHC = major histocompatibility complex; NOD = nonobese diabetic; PgR = progesterone receptor; RA = retinoid acid; SCID = severe combined immunodeficiency disease; SP = side population; TAA = tumour-associated antigen; TDLU = terminal duct lobular unit.

transfer, has the advantage of treating the putative cells of tumour origin and can be used in conjunction with current treatment regimes. For these treatments to become effective, cancer stem cells will need to be defined in terms of antigenicity and distinctions between cancer stem cells and stem cells must be found. This review will discuss stem cells and their role in mammopoiesis, cancer stem cells and their function in tumour formation, and the potential targeting of cancer stem cells for therapy with a focus on breast cancer.

Stem cells

Somatic stem cells are responsible for tissue homeostasis in the adult and have limited plasticity. They are responsible for tissue renewal and repair and can become activated in response to environmental signals such as hormones. Somatic stem cells are part of a hierarchy of cells in the tissue, including the slowly proliferating somatic stem cell, the more differentiated and proliferating transit-amplifying cell progeny, and the several lines of differentiated cells. While stem cells are mostly quiescent, they may asymmetrically divide to give rise to one transit-amplifying cell and another stem cell [4]. This provides continuation of the stem cell compartment while providing the starting material for production of differentiated cells. Somatic stem cells may also divide symmetrically to produce two stem cells. Understanding the role that the maintenance of cell division and differentiation of stem cells plays may lead to new insights into the signalling pathways involved in cancer progression and, ultimately, yield new approaches for cancer treatment.

Stem cells have basic defining and identifying properties. The ability for self-renewal, in which through cycles of cell division a stem cell gives rise to at least one daughter cell with the same characteristics of the parent, is a critical characteristic. The proliferative potential of cancer stem cells has been examined *in vitro*, in particular for stem cell-like neural precursors of human glioblastoma, and has been characterised as capable of exponential proliferation, long-term proliferation, self-renewal, and multipotency [5]. The plasticity of stem cells allows them, when activated, to renew several lineages of differentiated cells during homeostasis. In adult tissue, the stem cell population rarely divides, but when stimulated by hormones during development or by a loss of transit-amplifying cells they can be rapidly activated to undergo asymmetric cell division to renew the tissue compartment. Transit-amplifying cells can expand rapidly, providing progeny that differentiate into mature cells of varying lineages. Nonproliferative differentiated cells make up the bulk of the tissue and undergo apoptosis after a finite life span. In this way, under most conditions, the total number of cells in a tissue is maintained in equilibrium, with the number of differentiated cells dying being equalled by the number of progenitor cells dividing.

Stem cells have a relatively lineage-negative (Lin^-) phenotype with few cell surface markers identified. One property shared by normal stem cells and cancer stem cells is the expression of the ATP-binding cassette (ABC)-G2 transporter. The ABCG2 is a class of drug transporters capable of pumping out of the cell a variety of substrates, including cytotoxic drugs, by using ATP energy [6]. High expression of these transporters may help protect cancer stem cells from cytotoxic agents used for cancer treatment. The ABCG2 transporter has been shown to specifically pump out the DNA-intercalating dye Hoechst 33342 [6]. Activity of this transporter leads to the identification of a population of cells known as the side population (SP) by flow cytometric analysis. This functional property has been used to study mammary stem cells, which upon transplantation into cleared mammary fat pads have been shown to give rise to breast tissue [7,8]. However, other studies have called into question the use of the SP to isolate cells with potential to reconstitute a functional mammary gland [9].

The human breast and mammopoiesis

Mammopoiesis is the development of the cellular lineages and functional units of the mammary gland. These units are comprised of terminal ductules and alveoli, which together form the terminal duct lobular units (TDLUs). Collectively, TDLUs form the branches of a greater ductal-lobular system composed of an inner layer of polarized luminal cells and an outer layer of myoepithelial cells [10]. The adult human breast is composed of 15 to 20 lobes each with multiple lobules surrounded by adipose tissue. Additionally, the breast has a system of lymphatic vessels responsible for draining breast tissue leading to internal mammary lymph nodes and axillary regional lymph nodes. The human breast is a dynamic gland with tissue homeostasis occurring during early development, puberty, within menstrual cycles, during pregnancy and lactation, and eventual involution during menopause. It is believed that certain of these processes are brought about by the action of somatic stem cells.

The breast originates from the invagination of the epidermis into the underlying mesenchymal tissue during the 10- to 24-week period of gestation. This process gives rise to epithelial ducts, which in turn give rise to rudimentary lactiferous ducts. Unlike a variety of other organs, the human breast continuously undergoes morphological and functional changes well into adulthood, with a secondary onset during puberty and culminating with the greatest differentiation occurring during pregnancy and lactation. Oestrogen hormonal stimulation during puberty drives the ductal elongation of the breast with stem cell activity found in the terminal end buds [11]. Prolactin and progesterone drive ductal branching and formation of acini, leading to the formation of mature breast tissue [12]. Two major subclasses of cells appear at this time: the outer myoepithelial (or basal) cells and the inner luminal epithelial cells. Myoepithelial cells are characterised by expression of common acute lymphoblastic leukaemia antigen (CALLA) or CD10 [13], Thy-1 [10], alpha-smooth muscle actin [14], vimentin [15],

and cytokeratin (CK) 5 and CK14 amongst others [16]. Myoepithelial cells are contractile cells that form a sheath around the ductal network of the breast. Luminal epithelial cells are characterised by expression of MUC1 [17], epithelial surface antigen (ESA) also known as EpCAM (epithelial cell adhesion molecule) [18], and CK7, CK8, CK18, and CK19 [16] as well as oestrogen receptor (ER) and progesterone receptor (PgR). During periods of pregnancy and lactation, the breast goes through further rounds of development with an increase in cell growth and formation from the luminal epithelial lineage of functional milk-secreting alveoli. Following these periods and again during menopause, there is an involution through apoptosis of the breast tissue [19].

Mammary stem cells

In the 1950s, Deome and colleagues [20] performed one of the earliest demonstrations of the existence of adult mammary stem cells, using a limiting-dilution assay showing that clonal precursor cells are capable of forming functional mammary outgrowths in a murine transplantation model. This cleared fat pad assay consists of clearing the endogenous epithelium of mice followed by transplantation of a limited dilution of cells, fewer than 2×10^4, into the gland-free mammary fat pads of mice. Several additional lines of evidence have indicated that mammary stem cells are active in forming and maintaining breast tissue. X-chromosome inactivation studies have shown that entire areas of the breast, in particular individual TDLUs, are monoclonal in origin [21-23]. These areas are derived from one progenitor cell determined during early embryogenesis which has undergone random inactivation of one chromosome around day 16. Additional evidence indicating the clonal origin of areas of breast tissue has come from retroviral tagging of mammary epithelial cells. Single cells transplanted into the cleared fat pads of mice can produce progeny capable of forming a functional mammary gland [24]. More recently, it has been shown that a single cell with stem cell-like features is capable of forming a complete and functional mammary gland upon transplantation into the cleared fat pads of female mice [9].

Role of stem cells in tumourigenesis

Several models have been used to explain the origin and continued growth of tumours. Perhaps the most prominent model used to describe cancer is the clonal evolution of tumours theory. This model postulates that cancer originates from mutations occurring in a few cells or a single cell that eventually leads to uncontrolled and unlimited proliferation of a population of cells [25]. Genetic alterations continue to accumulate as the tumour progresses, leading to activation of proto-oncogenes into oncogenes and inactivation of various tumour-suppressor genes and ultimately giving rise to subtypes of cells in the tumour which have acquired several traits such as the ability to evade apoptosis, self-sufficiency in growth signalling, tissue invasion and metastasis, and limitless replicative potential [26]. This model further postulates that mutations in various cells would allow for a selection of cells to have a survival advantage over others, leading them to proliferate to a greater extent and allowing those cells to seed new tumours capable of further rounds of clonal expansion. The clonal evolution of tumours model is capable of explaining some of the key characteristics of cancer growth but is perhaps too simplistic. Building upon the clonal evolution theory, there are two different models for how tumours develop and progress through unlimited cell division: the stochastic and hierarchical models of tumour development. The stochastic model postulates that all cells in a tumour have equal potential to be tumourigenic (that is, any cell from that tumour has an equal probability to form a new tumour with characteristics similar to the primary tumour) [27]. The hierarchical model postulates that only a subset of the cells in a tumour have this tumourigenic capacity and that the rest of the tumour is populated by cells with varying degrees of differentiation which cannot regenerate the tumour on their own [27]. The latter model is in concordance with the cancer stem cell hypothesis, in which the cancer stem cell is the cell responsible for tumour self-renewal and not the differentiated cells that make up the bulk of the tumour.

Tumours have been characterised as heterogenous, composed of several types of differentiated and undifferentiated cells. What, then, is the cell type that can be transformed and give rise to both differentiated and undifferentiated cells? Epithelial cells in the breast do not proliferate greatly and are continuously being replaced and thus they do not have a considerable opportunity to be the target of mutation events. Though possible, it seems unlikely that well-differentiated cells could be the source of the transformation event or that they would then digress to form several undifferentiated cells as well as differentiated ones. Some experimental models of cancer have shown that tumours are initiated from cancer cell lines only when large numbers of cells are used (that is, using a larger amount of cells allows for the rare cancer stem cell-like cell to be included in the transplant). In these models, most of the cells in the original tumour or cell line lack the ability to establish cancer. In one experiment model of colon cancer, CD133, a stem cell antigen used for sorting cells with stem cell-like qualities, was used to show that the rare subpopulation in the tumour of $CD133^+$ cells was responsible for tumour maintenance. The *in vitro* growth characteristic of $CD133^+$ colon cancer tumour cells was compared with $CD133^-$ cells. $CD133^+$ cells grew exponentially as undifferentiated tumour spheres in serum-free medium *in vitro* and were more tumourigenic *in vivo* than $CD133^-$ cells [28]. These results indicate that there is an intrinsic difference in the growth rate of stem cell-like cells compared with other cell types in certain cancer models. Taken together, these results seem to indicate the presence of cancer stem cells at work driving tumour formation. Revising the clonal origin of cancer theory, tumours derived from a transformation event are seeded by a small subset of cells with cancer stem cell characteristics. These proliferating undifferentiated cells with

stem cell-like phenotypes can then give rise to the nondividing differentiated cells seen in tumours. Furthermore, mutations affecting the cancer stem cell compartment are the ones that are passed on to the descendant population and multistep tumour progression occurs through this small population of cells. This revision might also be too simplistic as the hierarchical and stochastic models of tumour formation might be working at the same time and might not be mutually exclusive; indeed, some tumours could be the products of transformation events in differentiated cells. Additionally, it is important to note that for therapeutic purposes it might be advantageous to identify cells capable of metastatic disease, an event that can occur early in tumourigenesis, rather than focus on populations that are responsible for initial tumour formation. In this case, the focus is on cancer stem cells that are able to seed new tumours.

Stem cells and cancer cells share a number of important characteristics. They both have the capacity for self-renewal and extensive proliferation. In the case of tumour cells, this takes the form of self-sufficiency in growth signalling and uncontrolled cellular proliferation, whereas for stem cells, this is a tightly controlled process that occurs during embryogenesis, organogenesis, and maintenance and repair of adult tissues. Both cell types are long-lived with active anti-apoptotic pathways and telomerase activity [29]. This feature makes stem cells more prone to accumulation of damaging mutations and genomic instability despite active DNA repair mechanisms. For tumours, there are often even higher risks of accumulating mutations as defects in DNA repair mechanisms are often present. Both cell types have a resistance to environmental toxins, chemotherapeutic and radiation agents, often as a result of multidrug resistance (MDR) via expression of ABC transporter proteins and selection by chemotherapy. Additionally, it should be noted that stem cells are relatively resistant to radiation because they are slow-cycling. It is thought that cells in a tumour which are able to withstand radiation might be cancer stem cells that have this slow-cycling quality. Stem cells and tumours also share the characteristic of being mobile, leading to migration and homing for stem cells and potentially to metastatic disease for tumour and cancer stem cells [30]. Anchorage independence is one of the most important characteristics of transformed cells (including metastatic cells) and is a property of normal stem cells. These stem cell characteristics that are common to cancer cells suggest that fewer or different steps might be involved for stem cells to transform into tumour-initiating cells in comparison with differentiated cells.

The evidence for the existence of adult stem cells responsible for the initiation and maintenance of cancer has been characterised for several tumours recently. During the 1990s, studies of acute myeloid leukaemia first confirmed that transformed stem cell-like cells were capable of being the origin of tumours [31,32]. These results were then confirmed in relation to the brain [5,33] and the breast [34,35], demonstrating that stem cells are involved in the initiation of leukaemias and solid tumours.

Breast cancer stem cells

Some indication that stem cells play a role in breast cancer comes from epidemiology data on breast cancer incidence following radiation exposure. Women exposed to radiation in their late adolescence following the Hiroshima and Nagasaki atomic blasts had the highest susceptibility of breast cancer 20 to 30 years later compared with women exposed at other age groups [36]. This suggests that adult mammary stem cells accumulate genetic changes leading to transformation over several years with the eventual development of solid tumours. A model of tumour formation and development which takes into account the role of stem cells as primary targets for mutation should be incorporated into the view of how breast cancer initiation occurs. Additionally, early or late progenitor cells could be the targets of transforming events. In this case, these progenitor cells would need to acquire, through mutations or epigenetic changes, the characteristics of stem cells such as self-renewal. Models of carcinogenesis are contentious, but the role that cancer stem cells play in tumour formation is beginning to be further defined. Recently, Al-Hajj and colleagues [35] showed that human BCSCs, identified on the basis of $CD44^+$, $CD24^{-/low}$, Lin^- expression, could form tumours when as few as 100 cells were injected into nonobese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice. These cells have some of the key characteristics of stem cells. When 20,000 cells without this phenotype were used, they were unable to form a tumour. These experiments indicate that tumour initiation could be driven by rare BCSCs.

Studying mammary stem cells and breast cancer stem cells

Advances in cell culture approaches have been important in identifying and studying mammary stem cells. The study of mammary stem cells *in vitro* has been based upon work identifying neural stem cells through a cell culture assay known as the neurosphere assay, which makes use of serum-free medium supplemented with epidermal growth factor and basic fibroblast growth factor [37,38]. Application of the neurosphere assay culture conditions has been used to identify undifferentiated human mammary stem cells grown in culture [39] known as mammospheres and to identify a candidate human BCSC [34]. These culture systems have shown that mammospheres exhibit stem cell-like functional properties of relative quiescence and phenotypic properties such as ESA, CK5, and α-6-integrin expression. The use of flow cytometry for phenotyping and isolating putative mammary stem cells and BCSCs has also been critical in studying these cell populations. In addition to identifying the SP, flow cytometry has been used to show expression of CD44 and ESA on human mammary progenitors [40] and human BCSC populations [34,35]. ESA expression has also been identified in both primary breast cancer tumours and

Table 1

Phenotypic characteristics of breast cancer stem cells

Factor	Characteristics	Reference(s)
Cell surface markers that are expressed by putative breast cancer stem cells		
ABCG2	ABCG2 (ATP-binding cassette G2) is a class of drug transporters capable of pumping cytotoxic drugs out of the cell.	[6]
CD44	CD44 is involved in cellular adhesion, motility, and metastases.	[34,35]
CD10	CD10 is a common acute lymphoblastic leukaemia antigen that is overexpressed on many tumours.	[39]
EpCAM/ESA	Epithelial cell adhesion molecule/Epithelial surface antigen is expressed on mammary tissue and tumours.	[39]
CD29 (β1-integrin)	CD29 is a membrane receptor involved in cell adhesion and metastatic diffusion of tumour cells.	[9]
CD49f (α6-integrin)	CD49f is involved in basal and endothelial cell distribution and is a candidate stem cell marker.	[39]
CD133 (prominin-1)	CD133 is a cell surface glyoprotein with an unknown function in cancer stem cells and its expression is documented for various types of cancer.	[116,117]
ALDH1	Aldehyde dehydrogenase-1 plays a role in the differentiation of stem cells and its activity predicts poorer clinical outcomes.	[112]
CXCR4	CXCR4 is a chemokine receptor involved in metastasis and its expression is increased in mammospheres.	[44,45]
ER	Oestrogen receptor is expressed on breast cancer cells, mammary progenitors, and breast cancer stem cells.	[54,59]
Signalling pathways that play a role in cancer stem cells		
Delta/Notch pathway	This pathway is involved in cell fate development and is expressed in stem cells and early progenitor cells.	[60]
Notch-4	Notch-4 plays a role in mammary development and its overexpression has been shown to promote mammary tumours.	[45,60,61]
Wnt signalling pathway	This pathway is involved in stem cell self-renewal and its overexpression can lead to epithelial and mammary tumours.	[27,63]
β-catenin	β-catenin is a downstream target of the Wnt pathway. A pro-oncogenic role has been described.	[63]
Hedgehog/Patched pathway	This pathway is involved in embryonic growth and cell fate determination.	[64]
PITCH	A receptor for the Hedgehog signalling family, PITCH has been connected to early embryonic tumourigenesis.	[64]
EGFR	Epidermal growth factor receptor signalling has been found to be upregulated in breast cancer stem cells and may be required for mammosphere formation.	[77,111]

secondary metastasis as well as a variety of other malignancies [41]. Flow cytometric analysis has been important in demonstrating that SP cells are present in the human breast [7,39]. The SP is further increased in mammosphere cultures compared with freshly isolated primary tissue samples [39]. Additional candidate stem cell markers are waiting to be identified. Table 1 lists some phenotypic characteristics of BCSCs.

Breast cancer and metastatic disease

Results from experiments using primary human cancer tissue have shown that not all cells in a tumour are equivalent; a minority tumourigenic cell population exists in solid tumours of both the breast and the brain which represents around 1% to 2% of the total tumour burden [35,42]. It should be noted that the cells that are capable of metastasising might have a phenotype different from the original tumour-initiating cells. Targeting cells that have the potential to metastasise will be an important application of the BCSC field as these are the cells that cause the majority of mortality from breast cancer.

Circulating tumour cells often can be detected in patients with both primary and metastatic disease and the presence of these cells often is associated with worse prognosis for survival [43]. Metastasis is a nonrandom and organ-specific process undertaken by certain cell types. In the case of

breast cancer, metastatic disease often follows a pattern involving metastases to regional lymph nodes and then to bone marrow, lung and liver, and brain [44]. Interestingly, it has been shown that homing and migration pathways of haematopoietic/leukocyte cells might be involved in BCSCs and metastatic disease. CXCR4, a chemokine receptor expressed by haematopoietic stem cells which binds CXCL12, has been shown to be increased by a factor of four in mammospheres and to be expressed in both metastatic breast cancer cells and neuroblastomas [44-46]. Additionally, the organs that form the main target of breast cancer metastasis have the highest expression of the ligand CXCL12 [44]. This indicates the importance of both the BCSC metastatic 'seed' and the 'soil' of the organ of metastasis for this process to occur. The CXCR4/CXCL12 pathway could provide a new target to specifically neutralise the cells in tumours that are capable of forming new metastases before metastatic disease occurs.

The cancer stem cell compartment is thought to be the reason for initiation of disease, resistance to treatment, and occurrence of metastatic disease. Hence, the identification and targeting of any of these cancer stem cell compartments, without toxicity to normal stem cells, will be an important goal of immunotherapy of breast cancer. This treatment will probably find most utility in the setting of targeting cells with a metastatic potential. However, disrupting the cancer stem cell compartment is made more difficult by the realisation that disruption of supporting cells around the stem cells, or differentiated tumour cells around cancer stem cells, could have deleterious effects by disrupting putative stem cell niches.

Stem cell niches

Stem cell niches are defined as locations in a tissue which specifically can support the existence of somatic stem cells. Niches allow the repopulation of the stem cell compartment from migrating stem cells or even from differentiated cells if the stem cell compartment is depleted [47-49]. It is possible that tumour therapy that disrupts the stem cell niche through ablation of the surrounding differentiated cells could lead to the subsequent death of the cancer stem cells. Alternatively, tumour therapy that depletes stem cells, but does not eradicate the stem cell niche, could lead to repopulation of the stem cell niche with additional cancer stem cells. Murine mammary stem cells have been shown to be resident in the peripheral caps of terminal end buds [50]. Identifying candidate stem cell niches in human breast tissue has been difficult. In humans, terminal end bud structures are not as prominent and identifying stem cell zones has had to rely on microdissection followed by cell sorting and functional characterisation of putative cells to determine stem cell niches in ducts and lobules. Villadsen and colleagues [51] recently identified a stem cell niche in the ductal tissue with cells with the characteristics of clonal growth, self-renewal, and bipotency and positive staining for putative stem cell markers K19 and K14. Identification of the properties of stem cell niches will be important for targeting BCSCs as it will be necessary to disrupt the inappropriate signalling that the stem cell niche may provide to achieve lasting clinical effects.

Hormone receptor status of the putative breast cancer stem cells

Breast cancer subtypes include at least two different cellular phenotypes, one reminiscent of basal lineages and the other of luminal lineages [52]. This has led to the idea that various breast cancer subtypes might arise via mutations in different compartments of stem cells [53,54]. *In situ* observations have identified candidate cells with stem cell-like feature of various phenotypes. Some of these observations have identified candidate stem cells that are ER^+. Indeed, ER^+ stem cells have been identified as being important in adult mammary gland homeostasis [54]. However, ER^- stem cells resident in the mammary tissue have also been identified and might represent the more primitive mammary stem cells. Recently, it has been shown that the mammary reconstituting cells are ER^- and PgR^- [55]. Recent experiments have demonstrated a role for *BRCA1* being involved in the differentiation of human ER^- stem/progenitor cells into ER^+ luminal epithelial cells [56]. The deletion of *BRCA1* results in the prevention of the transition of ER^- stem cells into ER^+ progenitor cells. Heterozygous mutations in the *BRCA1* gene predispose women to breast and ovarian cancer [57], with tumours often being of the basal-like phenotype characterised by a lack of expression of ER, PgR, and HER-2. This work suggests a model in which a block in *BRCA1*-mediated transition from stem cells to progenitor cells results in an increase in ER^- stem cells that can then be the pool of target cells for further mutation events. However, greater than two thirds of breast cancer tumours are ER^+ and the majority of these tumours are dependent on oestrogen for growth and thus can be treated with hormonal therapy [58]. A model of breast cancer origin has been proposed in which ER^+ tumours are derived from ER^+ stem cells or ER^+ early or late progenitor cells and ER^- tumours are derived from the more primitive ER^- stem cells [59]. Other models have postulated that ER^- stem cells, which rarely divide and are also resistant to hormonal therapy, can generate ER^+ short-term transit-amplifying cells that in turn give rise to ER^+ differentiated cells. These models suggest that the diversity seen in tumour types among patients could be a direct result of transformation events occurring in different lineages of stem cells or progenitor cells. Further defining the hormone receptor status of BCSCs will have important implications on the treatment of disease as hormone receptor status can dictate treatment options and is known to be an indicator of prognosis.

Pathways involved in self-renewal and differentiation

Among the most important characteristics of stem cells are the capacity for self-renewal and the regulation of the balance between self-renewal and differentiation. Signalling pathways,

such as Hedgehog (Hh), Wnt/β-catenin, and Notch, that play a role in embryogenesis and organogenesis also play a role in the maintenance of tissues in the adult through regulation of the balance between self-renewal and differentiation of stem cells. In the mammary gland, these three signalling pathways play a role in stem cell self-renewal and thus represent potential targets for therapy for BCSCs.

The Notch family of transmembrane signalling proteins are involved in cell fate development and are expressed in stem cells and early progenitor cells [60]. Notch-4 plays a role in normal mammary development as a constitutively active form of overexpressed Notch-4 has been shown to suppress differentiation of breast epithelial cells *in vitro* [61] and to suppress the development of normal mammary glands while promoting the development of mammary tumours *in vivo* [62]. This suggests that alterations in Notch-4 signalling might play a role in the transition of a healthy stem cell to a cancer stem cell. Additionally, expression of Notch family members has been found on mammospheres and Notch ligands are capable of affecting the self-renewal and differentiation capacity of healthy mammary cells, indicating a role for Notch in breast cancer development [45].

The Wnt pathway is involved in cell fate determination in many organs, including the developing mammary gland. A pro-oncogenic role for β-catenin, a downstream target of Wnt signalling, has also been described. Wnt signalling has been shown to play a role in haematopoietic self-renewal, and experimental evidence from transgenic mouse models has shown that activation of the Wnt signalling pathway in stem cells can lead to epithelial tumours [27]. Overexpression of Wnt in mouse mammary glands can also lead to increased mammary tumour formation [63]. Taken together, these data indicate the involvement of Wnt signalling pathway members and β-catenin in the deregulation of stem cells into cancer stem cells.

The Hh/Patched pathway is important for embryonic growth and cell fate determination during development. The PITCH membrane protein (product of the tumour suppressor gene *Patched*) is a receptor for the Hh family of signalling molecules and has been connected to early embryonic tumourigenesis [64]. Alterations in this pathway have been implicated in several types of cancer, including breast, prostate, and lung cancer.

Breast cancer stem cells as therapeutic targets

In the past two decades, more than 30 new anticancer drugs have been introduced, but survival rates have improved only marginally for many forms of cancer [65]. In contrast to most cancer cells, cancer stem cells are slow-dividing and have a lowered ability to undergo apoptosis and a higher ability of DNA repair, making them more resistant to traditional methods of cancer treatment such as radiation and chemotherapy. *In vitro* experiments comparing differentiated breast cancer cells grown under monolayer conditions with $CD24^{-/low}$ $CD44^{+}$ cancer stem cells grown under mammosphere conditions showed that the stem cell-like population was more resistant to radiation [66]. In addition, stem cells express ABC drug transporters, which protect the cell from cytotoxic agents and may lead to MDR [67]. Current anticancer therapy is effective at debulking the tumour mass but treatment effects are transient, with tumour relapse and metastatic disease often occurring as a result of the failure of targeting cancer stem cells. For therapy to be more effective, debulking of differentiated tumours must occur followed by targeting of the remaining surviving, often quiescent, tumour stem cells. This could be accomplished by differentiating BCSCs through differentiating therapy or eliminating them via immunotherapy.

Differentiation therapy targeting cancer stem cells

One way to target cancer stem cells is to induce the cancer stem cells to differentiate. Targeting the cancer stem cell pool to differentiate results in the loss of the ability for self-renewal, a hallmark of the cancer stem cell phenotype and the reason behind maintenance of the cancer stem cells. One differentiation agent used in the clinic is retinoid acid (RA) (vitamin A) [68]. RA and vitamin A analogues can promote differentiation of epithelial cells and reverse tumour progression through modulation of signal transduction. RA-based therapy followed by chemotherapy has found use in acute promyeloctyic leukaemia and could also find use in solid tumour therapy [69]. Recently, the use of bone morphogenetic protein (BMP)-4 has been described as a non-cytotoxic effector capable of blocking the tumourigenic potential of human glioblastoma cells [70]. This therapeutic agent is able to work by reducing proliferation and inducing expression of neural differentiation markers in stem-like tumour-initiating precursors. These findings are intriguing in light of the role that BMP-4 may play in some breast tumours [71]. Finding ways to specifically target BCSCs via differentiation therapy is an application that needs to be further defined.

Targeting stem cells for elimination

Much of cancer therapy research is focused on targeting specific markers on tumour cells that are overexpressed or mutated and that often represent essential genes/proteins or pathways thought to be important for the development of the tumour. For instance, traztuzamab (Herceptin®) targets the HER-2/*neu* (ErbB2) oncogene, a member of the epidermal growth factor receptor (EGFR) kinase family, a protein overexpressed on roughly 30% of breast tumours [72]. While these approaches have seen some clinical successes, the cancer stem cell model predicts that only by targeting the remaining cells left over after treatment, the putative cancer stem cells, will significant clinical remissions of the disease occur. It is important to note not only that tumours may be

driven by mutated proteins and inappropriate signalling, but also that epigenetic mechanisms of gene expression of genes involved in 'stem-ness' such as *Oct4*, *Nanog*, and *Sox2* could be behind tumour formation [73]. Reversal of these epigenetic switches of cancer stem cells could be one novel way to target cancer stem cells. New therapeutics aimed at eliminating cancer stem cells could also be achieved through a variety of methods: targeting the self-renewal signalling pathways critical for cancer stem cells, targeting the ABC drug transporters that cancer stem cells use to evade chemotherapy, or inducing the immune system to eliminate the cancer stem cells through various immunotherapeutic interventions.

Targeting of molecular signalling pathways and drug transporters

The use of the steroid-like molecule cyclopamine to inhibit the Hh signalling pathway has shown some promise in inhibiting the growth of medulloblastoma and could be used in treatments of other tumours [68]. The Wnt pathway can also be inhibited through a variety of mechanisms. Targeting of β-catenin has received a lot of attention as RA has been shown to inhibit β-catenin activity [74] and tyrosine kinase inhibitors such as imatinib (Gleevac®) have been shown to down-regulate β-catenin signalling [75]. Finally, the Notch pathway has also been investigated as a target. An antibody capable of blocking Notch-4 has been used *ex vivo* to block the formation of mammospheres from primary human specimens [76]. This indicates the potential to block the self-renewal capacity of BCSCs in the patient with this antibody and opens up the use of other antibody therapies in the elimination of BCSCs.

In vitro experiments have shown the resistance of BCSCs to chemotherapy and radiation. Recent clinical evidence has established that tumourigenic breast cancer cells with high expression of CD44 and low expression of CD24 are resistant to chemotherapy [77]. Breast cancer patients receiving neoadjuvant chemotherapy had an increase in the $CD44^+/CD24^{low}$ population of cells following treatment. These cells retained the capacity to form mammospheres (demonstrating self-renewal) and had an enhanced propensity for forming tumours in SCID/Beige mice compared with pretreatment samples, increasing from 4 of 14 (29%) to 7 of 14 (50%) patient samples transferred. Treatment of patients with HER-2-positive tumours with lapatinib, an EGFR and HER-2/neu (ErbB-2) dual-tyrosine kinase inhibitor, resulted in nonstatistically significant decreases in the percentage of $CD44^+/CD24^{low}$ population and in the ability for self-renewal as assessed by mammosphere formation. Thus, inhibition of regulatory pathways involved in self-renewal may confer improved clinical outcomes by targeting BCSCs.

The high expression of ABC transporters such as breast cancer resistance protein (BRCP-ABCG2) and MDR-associated protein-1 (ABCB1/MDRR1) is a property of stem cells which is also a feature of cancer stem cells [6]. These transporters provide a protective mechanism against xenobiotic toxins and also are partially responsible for the resistance of cancer stem cells to traditional therapies. Pheophorbide, a chlorophyll catabolite, is a specific probe for ABCG2 which causes inhibition of ABCG2 efflux properties [78]. The combined use of ABC transporter inhibitors and chemotherapy could be used to increase the efficiency of chemotherapeutic drugs to kill cancer stem cells [67]. ABC transporter inhibitors also cause inhibition of normal stem cells, leading to potential toxicity in the bone marrow, and play a role in the maintenance of the blood-brain barrier [79]. However, effective targeting of this molecule could be vital since it plays a significant role in the resistance of cancer stem cells to treatment.

Immunotherapy targeting breast cancer stem cells

Immunotherapy aimed at stimulating the immune system to recognise and eliminate tumours has been explored for many years but recently has gained renewed interest. Many vaccines targeting solid tumours have been employed with varying success both preclinically and clinically in the treatment of cancer [80]. Interest in these vaccines has been bolstered by increased understanding of the role that the immune system plays in cancer and by the molecular identification of tumour-associated antigens (TAAs) that can be used as targets for therapy. In the case of breast cancer, evidence is now coming to light that the immune system is involved in the surveillance of cancer, is impaired by tumours during the progression of cancer, and can recognise and eliminate cancer. DCs are central to these processes as a result of their role in innate immunity and in generating humoral and cellular immune responses. DCs are professional antigen-presenting cells and initiators of adaptive immunity through processing antigens and presenting epitopes in the context of major histocompatibility complex (MHC) to T cells [81]. DCs are capable of stimulating cytolytic T-cell responses (CTLs) to TAAs on tumours and are equipped with all of the necessary co-stimulatory and cytokine signals needed to drive an effective immune response to tumours.

Evidence for the protective role of the immune system against cancer is seen in the increased incidence of melanoma observed in renal transplant recipients [82]. The concept of tumour immunosurveillance has stimulated interest in both the interaction between tumours and the immune system and the potential power of using immunotherapy to target tumour. For breast cancer, it has been observed that DCs and lymphocytic infiltrates in tumours are associated with better prognosis and survival, independent of tumour size [83]. However, in breast cancer, it is also apparent that DC impairment correlates with tumour progression. For instance, co-stimulatory molecule expression and antigen presentation by DCs are decreased in breast cancer patients, with a

subsequent impairment in the capacity of these DCs to stimulate T-cell proliferation and secretion of cytokines [84]. DCs of breast cancer patients secrete less interleukin-12 in response to maturation signals [85]. Interestingly, it has also been shown that breast cancer can induce apoptosis in circulating DCs but that this process is reversible when the DCs are removed from the inhibitory cancerous environment [86]. Additionally, precursors to DCs can be isolated from the body before the start of treatment, cultured *ex vivo* with GM-CSF (granulocyte-macrophage colony-stimulating factor) and interleukin-4 to form DCs, and loaded with appropriate antigens to expand functional tumour antigen-reactive T cells [87]. These findings suggest a strong role for the immune system in both the progression of disease and as a potential tool to eliminate cancer. Immunotherapy in this context is dependent on finding strategies that will effectively target tumour, most importantly by identifying useful tumour antigens.

Breast cancer TAAs include epitopes from proteins that are involved in tissue differentiation (such as carcinoembryonic antigen and NYBR-1 [88]), are overexpressed in breast cancer (such as HER-2/*neu* and MUC1), or are shared among a variety of tumours (such as telomerase, survivin, and p53) [89]. Most TAAs have been identified on the basis of serological identification of antigens by recombinant expression cloning (SEREX) followed by a demonstration that patients have T cells capable of identifying the TAAs in an MHC-restricted fashion [90]. Breast carcinomas fall into various subtypes associated with different gene expression patterns, phenotypes, and clinical outcomes [91]. However, in a single patient, tumours are heterogenous, with individual tumour cells displaying different phenotypes and TAAs. This raises the possibility that no single antigen can be used to effectively target and eliminate all tumour cells as there will likely be a resistant cell not expressing the targeted antigen that is capable of repopulating the tumour. Targeting of the BCSC pool could potentially eliminate this population.

DC-based vaccination strategies encompass a variety of different approaches that can be divided into two groups: antigen-defined vaccines and polyvalent vaccines [80]. Preclinical mouse models have employed targeting of a single tumour antigen with some success. In one model, a DC-based vaccine prevented the outgrowth of a spontaneous breast tumour in a mouse model when used to specifically target a single differentiated tumour antigen, HER-2/*neu* [92]. This vaccine induced the production of anti-*neu* antibodies and interferon-γ expression by T cells. Despite preclinical evidence that DCs induce effective antitumour T-cell responses, clinical trials overall have been disappointing, with a lack of objective tumour response reported in 12 of 35 trials [80]. However, DC-based vaccines have no serious side effects [93]. Most clinical trials using DCs have examined the use of single antigen peptide-loaded DCs [94-97]. These peptides are generally either wild-type sequence or altered epitopes with better binding to the MHC and are generally restricted to HLA-A*0201 [98,99]. One trial using tumour necrosis factor-alpha-matured, monocyte-derived DCs pulsed with MUC1 or HER-2/*neu* elicited peptide-specific anti-tumour responses, but the overall clinical response was modest [100]. Another antigen-defined approach makes use of adoptive transfer of antitumour T cells that have been cultured *ex vivo* and identified to be active against antigens of interest on the tumour. The adoptive transfer method has already been used with some success to target cancer [101-103]. Recently, the examination of adoptive transfer of HER-2-specific T-cell clones clinically suggests the potential to use an antigen-specific therapy to eliminate specific single tumour cells but that additional treatments are needed to reduce the solid tumour due to the inhibiting effects of the stroma [104]. Targeting of a single tumour antigen may allow for regression of the tumour by gene deletion or downregulation or by outright failure to target cells in the tumour not expressing the targeted antigen [97,105]. Immunotherapy that targets single antigens may also fail to target the underlying cells responsible for cancer initiation or tumour metastasis, thus limiting the long-term success of this treatment. To avoid these problems, it is likely that targeting of either multiple antigens or essential antigens is going to be required. From the available information, it appears that several epitopes need to be targeted simultaneously for an effective therapy through the use of a polyvalent vaccine. One way to achieve this is to make use of whole tumours in the vaccine. Immunotherapy for stage IV melanoma using a DC/irradiated tumour vaccine has demonstrated a complete remission of disease in 3 out of 46 patients and a partial remission for an additional 3 patients [106]. Fusions of DCs and breast tumours have been shown to elicit CTLs against autologous tumour cells [107].

To induce long-lasting clinical responses using immuno-therapy, targeting cancer stem cells may be required. To achieve this, specific antigens expressed on the cancer stem cells but ideally not by normal stem cells must be found and targeted. While there are not yet antigens fulfilling this description, they are likely to be present considering the various pathways identified as different between these two cell populations. Hence, it is important to identify as many antigens as possible on BCSCs in order to develop a polyvalent vaccine approach targeting several antigens. Therefore, cancer stem cells need to be further defined in terms of gene expression that determines stem-ness and identifying molecules that are involved in regulating stem cell qualities. Gene expression comparisons have been conducted and can be used to identify what genes are expressed by the cancer stem cell compartment compared with normal stem cells [108,109]. Interestingly, recent work has demonstrated that the expression profile of BCSCs more closely resembles that of embryonic stem cells than that of adult stem cells. An embryonic stem cell-like gene expression pattern was found to be upregulated in the $CD44^{+}/CD24^{low}$ tumourigenic fraction of cancer cells [110]. Additionally,

mapping the transcriptional profile of embryonic stem cell-like genes in primary human breast cancer has revealed two classes of tumours: those with an embryonic stem cell-like activated program and those with an embryonic stem cell-like repressed program. Those tumours with an embryonic stem cell-like activated program were associated with poorer differentiated tumours that were more likely to progress to metastasis and death. The $CD44^+/CD24^{low}$ phenotype in human breast tumours has been found to be associated with basal-like tumours, and particularly *BRCA1* hereditary breast cancer, and has been linked to expression of CD49f, elevated expression of CK5/14 and EGFR, and low expression of ER, PgR, and HER-2 [111]. Basal-like tumours often have been linked to poorer prognosis. The occurrence of the $CD44^+/CD24^{low}$ phenotype was found to be lower in tumours of luminal type, and particularly $HER\text{-}2^+$ tumours, irrespective of ER status.

Mammospheres have shown expression of markers ESA, CK5, and CD49f (α6-integrin) among many others, which potentially could be used to identify or target BCSCs [39]. The activity of aldehyde dehydrogenase-1 (ALDH1), a detoxifying enzyme that may play a role in the differentiation of stem cells, has been detected in both normal and malignant human mammary stem cells and can be used as a predictor for poor clinical outcomes [112]. High activity of ALDH1 identified the cells capable of self-renewal and high tumourigenicity in NOD/SCID xenografts. As previously mentioned, another molecule used to identify or target BCSCs is CD44, which is a membrane receptor involved in cell adhesion, motility, and metastases and which along with P-glycoprotein (the product of the *MDR1* [ABCB1] gene of drug transporters) has been linked to MDR [113]. CD44 routinely has been used as a marker to purify and enrich BCSCs by selecting for cells that are $CD44^+CD24^{-/low}Lin^-$ and ESA^+ [35]. CD29 (β1-integrin) and CD49f (α6-integrin) expression has also been associated with murine mammary stem cells with a Lin^-CD24^+ phenotype [9,114]. Additionally, neither Sca-1 (stem cell antigen) expression nor the SP phenotype was found to be expressed in the mammary reconstituting $Lin^-CD29^{hi}CD24^+$ cell population [9]. These mouse data demonstrate that the use of Hoechst dye to identify an SP phenotype does not accurately enrich for cells capable of reconstituting a mammary gland when transplanted into cleared mammary fat pads and call into question the use of the SP phenotype to exclusively isolate mouse and human BCSCs, which are a heterogenous population of cells. However, one report using the human breast cancer line MCF7 has shown that the SP phenotype can be used to identify cells with characteristics of cancer stem cells that express the tumour antigen MUC1, supporting a role for the SP in further analysis of human BCSCs [115]. A recent report has shown that *BRCA1*-deficient murine breast tumours contain heterogeneous cancer stem cell populations [116]. In that report, some tumours contained cells with a $CD44^+/CD24^{low}$ phenotype, while cell lines derived from another tumour contained $CD133^+$ cells, a phenotype that is associated with other cancer stem cells for brain, prostate, and colon cancer [117] but that has not been described in breast cancer. Importantly, both populations of cells expressed the stem cell-associated genes *Oct4*, *Notch1*, *Aldh1*, *Fgfr1*, and *Sox1*. That study shows that, although cancer stem cell populations may be heterogenous, they in fact share a common set of characteristics such as expression of stem cell regulatory genes, expression of cell surface markers, mammosphere formation, and tumourigenicity in xenografts, which may be exploited for targeting cancer stem cells [118].

Understanding of the biology of BCSCs will help to determine the best way to target them. While we now have a broader understanding of the genes and signalling pathways involved in stem cells and putative cancer stem cells, the application of immunotherapy targeting these molecules might not necessarily be useful since they are often expressed by healthy stem cells and indeed by a variety of other cells. Determining what mutations are present in cancer stem cells, how these mutations aid either the stem cell-like phenotype or the tumourigenic phenotype of these cells, and how to best target these mutations is going to be a critical component of immunotherapy. This is made more difficult by the role that epigenetic regulation plays in cancer stem cells. Additionally, targeting universal TAAs such as human telomerase reverse transcriptase and inhibitor of apoptosis proteins might be important for effectively targeting tumours with immunotherapy, as will combining these treatments with ones that target unique stem-ness-related antigens [119]. Several additional markers and signalling molecules, such as the Hh/Patched pathway, as well as the ABCG2 drug transporters could be used as potential targets of therapy for breast cancer [79]. Additionally, markers that are used for migration of BCSCs, such as chemokine receptors, should be explored as potential targets.

It is preferable that any therapy developed is able to target metastatic disease before metastases occur. Circulating tumour cells have been detected in the blood of patients with metastatic and primary tumours and have been linked with a decrease in survival times [43]. Recent evidence has indicated that metastatic spread can be an early event in tumourigenesis [120-122]. Gene expression studies have shown that the profile of the primary tumour of breast cancer patients can be used to predict disease outcome, with a specific gene expression signature predictive of a short interval to metastatic disease [121]. The poor prognosis profile included genes involved in regulating the cell cycle and angiogenesis. Studies such as this have challenged the traditional view of metastatic cells arising late in disease and have stressed the importance of developing assays to identify disseminated disease early. The identification of molecular targets on disseminated tumour cells, targets that might also occur on BCSCs, could lead to better treatments. Preferably,

these treatments should be applied during early stages of tumourigenesis before overt metastasis occurs. Immunotherapy is certainly one approach to targeting these cells as it has the potential to target even single cells for cell death and has the power to target systemic disease. Another critical component of immunotherapy targeting BCSCs is the determination of the number of BCSCs residing in the tumour and the ability to eradicate them with the treatment, as tumour regression will be dictated by any escape of BCSCs. Additionally, any immunotherapy approach will likely require additional therapies such as cytotoxic T-lymphocyte antigen (CTLA)-4 blocking of the T-cell regulation to overcome tolerance of the immune system to cancer [123]. Finally, the identification of appropriate antigens expressed on BCSCs needs to be conducted along with identifying the best way to stimulate an immune response using these antigens.

Cancer/testis antigens and cancer stem cells

Cancer/testis (CT) antigens are derived from proteins that appear to be expressed only in germ cells and tumours [124]. A range of tumours are capable of making hormones such as chorionic gonadotropin that are trophoblastic in origin [125]. A model for the trophoblastic origin of some tumours has been postulated in which tumours arise from germ cells that fail to reach the gonads/ovaries during development [126,127]. CT antigens currently are being investigated for their role in tumour formation and as potential targets of tumour immunotherapy. CT antigens are ideal cancer antigens because they are expressed by a proportion of cells of the tumour and are largely absent from non-tumour tissue. Furthermore, immunogenic CT antigens have been shown to be present in tumours such as melanomas and breast cancer [128] and have been used as the targets of tumour vaccines such as targeting the MAGE-3A1 peptide for melanoma [97]. There are some intriguing characteristics shared between germ cells and tumour cells, including immortalisation, invasion, migration/metastasis, angiogenesis induction, and immune evasion through downregulation of MHC [127]. Immunohistochemical studies have shown that CT antigens are expressed on only a small proportion of cells in a tumour [129]. This small proportion of cells could represent the cancer stem cell compartment or the early progenitor cells. It has been postulated that CT antigens could serve as markers and potential therapeutic targets of cancer stem cells within tumours. Melanoma cell lines enriched for stem cells express various CT antigens and some of these antigens are present on the majority of stem cells [130]. The relationship between cancer stem cells and the expression of CT antigens needs to be further defined, and the exact role of CT antigens in both germ line and tumours remains a central question of research. A model for cancer arising from mutations in stem cells or early progenitor cells which gives rise to a phenotype of expression of CT antigens has been postulated [127]. While the exact role of the CT antigens themselves might not be fully understood, the potential to target them through immunotherapy is exciting because they are expressed on a proportion of cells in a tumour, possibly the putative cancer stem cells, and they have been shown to be immunogenic.

Conclusions

The cancer stem cell hypothesis is a new paradigm that could have a major impact on the treatment of disease by suggesting a new target for cancer therapy. Mammary stem cell biology needs to be understood in the context of both mammary development and as potential sources of the BCSCs. Transformed mammary stem cells have been identified as a potential source of breast cancer, tumour relapse, and tumour metastases; as such, they have gained prominence as potential targets for immunotherapy of cancer. Current treatments of cancer have shown efficacy in removing the bulk of differentiated cancer cells while failing to eliminate the cancer stem cells responsible for tumour relapse. Future therapies will need to effectively target the cancer stem cells to induce clinically significant remission of disease. Target antigens for BCSCs need to be further defined so that effective targeting of the BCSC compartment can be realised which spares normal stem cell niches but disrupts the cancer stem cell niche. New treatments typically will not be fully optimal by themselves and will need to be further developed and placed into combination therapy with existing treatments. Therapies targeting BCSCs might be employed after debulking of the differentiated tumour tissue. This would allow immune surveillance to more efficiently eliminate the few remaining cancer stem cells. Targeting BCSCs might be an attractive approach to treat breast cancer metastasis and relapse and could lead to significant increases in clinical remissions and quality of life for breast cancer patients when used in a multimodal treatment regimen.

Competing interests

The authors declare that they have no competing interests.

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