Breast Cancer Stem Cells

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Despite the progress made in the early diagnosis and treatment of breast cancer, around 30% of all patients will develop recurrence of the disease, mostly in the form of metastatic tumours [1]. If patients are diagnosed early, conventional chemotherapy can initially control metastatic disease, however most patients will relapse over time [2]. Determining which of the cells in the tumour mass are most likely to give rise to daughter cells of a metastatic phenotype is an area of considerable research interest. Recent progress in our understanding of cancer biology has led to the identification of a sub-population of cells within tumours which have stem cell like properties [3]. There is growing evidence these cancer stem cells (CSCs) contribute to treatment failure due to intrinsic resistance to conventional therapies [4]. Therefore, the development of treatments which specifically target breast CSC could provide a new therapeutic approach aimed at curing the disease [5].

The cancer stem cell hypothesis

Until recently tumours were generally accepted to propagate according to the stochastic, model whereby a transformed cell would acquire an increasingly tumourgenic phenotype by accumulation of genetic mutations [6]. In this theory all progeny derived from the transformed cell would retain the potential to form new tumours (Figure 1A), however this idea is currently being challenged as there is growing evidence to support an alternative hierarchical CSC model. This new theory suggests that cancer originates from a mutated stem or progenitor cell within a tissue that acquires tumourgenic properties and is responsible for the initiation and maintenance of the neoplastic lesion [3]. In this model only a sub-set of CSCs within the tumour have the capacity to initiate new tumour growth (Figure 1B).

Cancer stem cells are aptly named because of stem cell like properties include the capacity to divide producing either daughter CSCs or progeny which can divide and form mature tumour cell types. Stem cells are hypothesised to be the tumour initiating cells within tissues because of two unique properties. Firstly, they have longer lifespan within the body allowing them to accrue the multiple somatic mutations necessary for cell proliferation at the same time the mutations also alter/abrogate key enzyme activity in pathways important for programmed (apoptotic) cell death. A second key property of CSCs is their capacity to form the multiple different mature cell types that have often been observed within tumours [7]. Although there is growing evidence supporting the CSC hypothesis, a recent study of breast cancers suggests that CSCs can also arise from the de-differentiation of mature cancer cells [8]. This finding supports the stochastic breast cancer model; however, further research will be required to determine whether both models for cancer initiation exist for different types of cancer.

CSC identification

The first evidence supporting the CSC hypothesis came from work that had been carried out on leukaemia [9]. In this early work leukaemic cells were separated according to the expression of protein markers of normal healthy stem cells. Only the sub-fraction of cells that expressed stem markers were able to initiate cancer when the cells were engrafted into immunocompromised mice [9,10]. Leukaemic cells that did not express stem cell markers were unable to engraft in mice. The methodology adopted in this study is now regarded as the gold standard for identifying CSC and similar findings using different combinations

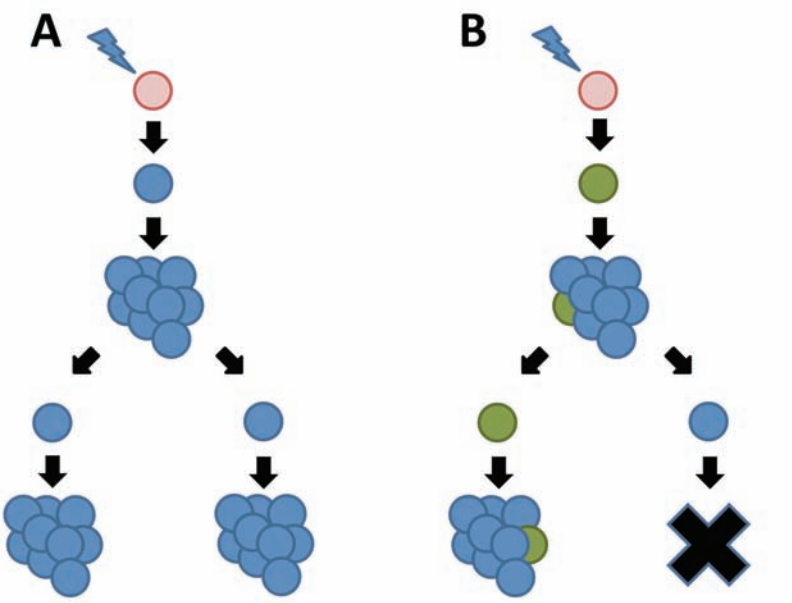

Figure 1: In the stochastic model of tumour initiation (A) all tumour cells have the same ability to initiate new tumours. In contrast the Cancer Stem Cell (CSC) model (B) proposes that a tissue stem cell becomes transformed into a CSC (green) which divides and differentiates to form a tumour mass (blue). Only the rare CSCs within the tumour mass are capable of initiating tumour growth at a new site.

of cell surface markers have been replicated in a wide range of solid cancers, including those from the brain [11], colon [12] and in osteosarcoma [13]. CSCs associated with breast cancer were first described in 2003 by Al-hajj, based on the surface markers $CD44^{+}CD24^{-/low}$ [14]. Since then other additional markers which can identify potential breast CSCs have been reported. These include the isolation of cells which exclude the Hoechest dye, the expression of the surface protein CD133 and the presence of the intracellular enzyme aldehyde dehydrogenase (ALDH) [15]. Additional research is, however, required to establish whether these markers identify the same sub population of CSCs within tumour specimens.

How CSC relate to breast cancer subtypes

Breast cancer is a heterogenous disease classified into a number of different subtypes, all with varying prognoses [16]. Breast cancer subtypes are often categorised on the basis of their expression of the oestrogen receptor (ER). ER positive tumours encompass the luminal subtypes with generally a more favourable prognosis, whereas the ER negative tumours include basal subtypes like HER2 and normal breast like tumours [16]. The clinical heterogeneity of breast cancer raises the question as to whether different subtypes contain distinct sub populations of CSCs responsible for the propagation and expansion of the tumour mass [17]. Studies with cancer cell lines indicate that this may be the case, as CSC expressing CD44 + CD24-/low are more closely related to basal like tumours [18]. In contrast, the intracellular enzyme ALDH was associated with CSCs from basal-like and HER2 positive tumours, whereas exclusion of Hoechest dye was associated with luminal type A CSCs [17].

CSC drug resistance

CSCs appear to have an intrinsic resistance to chemotherapy and radiotherapy. This is a property which has been implicated in treatment failure in a range of cancers [4,19]. In support of this theory is the enrichment of CSCs from tumour specimens resected after chemotherapy treatment of breast cancer patients [20]. The mechanisms enabling breast CSCs to resist the action of chemotherapy are only just starting to be understood. The expression of the membrane protein transporter ABCG2 in CSC is responsible for efflux of drugs from tumour cells, thus preventing the drugs from binding to their therapeutic targets [21]. In addition, the inhibition of the CSC marker ALDH sensitises breast cancer cells to chemotherapy and radiotherapy, suggesting that ALDH is also functionally important, allowing CSCs to become resistant to both of these treatments [22].

New therapeutic approaches which specifically target CSCs are beginning to show promising results. For example, a reduction in the CSCs of breast cancer was observed following neoadjuvant treatment with the tyrosine kinase inhibitor lapatinib [23]. Another drug showing promise as a treatment for CSC is salinomycin. This potassium ionophore resulted in a 100 fold greater cell death than paclitaxel [24], however further studies are required to assess whether salinomycin also targets the healthy stem cell population.

Metastasis

Metastasis is a complex process which we are only just starting to understand. Only a minority of the cells within a tumour have the capacity to migrate to distant organs and establish a new tumour [25]. Recent evidence suggests that CSCs possess the ability to form secondary tumours away from the breast cancer [26]. CSCs characterised as $CD44^{+}CD24^{-/low}$ cells in breast cancer have an invasive phenotype which is associated with an increased risk of metastasis formation [27]. An additional complexity of breast cancer metastasis is the organ-selectivity, for example, ER + tumours commonly migrate to the bone whilst ER- tumours have been observed to migrate and establish new tumours in visceral organs [17]. Although the reason for this preference is unknown it is thought that CSCs may play a role. This is an area which requires further research and is likely to be an active area in breast cancer research during the next decade.

Conclusion

The cancer stem cell theory is now widely accepted and there is growing evidence implicating CSCs as the driving force behind the growth and spread of breast cancer as well as playing a key role in treatment failure. CSCs can be identified by specific surface markers but additional markers are required to fully understand the role of CSC in different breast cancer subtypes. The resistance of breast CSCs to therapeutic regimens may be a reason for breast cancer relapse. As our understanding of the CSCs increases we will be able to design new treatments which selectively target the CSCs. Using drugs to target both the CSCs and mature tumour cells should lead to improved long term survival of patients suffering from this disease. ■

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