

Breast Cancer Stem Cells: How to Target These Chameleons - Masters of Disguise

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Introduction

Breast cancer develops in ducts that carry milk to the nipple or in lobules that make milk. By corollary, breast cancer is more frequent in women. A few rare cases of men have also been diagnosed with this disease. It is estimated that 230,480 females and 2,140 males will be diagnosed with breast cancer while 39,520 (female) and 450 (male) will die of the disease in 2011-2012 [1]. These numbers are only expected to rise further as the mortality rates from cancer increases due to the relatively longer average life-spans, and higher exposure to environmental risk factors [2].

Conventional treatment options for breast cancer include surgical resection in combination with radiation therapy, chemotherapy (before or after surgery), hormone therapy, aromatase inhibitors or treatment with targeted biologic therapy e.g. with trastuzumab (Herceptin) or lapatinib (Tykerb). Unfortunately, first-line therapy produces responses in only 60% to 80% of primary tumours [3], and that too only for an average of 3-4 years. The longest recurrence-free survival averages 10 years [4]. However, despite optimal treatment, even in cases in which the tumour responds well to initial treatment, recurrence is inevitable and mostly fatal, with only few patients surviving beyond 5 years. Thus, despite the advances in the diagnosis, classification, and the treatment options, the clinical picture remains dismal. This has fuelled investigations into the possible existence of a small but significant proportion of human breast tumour cells that have high tumour-initiating and maintenance potential. These cells, also known as breast cancer stem cells (BCSCs), were first identified almost a decade ago [5]. Because of their intrinsic characteristics and their clinical relevance, it has become apparent over the years that for any breast cancer therapy to be effective, it must eradicate this pool of BCSCs without harming any other cell types in the body. Is such specific targeting of BCSCs achievable? To answer this question, one needs to understand whether a targetable population of BCSCs can be defined for each of the different sub type of breast cancer.

Do BCSCs Vary with the Subtypes of Breast Cancer?

Global gene expression profiling studies demonstrated that breast cancer is highly heterogeneous with many molecular subtypes. Specifically, using hierarchical clustering approaches, breast cancer has been classified into five major molecular subtypes: luminal A, luminal B, HER2+, basal-like, and normal breast like [6]. However, recent analysis of primary breast cancers using genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays has also demonstrated the existence of four main breast cancer classes [7]. Regardless, both approaches clearly confirm that breast cancer is widely heterogeneous. Additionally, histo-pathological studies have shown that each of the subtypes of breast cancer is associated with a peculiar natural history and treatment responsiveness. However, a consensus on how to characterize BCSC phenotypes using cell surface marker profiles for each subtype is still lacking. In fact, recent experimental evidences suggest that the idea of a universal marker or combination of markers to identify and isolate BCSC from all breast cancers may

even be quixotic [8]. Evidences showing that each histological subtype of breast cancer has different underlying molecular signature and consequently variable clinical presentation appear to further support these suggestions. Therefore, breast cancers present varied outcome responses [9]. In addition to the inter-tumour heterogeneity, there is also a high degree of intra-tumour diversity amongst breast cancer subtypes. Specifically, a single tumour at any given time can contain tumour cell populations with distinct molecular profiles and biological properties. This is true not just for advanced cancers but also early ones. In fact, intra-tumour diversity has been reported as early as at the stage of ductal carcinoma in situ [10,11]. Recently Park et al. [12] studied twelve immune-histochemical markers in almost 400 ductal breast cancers and concluded that the frequency of breast cancer cells that are positive for stem cell-like and more differentiated markers vary according to tumour subtype and histologic stages [12]. Clearly therefore, developing therapeutics that show robust effects on cancer stem cells (CSCs), across all subtypes of breast cancer is not easy, and in some respects, appear unachievable with our current understanding of the field [13].

Deviations that may Lead to Successful Targeting of Cancer Stem Cells in a Subset of Breast Cancers

Traditionally, search for cancer therapeutic targets mainly focused on the cell cycle machinery that controls proliferation and apoptosis pathways. However, the approach to identify drug targets has to be radically different for CSCs because these cells replicate slower than the mature cancer, and are inherently drug resistant. Another important consideration in target identification in CSCs is that many of the pathways involved in self-renewal, survival and proliferation of CSCs appear to be the same ones that are implicated in the self-renewal of normal stem cells. Notch, Wnt, Hedgehog and BMI-1 are excellent examples to learn from. These stem cell regulatory pathways are de-regulated in cancer stem cells but are highly regulated in the normal tissues [14,15]. Obviously, targeting such pathways raises the concern of potentially damaging the common and critical pathways that are required for normal stem cells of the organism. Luckily, data from our studies on breast cancer [11,16] and studies on other cancer types [17,18] indicate that there are therapeutic windows of opportunity that may be exploited to target stem cell pathways. For

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Received October 26, 2012; Accepted October 26, 2012; Published October 29, 2012

Citation: Chakravarty G (2012) Breast Cancer Stem Cells: How to Target These Chameleons - Masters of Disguise. J Mol Biomark Diagn 3:e114. doi:10.4172/2155-9929.1000e114

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instance, during normal mammary development, expression of a TGF β signaling pathway protein SOX9 is nuclear. Most of its expression is confined to the basal layer of the embryonic mouse mammary bud. However, during later development, its expression is undetectable in terminally differentiated luminal epithelial cells even though those cells are derived from the same basal layer through differentiation. In contrast, 20-25% of invasive ductal carcinomas and some human breast cancer cell lines; particularly the ones with a higher proportion of CSCs show cytoplasmic expression of this protein [16]. It is therefore conceivable that therapeutic strategies developed to target this protein in cancer cells are unlikely to affect the nuclear protein in normal stem cells. Our data indeed suggest that targeting developmental pathways like TGF β that influence the fate of stem-like cells, particularly in triple negative basal like tumours, may be a viable option. Initial evidence that such an approach may be effective is already emerging. In a recent report, Bruna et al. [19] have demonstrated that TGF β activates a specific network of genes to boost the number of breast cancer stem cells in claudin-low basal like breast cancers [19] and that these stem like cells can be targeted using TGF β inhibitors that are already in clinical trials. Dr. Carlos Arteaga's group at Vanderbilt University has undertaken similar studies to identify a clinically relevant therapeutic strategy, prognostic signature and novel therapeutic targets that will improve TNBC therapy by eliminating TGF β -mediated enrichment of CSCs. Successful completion of these studies will have important clinical implications. In particular, establishment of a clear connection between the increase in BCSCs and high TGF β levels after primary therapy might provide strong evidence to identify patients who may be at most risk of developing tumour metastases vs. who may benefit from treatment with TGF β inhibitors.

Challenges in Therapeutic Development

The logic of pursuing therapies that might zero in on cancer stem cells is compelling. However, the challenges of devising CSC drugs that are specific for breast cancer subtypes are enormous. In addition, the current methods to evaluate the effectiveness of such therapies or to personalize cancer treatments based on stem cell markers are not well developed. Without an array of appropriate biomarkers, it will be hard to conclude whether drugs that target cancer stem cells are properly functional.

But there is reason for hope. Genetic markers of cancer stem cells have started showing promise. Using blood samples from 16 patients with acute myeloid leukemia, John E. Dick and his colleagues at the University of Toronto identified a gene expression signature that signals the aberrant behaviour characteristic of cancer stem cells and a poor prognosis for patients [20]. Identification of such signatures and techniques may help assess therapeutic response of BCSCs in cancer. In addition, methods are being developed to measure gene expression in single stem cells [21]. These analyses will be particularly useful for studying cancer stem cells, because these stem cells are in the minority and the gene expression patterns can easily be masked when cancer cells are analyzed in bulk, rather than individually. Things are looking up for genetic analysis, but the poor reliability of cancer stem-cell-surface markers remains a challenging issue. Let's look at the marker CD133. For nearly a decade, biologists have known that antigens such as CD133 can be found on the surfaces of cancer stem cells. But these markers are not particularly specific as many normal tissues also express it. Thus latest technologies for monitoring circulating tumour cells via surface markers cannot be adapted to monitor cancer stem cell populations during clinical trials. This problem is more acute for solid tumours such as breast cancer, where the search for tissue and stage

specific stem-cell-surface markers is still in its infancy. Furthermore, even if one does narrow down a surface specific marker, due to the subtle differences of the tissue types, it can vary from one type of cancer to another or even from one cell within a tumour to another. Until better and more reliable and specific markers are discovered, the cancer stem cell field will remain embryonic.

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