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The cancer stem cell phenotype as a determinant factor of the heterotypic nature of breast tumors

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ABSTRACT

Gathering evidence supports the existence of a population of cells with stem-like characteristics, named cancer stem cells (CSC), which is involved not only in tumor recurrence but also in tumorigenicity, metastization and drug resistance. Several markers have been used to identify putative CSC sub-populations in different cancers. Notwithstanding, it has been acknowledged that breast CSC may originate from non-stem cancer cells (non-SCC), interconverting through an epithelial-to-mesenchymal transition-mediated process, and presenting several deregulated canonical and developmental signaling pathways. These support the heterogeneity that, directly or indirectly, influences fundamental biological features supporting breast tumor development. Accordingly, CSC have increasingly become highly relevant cellular targets.

Abbreviations: ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; ESC, embryonic stem cell; JAK, Janus kinase; mTOR, mammalian target of rapamycin; NCL, nucleolin; non-SCC, non-stem cancer cell; PI3k, phosphatidylinositol-4,5-bisphosphate 3-kinase; STAT, signal transducers and activator of transcription; TIC, tumor initiating cells; WNT, wingless-related integration site.

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In this review, we will address the stemness concept in cancer, setting the perspective on CSC and their origin, by exploring their relation and regulation within the tumor microenvironment, in the context of emerging therapeutic targets. Within this framework, we will discuss nucleolin, a protein that has been associated with angiogenesis and, more recently, with the stemness phenotype, becoming a common denominator between CSC and non-SCC for multicellular targeting.

1. Tumor microenvironment as a key player in cancer development

Cancer remains a stressful condition in the western world, having surpassed heart diseases in 1999 as the pathology with the highest mortality (Jemal et al., 2006). Globally, lung cancer stands as the leading cause of death amongst patients with tumors in the respiratory system, whereas colon cancer stands out in digestive diseases. If one accounts for gender, a substantially different reality emerges, revealing breast cancer as the leading cause of death, accounting for 23% of all cancer cases among women (Jemal et al., 2011). Epidemiologic data from the United States of America suggest that every 1 in 8 women will develop breast cancer over their life time (DeSantis et al., 2014; Siegel et al., 2013). Among those, some will derive from familial inheritance. In this respect, *BRCA1* and *BRCA2* mutations confer high risk of breast cancer development, accounting for 40% of the familial cases (Shuen and Foulkes, 2011). Over the years, such realities have unleashed a tremendous effort from the scientific community in order to address this enormous health problem. Such efforts have been unraveling novel insights about tumor biology, adding novel cellular and molecular players present in the tumor microenvironment that play a significant role in cancer development.

In fact, a multitude of different cell types, including cancer cells, fibroblasts, endothelial cells or cells from the immune system are identified within the tumor microenvironment. They carry dissimilar roles, thus contributing to the heterotypic nature of tumors (Hanahan and Weinberg, 2011). This layout suggests an intrinsic interaction between those cells, which ultimately provides a fostering ground for the acquisition of several features that support tumor development. Indeed, such features were rationally summarized by Douglas Hanahan and Robert Weinberg in a seminal manuscript from 2000 (Hanahan and Weinberg, 2000), which originally included six important hallmarks of the disease and that were further upgraded in 2011 (Hanahan and Weinberg, 2011): (I) sustained proliferative signaling, (II) evading growth suppressors, (III) enabling replicative immortality, (IV) resisting cell death, (V) angiogenesis induction, (VI) activation of invasion and metastization, (VII) immune system evasion, (VIII) deregulation of cellular energetics, and also enabling characteristics as (IX) genome instability and mutation and (X) tumor-promoting inflammation. These features account for the major known deregulated pathways in cancer/tumor cells, which control metabolism, including nucleic acid turnover, cellular proliferation and fate, and cell signaling (Hanahan and Weinberg, 2011).

2. Cancer stem cells, tumor initiation and progression

Along with those instrumental processes for drug resistance and tumor survival, the role of a rather illusive cell type, resembling cells from embryonic development has been unveiled. The acknowledgement of common signaling pathways between stem cells and subpopulations of tumor cells set developmental biology and cancer closer than one would think, and gave rise to the cancer stem cell concept (Reya et al., 2001).

2.1. Lessons from stem cells: cellular reprogramming

Pluripotent stem cells have the potential to differentiate into any type of cells of the three germ layers (endoderm, mesoderm and ectoderm) (De Miguel et al., 2010), besides displaying self-renewal capability (De Miguel et al., 2010; Evans and Kaufman, 1981; Thomson et al., 1998). In an attempt to identify pluripotent cells, such as embryonic stem cells (ESC), several markers have been established, including upregulated levels of *NANOG*, *OCT4* (a.k.a. *POU5F1*), *TDGF* and *GDF3*, which are strongly regulated developmental genes (Adewumi et al., 2007; De Miguel et al., 2010). NANOG and OCT4, as well as SOX2, are regulatory transcription factors essential for self-renewal and pluripotency maintenance of stem cells (Pan and Thomson, 2007; Stadtfeld and Hochedlinger, 2010). They control several downstream gene targets, including *STAT3*, essential for self-renewal (Boyer et al., 2005; Niwa et al., 1998; Stuart et al., 2014). Tight levels of OCT4, control the transition between pluripotency and differentiation (Radzisheuskaya et al., 2013). Takahashi et al. demonstrated that it is possible to reprogram somatic cells such as adult fibroblasts, first from mouse, and later from human, into a state of pluripotency. Upon promoting the expression of four key transcription factors – OCT4, SOX2, c-MYC and KLF4 (OSKM) –, induced pluripotent stem cells (iPS) were generated (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). These cells closely resembled ESC, showing similar expression patterns of stem cell markers, like *NANOG* or *GDF3*, and demonstrating oriented differentiation capacity (Takahashi et al., 2007).

Overall, this suggested that cells with self-renewal potential can be generated from terminally differentiated somatic cells, thus reverting hierarchical developmental organization. This guided reintroduction of stemness in somatic cells somewhat represents a gain of function, a feature often occurring during cancer development.

2.2. The stemness concept in cancer

Tumors are biological entities that can be interpreted as an aberrant dysfunctional organ initiated by a tumorigenic cancer cell with the capacity to proliferate indefinitely by acquired mutations (Reya et al., 2001; Visvader, 2011). Viewed as an organ, tumors present functional heterogeneity in the microenvironment demonstrated by the existence of different populations of cells, including cancer cells with diverse phenotype. In order to accommodate that functional heterogeneity, a hierarchical organization model of tumor development, known as the cancer stem cell model, was proposed. This model postulates the existence of a sub-population of stem-like cells (the *cancer stem cells* – CSC) in the tumor microenvironment that is responsible for sustained tumor growth (Kreso and Dick, 2014; Reya et al., 2001; Visvader, 2011; Visvader and Lindeman, 2008, 2012). CSC have been defined operationally by their capacity to generate new tumors in immunocompromised mice, upon isolation from an established tumor (Scheel and Weinberg, 2012). However, the observation that not all cells of a putative CSC population are able to seed tumors, led to the introduction of the concept of Tumor Initiating Cells (TIC). Their abundance (established by *in vivo* limiting dilution experiments)