The tumour microenvironment, long considered as determining cancer development, still offers research fields to define hallmarks of cancer. An early key-step, the "angiogenic switch", allows tumour growth. Pathologic angiogenesis is a cancer hallmark, as it features results of tumour-specific properties that can be summarised as a response to hypoxia. The hypoxic state occurs when the tumour mass reaches a volume sufficient not to permit oxygen diffusion inside the tumour centre. Thus tumour cells turn on adaptation mechanisms to the low PO2 level, inducing biochemical responses in terms of cytokines/chemokines/receptors and consequently recruitment of specific cell types, as well as cell-selection inside the tumour. Moreover, these changes are orchestrated by the microRNA balance strongly reflecting the hypoxic milieu and mediating the cross-talk between endothelial and tumour cells. MicroRNAs control of the endothelial precursor-vascular settings shapes the niche for selection of cancer stem cells.

Key words: angiogenesis, cancer stem cell, endothelial precursor cell, hypoxia, microRNA.

Hypoxia-shaped vascular niche for cancer stem cells

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Introduction

As cancer stem cell (CSC) is a term to describe the most resistant and quiescent subpopulation of cells that, among the growing tumour cells, may be resistant to treatments and are able to aggressively reconstitute the malignant disease, these cells are also called cancer-initiating cells. Deciphering the molecular mechanisms they to use to dedifferentiate and to resist the harsh conditions resulting from the therapies is a challenge for new anticancer approaches. The cancer stem cells are not highly different from other stem cells, which makes their targeting difficult. In this aspect, the place and conditions in which they originate inside the tumour mass as well as their microenvironmental milieu remain a question of controversy. As they often appear to be associated with endothelial cells of the micro-vessels, their niche is described as non-hypoxic while they display resistance to hypoxia [1]. They also are the hypoxia-resistant cells that recruit endothelial cells to form the tumour vasculature both from sprouting preformed vessels as well as from bone marrow and/or vessel wall mobilized endothelial precursors [2]. In that process, cancer stem cells act through the production of factors able to exert a paracrine chemoattractant effect towards responsive endothelial cells and especially precursor endothelial cells. This effect is insured mainly by the production of vascular endothelial growth factors (VEGFs) and their endothelial receptors; it also permits the conditioning of the pre-metastatic niches by activating the endothelial barrier in secondary sites [3].

Vascular endothelial growth factor production illustrates the response to hypoxia by the proangiogenic molecule cascade [4] that develops after hypoxia-inducible factors (HIFs) early production and transcription [5]. As tumour angiogenesis is not efficient, it is unable to restore the cell oxygenation thus maintains the VEGF production, and pathologic angiogenesis is pursued in a vicious circle [3].

The antiangiogenesis strategies for cancer treatment have been successful in destroying the tumour vessels and reducing the tumour size. They have, unfortunately, also resulted in the selection of resistant cancer cells surviving to deep hypoxia and escaping from most conventional treatments because they are not dividing and isolated from systemic access [3]. Consequently, the interactions between the endothelial cells and the cancer stem cells determine the status of the niche [6]. This review focuses on the characteristics of cancer stem cells, pointing out their relation to hypoxia, the hypoxia-mediated participation of endothelial precursor cells to the cancer stem cell niche, and the fine mechanisms of regulation between EPCs and...
CSCs that the non-coding microRNAs are tuning in the context of hypoxia.

The characteristics of cancer stem cells are linked to hypoxia

Because it was shown that metastatic potential might be attributed to stem cell-like tumour cells (also called cancer stem cells), which are resistant to chemotherapy and induce dormancy in tumours, their detection, isolation, and characterisation is a challenge for cancer-targeted therapeutic strategies. As they are quite similar to normal stem cells, it is critical to find specific markers allowing CSC identification for effective targeted therapy. A number of markers have been used to isolate CSCs from solid tumours of the colon, oesophagus, liver, breast, brain, cervix, and head and neck squamous cell carcinomas (HNSCC). As summarised [7], antigens should be combined in order to type and even isolate the CSCs from a whole tumour population. CD44 and CD133 are commonly expressed in oesophageal stem cell carcinoma, liver, breast, prostate, and colon carcinoma. In addition, Nanog Oct3/4, CD90, CD34, CD177 (stem cell factor receptor), or CD271 are helping to type CSCs in various head and neck localised cancers [8].

Zhang et al. (2009) first suggested, that Hoechst 33342 dye by the ATP-binding cassette transporter (ABC, multidrug resistance receptors) can also be used to identify the side-population of cells that possess stem-like properties [9].

Aldehyde dehydrogenase enzymatic activity is used to type, isolate, and study the cancer stem cells. In many tumour types the cells that display a high aldehyde dehydrogenase (ALDH) activity have stem-like properties in terms of spheroid formation and tumourigenicity [10], but it is not a general feature [11].

Consequently, a variety of cell surface markers are used to define CSCs from primary tumours and lines, commonly CD133, CD44, CD24, and CD166 [12], but no study has allowed definition of the CSC identity, since the CSC-phenotype may vary substantially across different tumours, as in the case of melanoma metastatic process [13].

An interesting feature that is usually not taken into account along with characterization and functional studies is the influence of hypoxia vs. physioxia [14]. Most of the cited antigens and putative markers assessed are regulated by the pO2 conditions. Indeed, CD133 promoter is activated by hypoxia-inducible factors (HIFs) [15] and its glycosylation is also hypoxia-dependent, which explains the variable results obtained by antibody detection [16]. CD44 was also shown to be regulated by hypoxia in expressing its variant isoforms in triple negative breast cancer [17], as similarly shown for CD271 in melanoma [18]. This hypoxia-mediated effect was clearly demonstrated on CD34+ chronic myeloid precursor cells operating differentiation [19] and on CD24 as an effector of HIF-1α-driven primary tumour growth and metastasis [20]. Although in the melanoma, ALDH was not correlated with aggressiveness [21], we showed that its expression is stabilized in hypoxia-selected cells and it was shown to regulate stemness in breast cancer by activating HIF-2α [22].

Consequently, considering hypoxia effects on antigen expression it might help to precisely identify the stem cell characteristics in tumours.

The contribution of endothelial precursor cells to tumour angiogenesis as a response to hypoxia

Similarly to cancer stem cells, normal stem cells are very reactive to hypoxia and hypoxia-mediated signalling, as shown in embryonic development and particularly for haemangioblast specification [23]. This "good" aspect of angiogenesis becomes "bad" as it occurs in tumours through the early 'angiogenic switch' due to hypoxia (Hanahan and Folkman, 1996). It allows tumour development [24] and sets the tumour microenvironment in terms of cytokines, enzymes, extracellular matrix, and cells [14, 25]. Tumour
angiogenesis promotes tumour growth and facilitates metastasis. In response to pro-angiogenic signals, as vascular endothelial growth factor (VEGF)-A or interleukin (IL)-8 released mainly from neoepithelial cells and stromal cells, such as Tie-2 expressing monocytes (TEMs) [26] and fibroblasts [25], endothelial cells are further actively recruited to participate to the pathologic angiogenesis into the tumour mass [25]. Similarly, the infiltrating immune cell subpopulations that are recruited, apart from tumour-antagonising CD8 cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, i.e. macrophages, mast cells, neutrophils, and T and B lymphocytes, act as tumour-promoting cells [2]. Defining the type and differentiation characteristics of the endothelial precursors that are specifically recruited into the developing tumour is an important aspect in the design of new angiogenesis-based treatments [27, 28] that are able to take over antiangiogenic treatments and avoid selection of cancer stem cells. The normalisation of tumour vessels, first suggested by Jain [29], represents an adjuvant strategy which not only permits a strong increase of the efficacy of chemotherapcy and radiotherapy, but also reduces the tumour growth and allows eradication of metastases [30]. Moreover, it clearly reduces the number of stem-like cells in the treated tumours where the remaining markers are CD133+ and CD34+ cells, attributable to EPCs [31], while Oct3,4+, CD24−, and CD271+ cells totally disappeared together with the expression of their mRNA [30]. Stable normalisation may thus be the alternative to antiangiogenic treatments [32, 33].

**MicroRNA contribution to the vascular cancer stem cell niche**

MicroRNAs mediate an important aspect of the endothelial cell-to-cancer stem cell cross-talk. They have various means to act on cells and regulate their gene expression. They are largely documented for their expression in response to hypoxia. The so-called hypoxia miRs are regularly induced in hypoxia, and some of them act on angiogenesis (angomiRs). In tumours, miR-210, the most typical hypoxia miR, is HIF-1α-dependent and also stabilises HIF-1α, thus controlling the hypoxic phenotype [34]. This process is of fundamental interest for further therapeutic applications involving radioresitisation. As miR-210 is proangiogenic, it promotes endothelial cell migration [35]. It operates through endosome transport to endothelial cells [36], which appears to be a common means of interaction [37]. Besides this classical example, the huge role played by miRs in tuning the tumour microenvironment is documented for immune cells [38, 39], the extracellular matrix and, enzymatic regulation, as well as angiogenesis [2, 38, 40]. In this instance HO-1 activity in angiogenesis and compensation by miR-378 expression was shown to occur through exosomes produced by tumour cells [38]. MiRs that normally regulate stem cell biology act on cancer stem cells as well. MiR-34a was found to be a key negative regulator of CD44+ prostate cancer cells, thus offering a therapeutic agent against prostate CSCs [41]. Similarly, the endothelial precursors recruited and responding to the hypoxic milieu of the CSCs niche are submitted to the identical miR regulation. Tumour endothelial cells were found to share the same abnormalities as found in cancer cells [42], which could be due to a common cancer/endothelial cell progenitor [43], to cancer-to-endothelial cell transdifferentiation [44], to fusion between cancer and ECs [45], or to cancer stem-like cells undergoing vascular mimicry. In contrast, tumour endothelial cells have unique properties [46] suggesting that oncogene-bearing circulating endothelial cell precursors might be one of the possible identities of cancer stem cells. As such, miRs that potentially normalize angiogenesis by regulating PTEN activity [2, 30] like miR-21 vs. miR-29b, might provide powerful targets for therapies linking vessel stable normalisation to tumour growth control [47].

**Discussion**

As CSCs and endothelial precursor cells cooperate closely in the achievement of vascular/cancer stem cell niche, they operate through very close mechanisms and responses. The notch signalling protein is a mediator exemplifying the convergence of hypoxic responses to promote signals that lead cancer stem cells via the epithelial-mesenchymal transition [48]. Although the HIF-1α-mediated EMT pathway still remains undefined as to the precise mechanisms of the molecular expression cascade, the similar pathways used by the CSC and EPC responses to hypoxia offer new insights. The endothelial cells perform angiogenesis through the tips cells/stalk cell notch signalling, and the concordance of the mechanisms led to the observation of the CSCs transdifferentiation into endothelial cells, thus achieving vascular mimicry [49].

Hypoxia and HIF-1α overexpression have been reported to promote the expression of EMT activators [50]. Notch signalling pathway is required to convert the hypoxic stimulus into EMT [51]. As recently shown [52], hypoxia upregulates c-Myc and OCT3/4, contributing to vascular mimicry formation. Hypoxia is also a regulator of CSCs and EMT through NFκB, PI3K/Akt/mTOR, NOTCH, Wnt/β-catenin, and Hedgehog signalling pathways [48].

Thus, the hypoxic microenvironment seems to rule the vascular mimicry mechanism of angiogenesis formation, which is a convergence of the diverse angiogenesis mechanisms through stemness maintenance and cooperation of endothelial precursor cells with cancer stem cells. At this level, the regulation operated by microRNAs [53] offers an opportunity to control and possibly transform the tumour microenvironment at the CSC/EPC niche.

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