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Review

Tumor and its microenvironment: A synergistic interplay

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ABSTRACT

The mutual and interdependent interaction between tumor and its microenvironment is a crucial topic in cancer research. Recently, it was reported that targeting stromal events could improve efficacies of current therapeutics and prevent metastatic spreading. Tumor microenvironment is a "complex network" of different cell types, soluble factors, signaling molecules and extracellular matrix components, which orchestrate the fate of tumor progression. As by definition, cancer stem cells (CSCs) are proposed to be the unique cell type able to maintain tumor mass and survive outside the primary tumor at metastatic sites. Being exposed to environmental stressors, including reactive oxygen species (ROS), CSCs have developed a GSH-dependent antioxidant system to improve ROS defense capability and acquire a malignant phenotype. Nevertheless, tumor progression is dependent on extracellular matrix remodeling, fibroblasts and macrophages activation in response to oxidative stress, as well as epithelial mesenchymal transition (EMT)-inducing signals and endothelial and perivascular cells recruitment. Besides providing a survival advantage by inducing *de novo* angiogenesis, tumor-associated vessels contribute to successful dissemination by facilitating tumor cells entry into the circulatory system and driving the formation of pre-metastatic niche. In this review, we focus on the synergistic effect of hypoxia inducible factors (HIFs) and vascular endothelial growth factors (VEGFs) in the successful outgrowth of metastasis, integrating therefore many of the emerging models and theories in the field.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world and one of the major causes of death worldwide [1]. The prevention and the early diagnosis are surely the most important approaches for reducing the burden of CRC, given the symptoms of early disease occur just in 5% of cases. A significant portion of patients who receive surgery and adjuvant therapy still develop recurrences and metastasis and this phenomenon seems to be driven in some cell subsets by the acquisition of resistance to conventional therapy, such as chemo- and radio-therapy [2].

Growing evidence indicates that a cellular subpopulation with stem cell like features, commonly referred to as cancer stem cells (CSCs), is critical for tumor generation and maintenance.

A recent study showed that within the tumor population it is possible to identify a heterogeneous population of cells with different biological roles [3]. Recent advances in stem cell biology are revealing that this cellular fraction shares many properties with normal adult stem cells, including dormancy (quiescence), active DNA repair machinery, the expression of several ABC drugs transporters and an intrinsic resistance to apoptosis [4]. As their normal counterpart, the colon CSCs reside in a specialized microarchitectonic structures or niches that respond to both local and systemic conditions providing also protection against conventional therapies [5].

Moreover, microenvironmental stimuli, such as those involved in the epithelial-mesenchymal transition (EMT) and hypoxia, indirectly contribute to chemoresistance by inducing in cancer cells a stem like-phenotype. Understanding the driving force of tumor progression and the relationship between cancer cells and microenvironment could be fundamental in developing innovative therapeutic strategies for a better and definitive response on patient treatments.

2. CRC, stem cell niche and colon CSCs

It is widely accepted that CRC progression is driven by the acquisition of 4–5 progressive mutations in oncogenes or tumor

Abbreviations: CSCs, cancer stem cells; CRC, colorectal cancer; EMT, epithelial mesenchymal transition; ECM, extracellular matrix; ROS, reactive oxygen species; MMPs, matrix metalloproteinase; CAFs, cancer-associated fibroblasts; CAMs, cancer-associated macrophages; GSH, reduced glutathione; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor.

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suppressor genes [6]. Some driver mutations frequently occur in the same gene sequences and are shared by most of the people affected by this cancer, whereas some mutations are different and responsible of the final cancer phenotype in individual patients [7]. Most of the information about CRC derives from the study of familial adenomatous polyposis (FAP), an autosomal dominant colon cancer syndrome caused by *APC* gene mutation [8]. APC is involved in the regulation of Wnt pathway that, as we will discuss later in this review, can regulate cell proliferation, differentiation, migration and apoptosis [9]. Tumor progression is also achieved by other mutations such as *KRAS*, *SMAD2/4*, *TP53* and deletion of chromosome 18q [10].

It was recently demonstrated that despite the great heterogeneity and biological diversity of CRC it is possible to distinguish three different subtypes. De Sousa et al. indeed showed that two of these subtypes have already been identified for chromosomal-instable and microsatellite-instable cancer. A third one, prognostically unfavorable, is characterized by microsatellite stability and relatively more CpG island methylator phenotype-positive, thus rendering it impossible to be identified on the basis of characteristic mutations [11].

The presence of a distinct population with stem cell characteristics among disseminated and circulating cancer cells may be of clinical relevance, not only for their putative role in metastasis formation and recurrence, but also for their role in resistance to conventional therapy. CSCs are likely to share many properties of normal stem cells as mentioned above, which may underlie their capacity to survive therapeutic protocols based on genotoxic agents targeting actively proliferating cells [12].

First invoked by Paget, the "seed and soil" hypothesis suggests that the successful growth of metastatic cells depends on the interactions and properties of cancer cells (seeds) and their potential target organs (soil). Additionally, new concepts include: (i) the role of cancer stem-like cells as putative cells of metastatic origin (the "seeds"); (ii) the mechanism of EMT in driving epithelial cell into the blood stream to avoid *anoikis*, or anchorage independent cell death; and (iii) the reverse process of EMT, or mesenchymal to epithelial transition (MET), which promotes conversion back to the parent cell morphology and growth of macrometastasis in the target organ, open a new broad of aspect on this issue [13].

The microenvironment plays a crucial role in maintaining the pluripotency of colon SCs at the base of colon crypts influenced by fibroblast, endothelium and inflammatory cells, cytokines and growth factors secreted by these cells (in particular HGF) thus finely regulating the balance between self-renewal and differentiation of the staminal population [14–16]. The most characterized pathway involved in the maintenance of colon stem cells is Wnt [17–19], and it is clearly highlighted by the different expression of Wnt members along the colon crypt [20], even if the maintaining of stemness and the differentiation pattern is actually the result of the fine collaboration with other important pathways, such as PTEN-PI3K-Akt [21,22], BMP [23], Notch [24] and Sonic hedgehog (Shh) [25].

3. EMT, pre-metastatic niche and metastasis formation

Metastasis formation is considered a complex multi-step process with sequential molecular and cellular events that permit transformed cells to gain access to the blood stream (intravasation), survive their journey through the blood stream, and ultimately traverse through the microvasculature of target organs (extravasation) to deposit, survive, and grow in a foreign tissue environment. The EMT represents the first step of this highly regulated cascade and it is an important biological process initially studied in normal tissues during the organogenesis and then extended in the pathogenesis of cancer diseases, particularly referred to the acquisition of migratory phenotype in CRC cells [26]. After extravasation from the circulation into the target organ, aberrant cells must implant, proliferate, and induce angiogenesis in order to survive and grow in a foreign and presumably "hostile" environment. These phenomena are driven not only by genetic and/or epigenetic alteration of cancer cells, but also by the non-neoplastic stromal cells [27].

The EMT is characterized by the loss of epithelial properties, including the apico-basal polarity and cell adhesion, the E-cadherin, occluding and cytokeratins expression, and at the same time the acquisition of N-cadherin, vimentin, fibronectin, Twist1, zinc-finger proteins (SNAIL, SLUG, ZEB) and matrix metalloproteinases (MMPs) expression, all events that lead to an increased cell mobility [28]. Moreover, EMT-inducing factors released by the surrounding microenvironment [29] can affect the invasive phenotype in epithelial malignancies initiation. Key regulators of this process are TGF-β (by the activation of Twist, SLUG and ZEB2), PI3K/Akt (increasing the mTOR kinase expression), Shh and Wnt [30,31].

Currently, dissemination and spread of cancer cells during the tumor progression are elective events underling the invasion through the tissue extracellular matrix (ECM). It was recently shown that tumor cells have two different modes of motility: (1) the acquisition of a mesenchymal phenotype, as previously described that identifies a mesenchymal motility mode and (2) the amoeboid migration [32]. The mesenchymal mode is characterized by the acquisition of an elongated morphology and activation of the small GTPase Rac [33]; the amoeboid motility is defined by a rounded or ellipsoid cell morphology and weak interactions with the surrounding matrix, driven by Rho expression, which induce membrane blebbing through Rho-associated protein kinase (ROCK)-dependent myosin II phosphorylation and consequent actomyosin contractility [34]. These two migration modes are interconvertible and regulated by microenvironmental influences. The possibility to switch from one mode to the other one highlights the cell plasticity that accomplishes movement from the primary tumor, establishment in an ectopic site, and survival therein [35].

The balance between high levels of activated Rac and Rho proteins regulates finely the motility mode. Moreover, Rac signaling inhibits amoeboid movement through its effector WASP-family verprolin-homologous protein 2 (WAVE2), and in the same way Rho/ROCK suppresses Rac by the activation of ARHGAP22, a GTPase-activating protein (GAP) [36].

Although *RHO* gene mutations are extremely rare, their altered expression has been assessed in many human cancers, including CRC. In particular, RhoA is frequently overexpressed and its induction is rapidly mediated by TGF-β [37], while depletion of Rac1 strongly correlates with the inhibition of lamellipodia formation, cell migration and invasion in carcinoma cells [38].

Furthermore, recent study established the independent contribution of *KRAS* and *BRAF* mutations, which rarely coexist in human tumors, to migration and invasion of CRC cells through Rho GTPases signaling. Although KRAS and BRAF are common members of the same pathway, Makrodouli et al. showed that *BRAF* mutation enhances cell migration through RhoA activation, and its effect is more pronounced compared to KRAS. These findings are expected to eventually result in tailor-made therapies against Rho pathway components, since it depends on the genetic background of the cancer patient [39].

4. Status redox and hypoxia: two sides of the same coin

In the absence of an aberrant microenvironmental stimuli, genetic and epigenetic alterations in tumor cells are insufficient to induce primary tumor progression [27]. Either through structure and function-based mechanisms, including ECM remodeling,