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Research review paper

Investigations into the cancer stem cell niche using in-vitro 3-D tumor models and microfluidics

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ABSTRACT

The concept of Cancer Stem Cells (CSCs) and the CSC Niche/Tumor Microenvironment (TME) as the central driving force behind tumor progression and maintenance has garnered much attention in recent years. Concomitantly, the widespread adoption of 3D tissue models, organotypic co-cultures, and the revolutionary microfluidic technology has resulted in a plethora of ground-breaking fundamental discoveries and has enabled investigations which were previously unfeasible. A large number of existing review papers concern themselves with either a broad look at the TME and CSC Niche, or on the studies undertaken on a particular niche component alone. In this article, we attempt to bring out a harmonic, expansive look at the concept of CSCs, the TME, and the various advancements in answering key biological queries enabled by these emerging new technologies. Our primary goal is to present a fundamental understanding of CSCs, as well as the CSC niche, and elucidate note-worthy examples of investigations being carried out with regard to each of the major TME components, along with our insights into the potential for further research. We hope that this serves as an impetus to new, as well as existing researchers in this area, to gain fresh perspectives on the CSC niche, as well as provide them with a glimpse at the kind of progress being made using 3D tumor models and microfluidic devices.

1. Introduction

Fundamental investigations into cancer biology are of prime importance due to their multiple beneficiary fields of research, most importantly, drug development and therapeutic targeting. Cancer research is a constantly evolving field of study, with a multitude of perspectives and approaches being dynamically adopted, pursued, or in some cases discarded over time. Decades of research have unearthed widely varying view-points on what constitutes the driving force behind tumor growth and progression – viruses, mutations, metabolic changes etc. As such, multiple models have been proposed for the same, with varying degrees of evidence and success. Herein, we are primarily concerned with two widely accepted, yet distinct approaches – the cancer stem cell model, and the clonal evolution model.

The concept of the Cancer Stem Cell (CSC) has gained a great deal of traction in recent years as a central feature of cancer research. At its heart, the idea is an intriguingly appealing perspective of core observations made over decades of study. The generally accepted interpretation suggests that in a population of tumor cells, a particular subset of cells, termed CSCs, are primarily responsible for tumor initiation, progression and recurrence (Visvader and Lindeman, 2008). CSCs are functionally similar to normal stem cells (SCs) in that they self-renew and differentiate. This implies that similar to normal tissue, tumor cells have an established organizational and functional hierarchy among themselves. Dick and colleagues in 1997 provided the first experimental evidence for this. In their study, they established the presence of a hierarchy in leukemic clones in acute myeloid leukemia (Bonnet and Dick, 1997). However, CSCs differ from regular SCs in that the processes of self-renewal and differentiation are highly deregulated and largely continuous in CSCs. This is a major factor allowing them to generate growing tumor populations. (Clarke et al., 2006; Dawood et al., 2014).

In relative contrast to this stands the clonal evolution model, which states that all tumor cells are originally equivalent. Subsequent genetic/epigenetic modifications occurring over time in these tumor cells can induce them to stochastically acquire the properties to metastasize, become resistant to therapy and promote tumor recurrence. Additionally, the more aggressive ones out of the lot are considered to be responsible for driving tumor growth and progression (Beck and Blanpain, 2013; Carnero and Lleonart, 2016; Ding et al., 2013; Greaves and Maley, 2012).

However, recently evolved perspectives suggest that the dichotomy

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between both the models is largely artificial, and that they complement each other as far as an actual collection of tumor cells is concerned (Carnero and Lleonart, 2016; Plaks et al., 2015). This can be explained using the concept of CSC plasticity, wherein a tumor cell acquires the ability to reversibly transition between non-stem and stem cell states. This effectively introduces an element of stochasticity into the hierarchical model of CSCs. (French and Clarkson, 2013; Kreso and Dick, 2014; Prasetyanti and Medema, 2017; Rich, 2016). The direct implication is that stochastic events can lead to de novo hierarchical populations, thereby indicating that a dynamic equilibrium exists between stem and non-stem states.

CSCs also help explain the observance of tumor heterogeneity in terms of morphological/physiological properties. CSCs contribute to tumor heterogeneity via the production of various genetically similar tumor cell types by differentiation processes. (Pietras, 2011). Evidence suggests that genetically distinct clones can exist within the same tumor as well (Marusyk and Polyak, 2011). Using mathematical models, CSCs have been shown to stochastically divide into either of three cell fates: symmetric renewal, symmetric differentiation, or asymmetric self-renewal, in a manner similar to SCs. Out of these, asymmetric cell division seems to be the major factor which helps maintain tumor heterogeneity at the population level (Beck and Blanpain, 2013).

CSCs are also believed to play a pivotal role in metastasis – specifically, in the progression of the EMT (epithelial mesenchymal transition) pathway, which reprograms epithelial cells to achieve a mesenchymal phenotype (Kalluri and Weinberg, 2009; Morel et al., 2008). This conversion involves secretion of transcription factors such as TWIST and SNAIL that suppress E-cadherin (Berx et al., 2007; Kang and Massagu?, 2004). This suppression is characterized by the loosening of cell-cell contacts and the promotion of increased extracellular matrix interactions via integrins and FAK (focal adhesion kinase) (Desgrosellier and Cheresh, 2010; Golubovskaya et al., 2009; Hood and Cheresh, 2002; Seguin et al., 2015; Yoon et al., 2015). More importantly, the genes responsible for regulating EMT can also regulate normal stem cell and CSC properties, thereby illustrating the intricate connection between CSCs and metastasis (Hermann et al., 2007; Mani et al., 2008).

CSCs have been shown to be resistant to therapy in many cases. Also, they have been linked to the phenomenon of tumor recurrence and drug resistance. This may be due to their properties like overexpression of anti-apoptotic molecules, enhanced DNA repair ability, production and utilization of drug efflux transporters etc. (Rochat, 2009; Wilson et al., 2008). In fact, the percentage abundance of CSCs in a sample has been shown to get enriched in mouse xenografts following chemotherapy (Dylla et al., 2008). The increased radiotherapy resistance shown by breast and prostate CSCs has been attributed to the low expression rates of ROS (reactive oxygen species) in them, as well as their enhanced DNA repair mechanism. The low ROS levels is believed to be mediated by an enhanced free-radical scavenging machinery expression in these cells (Cabarcas et al., 2012; Diehn et al., 2009; Kim et al., 2013).

It is clear that therapies targeted at CSCs can achieve significant clinical pay-offs by mitigating tumor recurrence after therapy. This calls for a deeper understanding of tumor biology and the associated processes, in particular, those key to CSC survival and maintenance. Over the past few years, the concept of a CSC Niche, or the Tumor Microenvironment (TME) – the primary focus of this article - has established itself as an indispensable accessory of CSCs, and one which plays an enormous role in maintaining, regulating, and dictating the tumor progression at the most fundamental biological level. In this article, our primary goal is to present a discussion on the relevance of the CSC niche and the niche's major components, in particular, the investigations being conducted into these using 3D models and microfluidics. Such a niche component-specific classification of investigations and their results is lacking in the literature. We have attempted to lay greater emphasis on the biological ramifications and potential of these results, rather than the technical details of the models/devices used. This review will place existing observations and results in a more practically relevant perspective and provide motivation and direction for future research work in the area of CSCs and TMEs.

2. The niche

SCs are usually localized in specific microenvironments, which are regarded as essential for the maintenance of the balance between self-renewal and differentiation processes. Similarly, the CSCs are thought to occupy localized tumor microenvironments called CSC niches. These niches can greatly influence the tumorigenic potential of the cell as well as determine cell fate. Characteristic features of CSCs like self-renewal, differentiation, cellular plasticity etc. are believed to be regulated by specific components of this tumor microenvironment. (Borovski et al., 2011; Plaks et al., 2015). The broad structure of the classification we adopt here is along similar lines to the one provided by Plaks et. al. in their 2015 review paper.

Niches can be defined as “anatomically distinct microenvironments contained within an overall tumor microenvironment” (Plaks et al., 2015). Typically, a CSC niche hosts a number of inhabitant cellular components like cancer associated fibroblasts (CAFs), immune system cells, multipotent stromal cells (MSCs), endothelial cells (ECs), in addition to a prevailing hypoxic environment. The extra-cellular matrix (ECM) is also a key factor affecting properties of the niche. We commence our discussion by providing a brief overview of the niche components (Fig. 1).

2.1. CAFs

Regular fibroblast cells have been shown to transform into what are called CAFs (variously termed as myofibroblasts/a subset of

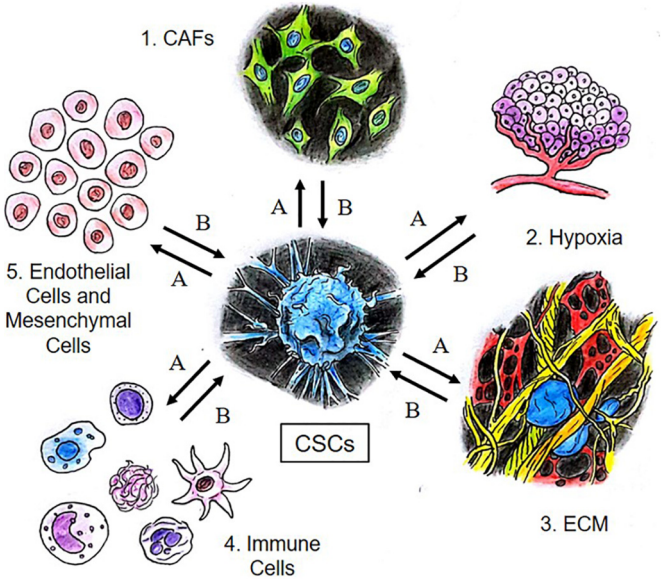

Fig. 1. The bi-directional relationship between CSCs and various cellular components of the CSC Niche. Arrows labelled “A” designate the effect CSCs have on the component, whereas arrows labelled “B” signify the influence on CSCs by the particular component. The details regarding these have been discussed at length in the text. 1.CAFs: A. Fibroblast transformation. B. Wnt and NOTCH pathway activation, VEGF-A production. 2.Hypoxia: A. Maintenance of hypoxic conditions in the CSC Niche. B. HIF secretion, TGF-β production and decrease in ROS levels. 3.ECM: A. ECM Remodeling process. B. Maintenance of cell-cell contact, physical barrier to drug delivery. 4.Immune cells: A. Evolution of TAMs and TANs. Suppression of NKs, CD8+ T-cells. B. Exosomes produced by TAMs, impetus given to metastasis-related processes by immune processes. 5.Endothelial cells and mesenchymal cells: A. Uptake of survival genes helping in apoptosis evasion. B. Activation of NOTCH pathway, production of cytokines like Gremlin-1, and inhibition of CSC differentiation.