Montagna Symposium on the Biology of Skin

Cancer Stem Cells in Squamous Cell Carcinoma

Zhe Jian[1,2], Alexander Strait[1], Antonio Jimeno[3] and Xiao-Jing Wang[1]

Cancer stem cells (CSCs) are found in many cancer types, including squamous cell carcinoma (SCC). CSCs initiate cancer formation and are linked to metastasis and resistance to therapies. Studies have revealed that several distinct CSC populations coexist in SCC and that tumor initiation and metastatic potential of these populations can be uncoupled. Therefore, it is critical to understand CSC biology to develop novel CSC-targeted therapies for patients with SCC with poor prognoses. This review compares the properties of CSCs in SCC with normal stem cells in the skin, summarizes current advances and characteristics of CSCs, and considers the challenges for CSC-targeted treatment of SCC.

Journal of Investigative Dermatology (2016) ■, ■–■; doi:10.1016/j.jid.2016.07.033

INTRODUCTION

Nonmelanoma skin cancers, including basal cell carcinoma and squamous cell carcinoma (SCC), are the most common skin cancer types and have increased dramatically worldwide in recent years (Moore et al., 2015; Narayanan et al., 2010). SCC can metastasize to ectopic sites (Klein, 2013), and advanced SCCs have high mortality rates and are often refractory to conventional therapy (Geissler, 2015). SCCs contain subpopulations of cells with cancer stem cell (CSC) properties that are linked to SCC initiation, metastasis, and resistance to chemo- and radiotherapy (Biddle et al., 2011; da Silva-Diz et al., 2016; Oshimori et al., 2015; Schober and Fuchs, 2011; White et al., 2013; Zhang et al., 2010). Therefore, characterizing SCC CSCs will provide new insights into SCC treatment. This review covers similarities and differences between SCC CSCs and normal stem cells (SCs) in the skin and discusses therapeutic strategies to target CSCs.

[1]*Department of Pathology, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colorado, USA;* [2]*Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi, China;* and [3]*Department of Medicine, Division of Medical Oncology, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colorado, USA*

Correspondence: Xiao-Jing Wang, Department of Pathology, University of Colorado Denver, Anschutz Medical Campus, Building RC1-N, RoomP18-5128, Mail Stop 8104, Aurora, Colorado 80045-0508, USA. E-mail: xj.wang@ucdenver.edu

Abbreviations: ABC, ATP binding cassette; ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; K15, keratin 15; SC, stem cell; SCC, squamous cell carcinoma; SP, side population

Received 25 January 2016; revised 11 July 2016; accepted 31 July 2016; corrected proof published online XXX XXXX

SCs VERSUS SCC CSCs IN THE SKIN

SCs are responsible for regenerating and maintaining tissues and have unique defining characteristics (Figure 1). First, normal SCs are capable of self-renewal. Each SC typically undergoes asymmetrical cell division to produce two daughter cells: one SC and one differentiating cell. Second, normal SCs are usually slow cycling with low proliferation rates, retaining tritium thymidine or BrdU labeling for long periods of time (also known as label retaining cells), yet maintain the capacity for clonogenic growth (Bickenbach, 1981; Morris and Potten, 1994). Third, they are rare in most tissues. Fourth, they are undifferentiated but can give rise to one or more cell lineages (multipotency or pluripotency). Fifth, normal SCs have a much longer lifespan than their progeny. Finally, normal SCs often have specific locations determined by their microenvironment (niche).

Epidermal SCs are located in the bulge of hair follicles, the basal layer of the interfollicular epidermis, and the base of the sebaceous gland (Levy et al., 2005). Hair germ cells, thought to arise from bulge cells, also contain BrdU label retaining cells (Ito et al., 2004). Although distinctive, the pattern of gene expression in hair germ cells is more similar to bulge cells than to transiently amplifying follicular matrix cells (Greco et al., 2009). Studies suggest that bulge cells and possibly hair germ cells contain multipotent follicular SCs that normally generate hair follicles, but can also regenerate the epidermis and sebaceous glands in response to skin injury (Ito et al., 2005; Jaks et al., 2008; Levy et al., 2005, 2007; Morris et al., 2004). Under normal conditions, SCs in the interfollicular epidermis and sebaceous glands are lineage specific, and generate their respective tissues without recruitment of SCs from the bulge (Claudinot et al., 2005; Clayton et al., 2007; Horsley et al., 2006; Ito et al., 2005; Levy et al., 2005; Morris et al., 2004).

CSCs are certain tumor cells exhibiting stem cell-like properties. Whereas normal SCs have several distinct characteristics as described above, CSCs are primarily defined by one criterion: the ability to initiate tumors, and the term CSC is often used interchangeably with "tumor-initiating cell." CSCs can be derived from SCs (Morris et al., 1986) or from nonstem cells that acquire the capacity to self-renew (Jamieson et al., 2004). Unlike normal SCs, CSCs may not be multipotent, leading to single lineage tumors, such as SCC (epidermal lineage), various follicular tumor types (hair follicle lineage), or sebaceous gland tumors (sebaceous lineage). Furthermore, CSCs may not be quiescent. For example, normal slow cycling bulge SCs can acquire genetic mutations, such as Kras mutations or Smad4 deletions, that drive them into hyperproliferation (White et al., 2013). Finally, the number of CSCs varies widely, ranging from ≤1% to approximately 20% in SCCs, depending on tumor types and

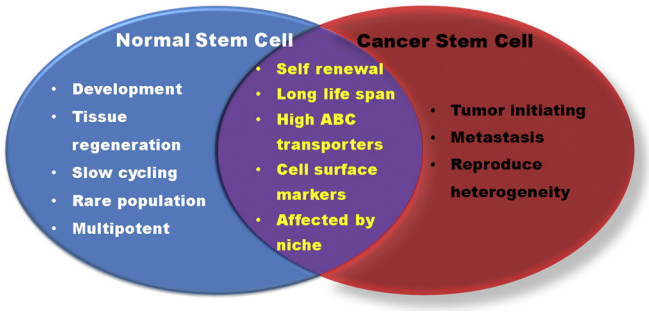

Figure 1. Venn diagram showing stem cell and cancer stem cell characteristics. ABC, ATP binding cassette.

experimental models used to assess tumor initiation, such as the severity of immune suppression of recipient mice in xenografts (Quintana et al., 2008; Song et al., 2010; White et al., 2013). For example, in our SCC mouse model, CSCs were rare in primary SCCs, but their numbers dramatically increased in metastatic SCCs and SCCs with epithelial to mesenchymal transition (White et al., 2013).

SCs and CSCs also share characteristics, such as the capacity for self-renewal, high levels of ATP binding cassette (ABC) transporters, certain cell surface markers, and being influenced by their niche. Also, CSCs share certain regulators with normal SCs. For example, SCC CSCs express factors that regulate self-renewal in embryonic SCs, such as SOX2, MYC, and OCT-4 (Bose and Shenoy, 2014; Boumahdi et al., 2014; Lim et al., 2014). Similarly, some common "stemness" pathways are activated in follicular SCs and CSCs, such as Wnt signaling (Malanchi et al., 2008; White et al., 2013).

SORTING CSCs

CSCs can be sorted by putative cell surface markers that can be either shared with or distinct from normal SCs depending on tumor type. CD34, a cell surface marker for mouse bulge SCs (Trempus et al., 2003), also serves as a CSC marker in mouse SCCs (Trempus et al., 2007) but is not expressed in human bulge SCs (Ohyama et al., 2006). CD200, a cell surface marker expressed in both mouse and human bulge SCs (Ohyama et al., 2006), is also enriched in metastatic SCC (Stumpfova et al., 2010). CD49f is a surface marker of quiescent label retaining cells, including bulge and interfollicular SCs (Blanpain et al., 2004; Jiang et al., 2010; Terunuma et al., 2003), and also serves as an SCC CSC marker (Schober and Fuchs, 2011; White et al., 2013). CD44 is high in SCC CSCs (Lapouge et al., 2012; Malanchi et al., 2008; Prince et al., 2007). CD133, a cell surface marker specific for hematopoietic SCs (Yin et al., 1997), was the first cell surface marker used to define tumor-initiating cells in human cutaneous SCC (Patel et al., 2012). In addition to cell surface markers, CSCs in SCCs can be sorted based on their aldehyde dehydrogenase (ALDH) and ABC transporter activity (Clay et al., 2010; Yang et al., 2014). The side population (SP) assay identifies stem-like cells based on their ability to pump out Hoechst dye and chemotherapeutic drugs via ABC transporters (Goodell et al., 1996; Zhang et al., 2009), and has been used as a marker for both normal skin SCs and SCC CSCs (Larderet et al., 2006; Song et al., 2010; Tabor et al., 2011; Wan et al., 2010; Zhang et al., 2009). SP cells are distinct from CD4f+ keratinocyte stem cells (Terunuma et al., 2003) and SCC CSCs (White et al., 2013). As discussed below, CSC markers can be altered by CSC plasticity and interactions with their niche. For instance, $CD44^+$ SCC CSCs express other CSC markers with a high degree of variability (Krishnamurthy et al., 2010).

DETERMINANTS OF SKIN SC AND CSC BEHAVIOR

Genetic and epigenetic modification

Within the same SC compartment, different genetic mutations have distinct effects on CSC behavior. For example, a $Kras^{G12D}$ mutation in keratin 15 $(K15)^+$ bulge SCs initiates benign papillomas in genetically engineered mouse models, but requires the loss of an additional tumor suppressor to induce SCC (Lapouge et al., 2011; Nassar et al., 2015; White et al., 2013). When combined with radiation, a heterozygous *Ptch* deletion is sufficient to induce basal cell carcinoma in $K15^+$ cells, and is exacerbated by the additional loss of *p53* (Wang et al., 2011). We found that a $Kras^{G12D}$ mutation in combination with *Smad4* deletion not only caused metastatic SCCs from $K15^+$ cells, but also produced tumors of other lineages, such as basal cell carcinomas, trichoepitheliomas, and sebaceous adenomas (White et al., 2013). However, because the K15 promoter could create leaky Cre expression, targeted mutations may not be limited to bulge stem cells. Nevertheless, not all genetic mutations caused multilineage tumor types despite being driven by the same K15 promoter, suggesting that specific stem cell mutations play an important role in determining tumor lineages. To validate if bulge stem cells are the source of tumor-initiating cells, additional bulge stem cell markers described in the Sorting CSCs section are used in studies summarized in Table 1.

Epigenetic regulation, including DNA methylation, histone acetylation, and miRNA expression, also plays an important role in skin SC and CSC behaviors. For example, enhancer of zeste homolog 2 is a major epigenetic component of polycomb repressive complex 2 and is required for epidermal CSC survival, migration, invasion, and tumor formation (Adhikary et al., 2015; Banerjee et al., 2011). miRNAs can also maintain SC populations. For example, miR-205 enhances phosphoinositide 3-kinase (PI3K) signaling and is required for the expansion of neonatal skin SCs (Wang et al., 2013). miR-203, the most abundant miRNA in normal skin, is downregulated by the Hras oncogene, and silencing of miR-203 is an early event in mouse and human SCC (Riemondy et al., 2015). miR-203 limits cell division in both early embryonic skin development and SCC CSCs, and its loss caused an expansion of CSCs, resulting in increased tumorigenesis in an experimental skin carcinogenesis model (Riemondy et al., 2015). Furthermore, we found that miR-9 overexpression contributes to the expansion of metastasis-associated CSCs by inhibiting α-catenin and subsequent Wnt activation (White et al., 2013).

Location and microenvironment

As discussed above, mutations in hair follicle bulge SCs potentially cause tumor formation representing lineages of the epidermis, hair follicles, and sebaceous glands, whereas lineage-committed mutant SCs only generate tumor types from that lineage. For instance, mutant interfollicular SCs