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Review

Cellular reprogramming in skin cancer

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ABSTRACT

Early primitive stem cells have long been viewed as the cancer cells of origin (tumor initiating target cells) due to their intrinsic features of self-renewal and longevity. However, emerging evidence suggests a surprising capacity for normal committed cells to function as reserve stem cells upon reprogramming as a consequence of tissue damage resulting in inflammation and wound healing. This results in an alternative concept positing that tumors may originate from differentiated cells that can re-acquire stem cell properties due to genetic or epigenetic reprogramming. It is likely that both models are correct, and that a continuum of potential cells of origin exists, ranging from early primitive stem cells to committed progenitor or even terminally differentiated cells. A combination of the nature of the target cell and the specific types of gene mutations introduced determine tumor cell lineage, as well as potential for malignant conversion. Evidence from mouse skin models of carcinogenesis suggests that initiated cells at different stages within a stem cell hierarchy have varying degrees of requirement for reprogramming (e.g. inflammation stimuli), depending on their degree of differentiation. This article will present evidence in favor of these concepts that has been developed from studies of several mouse models of skin carcinogenesis.

1. Introduction

One of the core questions in cancer biology relates to the identity and nature of the cancer "cell of origin," the target cell in which the first oncogenic driver mutation occurs and leads to tumor initiation [1]. With the emerging concepts of "cancer-stem-cells", i.e. that sub-population of cells with tumor-initiating capacity in serial transplantation assays, it has been hypothesized that the primitive early stem cells might be the cancer cells of origin, because of their intrinsic features of self-renewal and longevity. However, evidence for the plasticity of completely normal cells, demonstrating their ability to acquire stem cell characteristics [2] raised the possibility that more committed progenitor cells can also serve as target cells for initiation, as long as they maintain or can re-acquire stem cell-like features. Among the questions that arise in light of these new concepts, are the following: (1) do tumors arise from stem cells, or committed progenitor cells that require reprogramming through inflammation or biological stress? (2) How does the cell of origin (stem cell or committed progenitor cell) affect malignant potential? (3) Do cancers of different histological subtypes arise by reprogramming of the same target cells?

In general, cellular reprogramming refers to the concept of rewiring the epigenetic and transcriptional network of one cell type to that of a different cell type [3,4]. In this review, we refer to "reprogramming" as those additional genetic, epigenetic, and micro-environmental alterations that are required for target cells to initiate and maintain/propagate a tumor.

2. Stem cell hierarchy and malignant potential

Increasing evidence suggests a surprising role for normal committed progenitor cells as a backup reservoir for adult stem cells after reprogramming in response to stress conditions. Using a method to trace the lineage of quiescent label-retaining cells (LRCs) in vivo in the intestinal crypt, it has been demonstrated that upon wounding, a population of LRCs that normally gives rise to Paneth cells can be reprogrammed to repopulate the stem cell niche and contribute to the regeneration of all intestinal cell lineages [5]. Similar results have been obtained by lineage tracing of Bmi1-positive quiescent stem cells exposed to radiation damage [6]. Another study, also using in vivo lineage tracing in mice, has demonstrated that upon depletion of airway stem cells, differentiated luminal secretory cells can be de-differentiated into basal stem cells [7]. This capacity of committed cells to function as reserve stem cells via

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reprogramming is very relevant to the tumor cell of origin question, as these studies support the idea that terminally differentiated cells can also become tumor-initiating cells. Similarly, it has been shown that inflammatory tumor promoting environments mediated by enhanced NF-κB activity and subsequent *Wnt* pathway activation induced intestinal epithelial non-stem cells to acquire a stem-cell like fate and function as tumor-initiating cells [8]. A mouse model of brain cancer also has shown that aggressive glioblastoma can originate from a range of different cell types including astrocytes and mature neurons in the nervous system via direct reprogramming [9].

These studies support the notion that tumors can originate from progenitor cells as well as stem cells. It is likely that there is a continuum of potential cells of origin ranging from early primitive stem cells to committed progenitor or even terminally differentiated cells, and the combination of the nature of the target cell and the specific types of gene mutations introduced determine their malignant potential [1]. For example, tumors that arise from early epidermal stem cells within the bulge region of the hair follicle might intrinsically possess more aggressive properties than tumors with the same genetic mutations but arising from a more differentiated, committed cell type in the epidermal lineage. Conversely, initiated cells within the more differentiated cell compartment would have the greatest requirement for reprogramming, in order to facilitate self-renewal and malignant progression (Fig. 1). Evidence in favor of these concepts has come from studies of the skin model system, as discussed later in more detail [10,11].

3. Inflammation and multistage skin tumorigenesis

Inflammation is a highly complex process involving cellular and humoral components of the immune system that exhibit both pro- and anti-tumor properties during cancer development [12,13]. Since the initial study in 1863 by Virchow [14], who observed the presence of a leukocytic infiltrate in tumor tissues and hypothesized a connection between the sites of inflammation and tumorigenesis, critical roles for inflammatory stimuli have been demonstrated in initiating, maintaining, and advancing tumors. These insights have led to various therapeutic strategies targeting inflammation for the prevention and treatment of cancer [12,13,15–18].

A critical role for inflammation in skin cancer is well characterized from studies using the classical two-stage DMBA/TPA chemical carcinogenesis model. This involves a single topical application of a mutagen, such as DMBA (7,12-dimethylbenz-[a]-anthracene) to introduce an initiating mutation, followed by prolonged exposure to a tumor promoter, such as TPA (12-O-tetradecanoyl phorbol-13-acetate) for 20 weeks. The predominant types of tumors that arise in this model are benign squamous papillomas, and a small portion of these tumors progress to malignant squamous cell carcinomas (SCCs). Some of these SCCs further undergo epithelial-mesenchymal transition (EMT) and become a more aggressive form of poorly differentiated carcinomas known as spindle cell carcinomas [19]. In the DMBA/TPA protocol, DMBA can form covalent adducts with the DNA of all epidermal target cells and causes tumor initiation commonly through a specific mutation in codon 61 of *Hras* (*Q61L*) [20–22]. However, the initiating *Ras* mutation by itself is not sufficient to form tumors in most experimental models, emphasizing the need for a tumor promoter in the second stage. The type of promoter as well as the duration and frequency of the tumor promoter treatment profoundly affect the incidence of papillomas in two-stage carcinogenesis experiments [23,24].

The tumor-promoting role of TPA in the DMBA/TPA model is not simply limited to stimulation of cell proliferation. TPA regulates activation of protein kinase C (PKC) isoforms and induces a pleiotropic tissue inflammatory reaction by the production of a wide range of cytokines such as Tgfβ, Tnfα and Il-1α that are crucial mediators of wound inflammatory responses [25–29]. This wound inflammatory reaction is critical in tumor promotion in that (1) other promoters that are equally potent as TPA in enhancing cell proliferation but without inducing cutaneous inflammation do not cause papilloma development, and (2) TPA treatment can be replaced by repeated wounding as well as by the injection of wound growth factors such as TGF-β with resultant papilloma formation [25,30–32].

Skin carcinogenesis induced in the DMBA/TPA model was presumed to proceed in a linear fashion through distinct stages including development of inflammation-dependent benign papillomas, conversion into malignant squamous cell carcinomas (SCCs), and progression of some of SCCs to undifferentiated spindle cell carcinomas via EMT [19]. There is however evidence that some aggressive spindle cell carcinomas arise by a separate route that is distinct from the classical pathway that is heavily dependent on inflammatory stimuli and *Hras* mutation [11]. Detailed analysis of carcinomas by gene expression profiling and histology revealed two distinct categories representing pure SCCs or those with only a minor spindle cell component (Class A), or essentially pure spindle cell carcinomas (Class B). Unlike the Class A carcinomas, the Class B carcinomas exhibit characteristics of EMT including (1) up-regulation of *Snai1*, *Zeb1* and *Vimentin*, (2) down-regulation of *E-cadherin*, *Krt5*, and *Krt14*, and (3) "Claudin low signature," which denotes stem cell-like features that have been shown in a subset of human breast cancer [33,34].

Striking differences between the Class A and Class B carcinomas were seen by analysis of (1) the integrity and expression of the *Ink4a/Arf* locus, and (2) their dependency on inflammation and *Hras* signaling, suggesting that Class B tumors may arise via a separate route involving distinct genetic and molecular reprogramming mechanisms. The fact that the Class B tumors frequently undergo loss of the *Ink4a/Arf* locus is notable. Three genes, *p16/Cdkn2a*, *p15/Cdkn2b*, and *p19/Arf*, are encoded from the deleted regions in the Class B carcinomas, and *p16* and *p19* have been specifically implicated in hindering self-renewal of neural and hematopoietic stem cells and blocking direct reprogramming of somatic cells to iPSCs [35–37]. This is reminiscent of the mechanisms by which p53 modifies the stem-like state and malignant potential of target cells (discussed later).

Exposure of mice to different levels of inflammation by modulating the length of TPA treatment revealed a differential dependence of these two tumor classes on inflammatory responses. While reduced exposure to inflammation dramatically decreased overall yields of papillomas and Class A carcinomas, more Class B carcinomas with high frequency of *Ink4/Arf* genetic alterations were observed. Mice treated with an abbreviated duration of TPA treatment of 5 weeks, rather than the usual 20 weeks, showed the highest relative proportion of Class B to Class A carcinomas. This observation is reminiscent of the "high-risk papillomas" reported by *Hennings et al.* three decades ago, which gave rise to carcinomas with a rather high 20% conversion rate after an abbreviated TPA treatment of 5 weeks [38]. We conclude that most papillomas and Class A SCCs seem to have a greater requirement for reprogramming by exposure to inflammatory agents, while the Class B carcinomas, which express high levels of Cd34 and some other known bulge stem cell markers, have a reduced requirement for this reprogramming stimulus. Intriguingly, mutant *Hras* signaling was downregulated in Class B spindle carcinomas prompting a study of skin tumor development in mice lacking the *Hras* target gene. Despite the fact that very few papillomas developed in *Hras*-deficient mice, surprisingly, the majority of null mice developed Class B carcinomas, most of which harbored mutations in *Kras* and deletions of the *Ink4/Arf* locus. All these data suggest the Class B