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WNT signaling in stem cell differentiation and tumor formation

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Embryonic stem cells (ESCs) hold great therapeutic promise for the regeneration of functional cell types and clinical applications. However, tumorigenic potential of stem cells in a transplanted host remains a major obstacle. In this issue of the *JCI*, Cui and colleagues identified TCF7-mediated canonical WNT signaling as a critical determinant of both the tumorigenicity and therapeutic function of ESC-derived retinal progenitor cells (ESC-RPCs). Their findings suggested that addressing key extracellular signaling and related intrinsic factors will be essential for the successful use of ESC-derived progenitor transplantation.

Photoreceptor degeneration underlies major causes of blindness, including macular degeneration and retinitis pigmentosa, affecting tens of millions of people worldwide. In theory, vision of affected patients may improve if the diseased cells (rods and cones) are replaced with new, healthy cells that form appropriate connections with the host retina. Embryonic stem cells (ESCs) possess unlimited self-renewal capabilities and the ability to differentiate into any adult cell type (1). These unique features make ESC-based therapy appealing for the treatment of various degenerative disorders but may also cause unwanted serious side effects. It has been previously demonstrated that ESCs or ESC-derived progenitors spontaneously form tumors upon transplantation in vivo, even when the cells are predifferentiated or presorted (2). Despite this issue, successful cell replacement of human ESC–derived (hESC-derived) retinal cells for vision restoration in animal models of photoreceptor degeneration has been reported recently. Photoreceptor precursors derived from hESCs have been shown to migrate into Crx-deficient mouse retina following intraocular injection, express appropriate markers for both rod and cone photoreceptors, and subsequently restore some light responses (3). In another study, subretinal transplantation of hESC-derived retinal pigment epithelium in patients with Stargardt's macular dystrophy and dry age-related macular degeneration improved visual acuity, with no signs of hyperproliferation or tumorigenicity after 4 months (4). These examples illustrate purity, stability, and proper localization of transplanted cells in vivo and prompted the development of numerous differentiation protocols. Still, the risk of tumor formation remains a barrier, because there are no parameters to quantify safety factors and because the appropriate stage of differentiation at which ESC-derived progenitors should be used has not been well evaluated. In this issue, Cui and colleagues attempted to address the above concerns by identifying major extracellular signaling and intrinsic factors controlling tumorigenicity and therapeutic potential of ESC-derived retinal progenitor cells (ESC-RPCs) (5).

ESC tumorigenic potential

The best proof of pluripotency of ESCs is their ability to form teratomas in which the stem cells differentiate to various tissue types of the embryo in a disordered fashion following transplantation into immunosuppressed mice. Teratomas usually contain all three germ layers and have typical tumor characteristics (6). In other words, ESCs are naturally tum-

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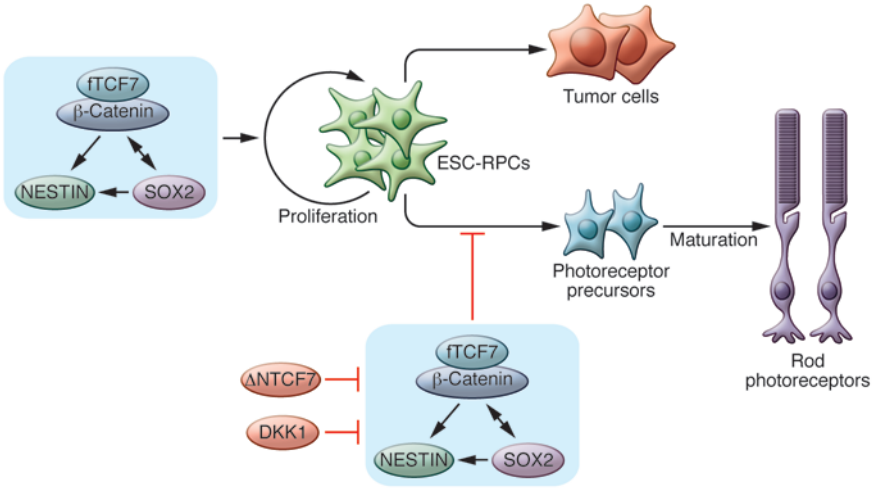

Figure 1
Canonical WNT signaling determines the proliferation and differentiation state of ESC-derived progenitors via full-length TCF7 (fTCF7). SOX2 and NESTIN act as direct targets of fTCF7 in vitro. Blocking the pathway by deleting TCF7 or treating cells with the WNT inhibitor DKK1 could reduce tumorigenicity and improve therapeutic efficiency. ΔNTCF7, naturally truncated form of TCF7.

origenic: like cancer cells, they are capable of unlimited proliferation potential and clonal propagation without anchorage dependence. Indeed, tumor formation is commonly considered to be a consequence of hyperproliferation of residual ESCs or precursor cells. Accordingly, purification of tissue-committed progenitor cells from undifferentiated ESCs or removal of undifferentiated ESCs before transplantation becomes a necessary prerequisite in order to reduce the risk of tumor growth. Several strategies have been implemented to address this critical issue, including the selection of specified cells based on their surface markers (e.g., by FACS or magnetic-activated cell sorting), isolation of lineages using genetic manipulation (e.g., lineage-promoter-driven antibiotic resistance), or the knockdown of or pharmacological interference with intracellular signaling pathways that increase tumorigenicity.

Role of WNT signaling in tumorigenesis and differentiation

Cui et al. demonstrated that ESC-RPCs had a high propensity for neural tumor formation following ocular transplantation (rate 60.58%); however, transplantation of primary retinal progenitor cells (P-RPCs) from neonatal mice resulted in efficient integration to host retina with no evidence of tumor development. Transcriptomic profiling revealed increased activity of the WNT signaling pathway in ESC-RPCs compared with that in P-RPCs (5). WNT signaling is one of the key signaling pathways in regulating cell proliferation, motility, and differentiation as well as tumorigenesis, and upregulation of β-catenin, an intracellular mediator of the pathway, is frequently associated with the differentiation potential of ESCs in teratomas (7). To determine whether WNT activation was related to the increased tumorigenicity of ESC-RPCs, Cui and colleagues treated the ESC-RPCs with DKK1, an extracellular inhibitor of WNT signaling, prior to transplantation. Expression profiling revealed that neural progenitor markers and cell proliferation markers were repressed, whereas large number of committed retinal markers were upregulated by WNT inhibition. The pretreatment with DKK1 dramatically suppressed tumor formation and improved integration of ESC-RPCs into the transplanted host retina (5).

Cui et al. further demonstrated that upregulation of the direct transcriptional targets of canonical WNT signaling, NESTIN and SOX2 was critical to the tumorigenicity of ESC-RPCs following transplantation (Figure 1). The authors also confirmed a previous study demonstrating the importance of the WNT pathway in eye development, in which a high percentage of embryonic eye field–specific cells were generated from hESCs by upregulation of DKK1, and the addition of WNT3A and BMP4 to cultures abolished the expression of PAX6 and RAX (8). Notably, a report that WNT/β-catenin signaling promotes dedifferentiation and proliferation of Muller glia-derived retinal progenitors and neural regeneration after damage (9) also suggested that sustained activation of the WNT pathway may maintain the proliferation ability of ESC-RPCs and contribute to the potential of tumor formation upon transplantation.

Differentiation of ESCs into retinal progenitor cells

Despite the concern of tumorigenic potential, stem cell–based therapy still holds the most promise to restore lost vision in patients with retinal diseases, due to our extensive knowledge of retinal development (10). To date, a number of protocols have been developed for differentiation of mature retinal cells, including photoreceptors, by a stepwise treatment with defined factors (3, 8). Notably, the rates of tumor formation and successful host integration vary among different reports, even using a same selective marker. Cui et al. selected SOX1.EGFP–positive neural progenitor cells first and then obtained ESC-RPCs and subsequent photoreceptor precursors by adding small molecule inhibitors. Unlike most other groups, they did not choose DKK1 as a common factor to direct ESCs to retinal progenitors and found a high rate of neural tumor formation following ocular injection as a result (5). Thus, a more in-depth comparison between P-RPCs and ESC-RPCs, such as assessment of epigenetic signatures using genome-wide tools (11), will be required to optimize differentiation conditions and to make progress toward translational applications.

As an alternative to ESC-RPCs or P-RPCs for cell therapy, pluripotent stem cells could be differentiated into more mature, lineage-restricted stem cells, such as neural stem cells, to reduce tumorigenicity (12). However, as Cui et al. discuss, donor cells might lose desired functions and the ability to integrate to host retina by prolonged differentiation. Thus, it will be critical to identify the appropriate stage of ESC-derived cells and ensure the balance between safety and efficiency (13). It remains to be

seen how broadly the approach to retinal progenitor cell differentiation by the WNT pathway modulation can be applied to differentiation of other tissue or cell types. Nevertheless, predifferentiation of ESCs in vitro to a desired cell population before transplantation both to ensure efficiency of transplantation as well as to minimize the risk of tumor formation may be a good general strategy in stem cell therapy.

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Striking the target in iron overload disorders

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The liver, a major site of body iron stores, mediates key responses that preserve systemic iron homeostasis. In this issue of the *JCI*, Guo et al. demonstrate that administration of antisense oligonucleotides that reduce expression of Tmprss6, a hepatic protein that plays an essential role in maintaining iron balance, can attenuate disease severity in mouse models of human iron overload disorders. These data reveal the potential of novel TMPRSS6-targeted therapies for the treatment of clinical conditions such as hereditary hemochromatosis and β-thalassemia.

Hepcidin and the regulation of systemic iron balance

The majority of iron required daily by the adult human body is used to meet the demands of hemoglobin synthesis. Most of this iron is obtained through the recycling of senescent erythrocytes by macrophages in the spleen, liver, and bone marrow, while a small amount is absorbed from the diet in the duodenum. Hepcidin, a small circulating peptide released by the liver, regulates iron balance by limiting both the absorption of iron from the diet and the release of iron from macrophage stores (1). Hepcidin mediates these effects by triggering the internalization and degradation of ferroportin, a cellular iron exporter that is highly expressed at the basolateral membrane of enterocytes and the cell membrane of macrophages (Figure 1). In hepatocytes, hepcidin transcription is modulated by an intracellular signaling cascade that is activated by binding of BMP ligands to a cell-surface receptor complex (Figure 2). The liver, a major site of iron storage, increases production of the BMP family member BMP6 in response to rising local iron stores; this leads to increased signaling for hepcidin production, which in turn limits further dietary iron absorption. Appropriate regulation of intestinal iron absorption is critical, as there is no regulated mechanism for eliminating surplus iron from the body.

Hepcidin insufficiency in iron overload disorders

Inherited forms of iron overload (hemochromatosis) result from mutations in gene products that are required locally in the liver for hepcidin production. In these disorders, the resulting hepcidin insufficiency leads to gastrointestinal iron absorption that exceeds the body's needs. The accumulation of excess iron promotes oxidative damage to tissues, which can ultimately lead to failure of organs such as the heart, liver, and endocrine glands. Hepcidin levels are inappropriately low relative to body iron stores in another class of clinical disorders associated with systemic iron loading: congenital anemias that are characterized by ineffective erythropoiesis (IE) (2). IE describes a defective form of erythroid maturation characterized by an increased proportion of erythroid precursors, which, due to excessive apoptosis, fail to produce the normal complement of mature erythrocytes. In β-thalassemia, the most common inherited form of IE, the primary genetic defect leads to reduced synthesis of the β-globin component of adult hemoglobin. The result is an excess

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