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SOX2 for Stem Cell Therapy and Medical Use: Pros or Cons?

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Abstract

Stem cell transplantation is a fast-developing technique, which includes stem cell isolation, purification, and storage, and it is in high demand in the industry. In addition, advanced applications of stem cell transplantation, including differentiation, gene delivery, and reprogramming, are presently being studied in clinical trials. In contrast to somatic cells, stem cells are self-renewing and have the ability to differentiate; however, the molecular mechanisms remain unclear. *SOX2* (sex-determining region Y [SRY]-box 2) is one of the well-known reprogramming factors, and it has been recognized as an oncogene associated with cancer induction. The exclusion of *SOX2* in reprogramming methodologies has been used as an alternative cancer treatment approach. However, the manner by which *SOX2* induces oncogenic effects remains unclear, with most studies demonstrating its regulation of the cell cycle and no insight into the maintenance of cellular stemness. For controlling certain critical pathways, including Shh and Wnt pathways, *SOX2* is considered irreplaceable and is required for the normal functioning of stem cells, particularly neural stem cells. In this report, we discussed the functions of *SOX2* in both stem and cancer cells, as well as how this powerful regulator can be used to control cell fate.

Keywords

SOX2, cell transplantation, stem cells, cancer, stemness

Introduction

Stem cell transplantation is a well-established technique that has been part of the clinical treatment strategies for both malignant (e.g., acute myeloid leukemia and Hodgkin's lymphoma) and nonmalignant (e.g., thalassemia and sickle cell anemia) diseases and disorders since 1959[1]. In response to the increasing medical need, stem cells are now being isolated and transplanted from a variety of sources, including umbilical cord blood, placenta, amniotic fluid, dental pulp, and adipose tissue[2–4]. In addition, the discovery of induced pluripotent stem cells (iPSCs) has dramatically broadened the field[5]. These improvements are a reflection of the unmet medical need of regenerative medicine and the limitations of drugs and medical devices[6]. Despite organ transplantation having been successfully performed for the heart, kidney, liver, lung, pancreas, intestine, and thymus, organ sources are in very limited supply[7,8]. Even if a patient receives an organ and the transplantation is considered a success, the patient is required to take antirejection drugs, which have the risk of severe side effects. As such, the development of artificial organs may represent a promising solution; however, effective and efficient techniques for tissue engineering pose numerous challenges[9].

Stem cells and/or progenitor cells may be a good choice for compensation of the functions of target tissues and for the secretion of appropriate cytokines and growth factors, including those that are considered immunomodulatory[10–12]. However, the majority of studies have revealed that delivered cells rarely transdifferentiate into their target type and the survival time remains insufficient[13,14]. Nonetheless, while the mechanisms remain unclear, most experimental and

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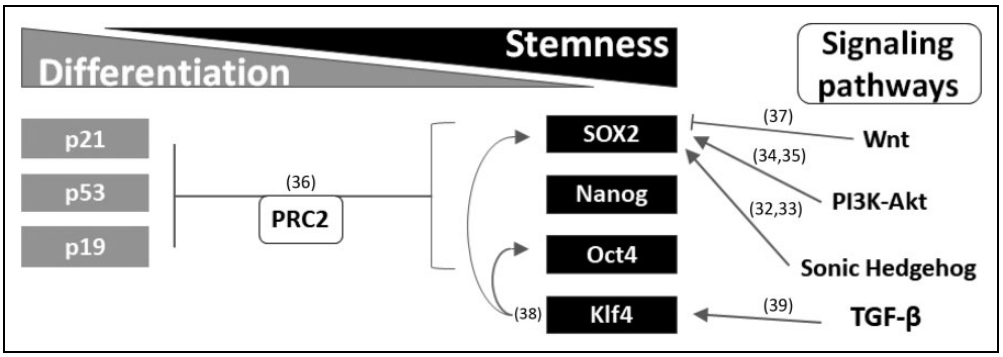

Fig. 1. Molecular interactions illustrate various SOX2 signaling partners.[32–39]

clinical results have shown positive results[15–17]. As such, the technologies underlying stem cell therapy continue to improve regarding cellular function and survival duration. The most important breakthrough has been the reprogramming of somatic cells into pluripotent stem cells with the exogenous expression of certain transcription factors[18]. The potential applications of stem cells, including cellular transplantation and organ development, have been tremendously enhanced after the discovery of iPSCs[19,20].

As a member of the *SOX* gene family and *SOXB* group, which includes *SOX1*, *SOX2*, and *SOX3*, *SOX2* (sex-determining region Y [*S*RY]-b*ox* 2) encodes a 34.3 kD protein[21]. As a key regulator of self-renewal, SOX2 protein binds to octamer-binding transcription factor 4 (Oct4) and enhances the expression of Nanog[22,23]. However, Tanaka et al. indicated that SOX2 is unnecessary as an enhancer, suggesting that it modulates the expression of Oct4[24–26]. The coupling of SOX2 to paired box protein 6 (PAX6) and BRN2 (encoded by *POU3F2* in humans) has been shown to regulate eye and neural primordial cell functions[27]. Interestingly, SOX2 and/or the partner protein are not considered sufficient for transcriptional activation, but this complex is[28]. Once the complex is formed, downstream genes such as undifferentiated embryonic cell transcription factor 1 and fibroblast growth factor 4 activate and enhance embrionic stem cell development and survival[29]. Accordingly, the knockdown of *Sox2* expression in mouse embryonic stem cells (ESCs) results in the failure of this self-renewal property and leads to differentiation[22]. In contrast to tumorigenesis, the expression level of SOX2 correlates with lower survival and treatment resistance[30]. Therefore, we evaluated the relationship between SOX2 and its functions in both stem and cancer cells and discovered a potential approach for improving stem cells and deteriorating cancer cells.

SOX2 Is Associated With an Enormous Expression Network

The characteristics of stemness are associated with the target genes of SOX2. In addition, stem cells possess regulatory mechanisms to maintain the appropriate expression of SOX2. For mouse ESCs, the exogenous elevated expression of *Sox2* leads to differentiation of ESCs into a wide range of cell types, including neuroectoderm, mesoderm, and trophectoderm (TE)[31]. Moreover, feedback regulation involved in the Akt pathway reactivates endogenous Sox2 expression and serves to retain cellular stemness (Fig. 1)[40]. However, in comparison with iPSCs, the expression of SOX2 is artificial and lacks interactive control. Nevertheless, to reprogram cells into iPSCs, four genes, namely, Oct4, Klf-4, SOX2, and c-Myc (abbreviated to OKSM), are exogenously activated and these genes need a specific ratio to work adequately. Since the OKSM is necessary for pluripotency, other accessory factors such as Nanog and Sal-like protein 4 can only increase the efficiency of reprogramming and cannot replace SOX2 or OCT4[41,42]. For example, a ratio increase of Klf4 is recommended in one of the commercial cellular reprogramming kits. Moreover, the expression of SOX2 is activated by the VP16 transactivator and further improves reprogramming efficiency[43]. These findings indicate that the OKSM acts as a driving force in the fertilization stage and should be tightly restricted or the cells may get out of control. Thus, the upstream and downstream regions of the *SOX2* coding sequence contain large untranslated regions (UTRs) or the so-called gene deserts to prevent mutations and false binding[44]. Certain enhancers, including miRNA, long-noncoding RNA, and posttranslational modification have been shown to reversely regulate both transcriptional and posttranslational activity. At least 19 miRNAs, including miR-200b and miR-145, can influence the expression of SOX2[45]. Indeed, miR-200 facilitates the reprogramming of fibroblasts into iPSCs in the presence of OKSM[46]. Moreover, miR-145 targets the 3′-UTR of SOX2 and inhibits self-renewal in human ESCs and iPSCs[47]. In other words, simply elevating the expression of SOX2 serves to attract negative regulation molecules such as the cyclin-dependent kinase inhibitor 1A ($p21^{Cip1}$)[48]. Conversely, the zygote has a massive demethylation pattern compared with reprogrammed cells[49]. Although existing techniques, including single-cell RNA-Seq, methylation array, and bioinformatics, have confirmed that the methylation state changes in zygotes

and somatic cells, the secrets of dedifferentiation remain a mystery[50].

SOX2 Utilization in Stem Cell Therapy

Except for the stemness regulation of *SOX2*, the existence for cell differentiation and development is necessary. Notably, in the development of ESCs, when the blastocyst is formed and then divides into the TE and inner cell mass, the expression of SOX2 is decreased. However, the TE will not form if *SOX2* is impaired or knocked down by siRNA[51]. This change is due to the complex formed with Oct4 and Nanog. For example, Oct4 and Nanog bind to *SOX2* and regulate its functions of self-renewal and differentiation inhibition[52]. In adult humans, the olfactory nerve proliferates and is replaced every 3 to 4 weeks. The SOX2/PAX6-expressed epithelium plays an important role in maintaining the multipotency of the olfactory nerve[53]. These findings suggest further applications in the transplantation from iPSC-differentiated neural stem cells (NSCs). In particular, the in vitro-transcribed mRNA of *SOX2* has been shown to induce NSC morphology in human dermal fibroblasts[54]. In addition, another study revealed that exogenous Sox2 expression in rat bone marrow–derived stem cells (BMSCs) benefits the cell transplantation treatment in a rat traumatic brain injury (TBI) model[55]. Especially, BMSCs retain their self-renewal property via the expression of Sirtuin1 (SIRT1)[56]. SIRT1 is a lysine deacetylase that contributes in maintaining SOX2 content by avoiding the acetylation and ubiquitination of SOX2[57]. Moreover, proliferation and differentiation potential is conferred by the forced SOX2 expression of BMSC[58]. Using MRI tracking, Jiang et al. found that NSCs migrate into the injury site of rats with TBI[59]. Therefore, the existence of SOX2 is essential for the maintenance of self-renewal and multipotency. These studies suggested that Sox2-positive cells may play a role in neuron regeneration, enhancing neural functions after brain injury[60].

Direct Evidence of *SOX2* Initiating Tumorigenesis

SOX2 is generally considered an oncogene; however, its role in tumorigenesis remains controversial[61,62]. As part of the same lineage of breast cancer cells, the *SOX2*-positive population shows a greater colony-forming ability and would be abolished by *SOX2* knockdown[63,64]. *SOX2* is amplified in patients with cancer, and it contributes to the same stemness property observed in stem cells of patients with lung, brain, breast, and colon tumors[65].

The clinical implications of SOX2 and cancers vary depending on the type of cancer, influencing patient survival and prognosis[66]. These molecules and pathways include VEGF, MAPK, Notch-Shh, BMP, Jak-STAT, and others, depending on the types of tumors[67–69]. In brief, SOX2 regulates downstream genes and microRNAs by direct DNA binding, resulting in the alteration of thousands of genes and hundreds of microRNAs[35]. Moreover, a glioma cell subset with high levels of *SOX2* has been shown to be resistant to platelet-derived growth factor (PDGF)- and insulin-like growth factor 1 (IGF-1)-receptor inhibitors[70]. Conversely, *SOX2* may play a role in the maintenance of PDGF and IGF-1 pathways in cancer cells as well as in stem cells and may produce dysplasia or tumor cell initiation. However, in patients with gastric cancer, the overall survival rate is lower with *SOX2* methylation than with unmethylated *SOX2*. Moreover, the exogenous expression of *SOX2* results in cell cycle arrest through cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$) and Rb phosphorylation[71]. Indeed, *Sox2* expression in the mouse respiratory epithelium does not cause pulmonary tumors but induces the cellular proliferation of respiratory epithelial cells[72]. Direct induced-NSCs can also be obtained by *SOX2* expression in human and mouse fibroblasts without tumorigenesis[73].

Future Prospects of SOX2 Utilization

Due to the close relationship between *SOX2* and cancer, studies that have investigated *SOX2*-dependent gene manipulation are limited. However, cell differentiation and proliferation has been achieved, including *SOX2*-expressing dental pulp stem cells, which lead to the differentiation of odontoblasts[74]. *SOX2* also cooperates with various cofactors, including Oct4 for stemness, BRN2 for neural differentiation, and PAX3 for melanocyte maturation. These studies suggested that *SOX2* (and possibly other members in the family) is one of the masters regulating cell fate and is associated with different kinds of cofactors[75].

An urgent question we would ask is how to take advantage of *SOX2* without eliciting detrimental effects? For the nerve system, *SOX2* may prove to be a useful regulator for the maintenance of progenitor characteristics, allowing the cells to retain their ability to self-renew and differentiate into neurons, astrocytes, and oligodendrocytes[76,77]. Moreover, Hagey and Muhr found that decreased expression of *Sox2* resulted in the differentiation of radial glia cells into their more developed progeny, intermediate progenitor cells[78]. However, mouse NSCs and progenitors have reduced differentiation ability and *SOX2* expression with the loss of the E2f3a transcription factor, indicating that the expression of *SOX2* is essential for neurogenesis[79]. These studies indicated that *SOX2* is required for stemness but is unfavorable for differentiation. In other words, the expression of *SOX2* should be controlled or there is a risk of the cells going corrupt.

Conversely, the *SOX2*-induced stemness ability is devastating and problematic when it appears in tumor cells. Since the existence of *SOX2* is associated with the cell membrane, specific antibodies are not effective for disrupting its function. To control the stemness and metastatic properties, Tuhin et al. found that the downregulation effect of actinomycin D induced the cell death of breast cancer stem cells[80]. Moreover, *SOX2* knockdown or small molecules reduced

Table 1. A Summary Table of SOX2 Potential in Medical Use and Their Main Concerns for Cancer Therapy and Stem Cell Transplantation.

Potential in Medical Use	Main Concerns	Ways to Breakthrough
Manipulate cell fate	The complicated upstream and downstream effectors of SOX2	Use the necessary domain of SOX2[66]
Prolong stem cell lifespan during transplantation	Risk of neoplastic or teratoma growth	Use an additional molecular beacon that indicates abnormal SOX2 expression[89]
Retains stem cell potential	May be involved in tumorigenesis	The development of tumor-specific siRNA or antibodies[90]
Target as a tumor marker	Constant expression in normal cells	

SOX2 expression and inhibited the stemness and metastatic properties[61,81]. A significant factor in patients with cancer is the selection of *SOX2*$^+$ cells after anticancer therapies, including radiotherapy and chemotherapy, and *SOX2*-induced drug-resistant genes have been characterized in numerous studies[82,83]. These *SOX2*$^+$ cells then form a new tumor bulk, with the most well-studied type being the quiescent Sonic Hedgehog subgroup medulloblastoma, which is activated into medulloblastoma-propagating cells after antimitotic drug treatment[84]. Moreover, the inhibition of the *SOX2*-driven transcriptional network arrests GBM growth by treatment with mithramycin, which is an antineoplastic antibiotic[85]. Indeed, SOX2 antigen and antibody were found in small-cell lung cancer (SCLC) cell lines and sera in SCLC patients. However, neither the antigen nor the antibody of SOX2 or other SOX group B genes exist in normal sera, suggesting that SOX2 might be a potential tumor target or marker[86]. However, these effects should be precisely targeted toward tumor cells to take advantage of the attributes of *SOX2*.

Summary

With the use of iPSC-derived retinal pigment epithelial cells transplanted in clinical trials, a large research and development effort has been undertaken to not only evaluate the effectiveness and safety but also improve the associated techniques[87,88]. In this report, we discussed the stemness-prone properties and some possible risks of *SOX2* in Table 1. In addition, the safety issue in iPSC-derived cell therapy is the most important topic; therefore, Larsson et al. established a molecular beacon to compliment SOX2 mRNA, displaying the fluorescent signal when SOX2 is expressed[89]. Although the interaction networks remain incomplete, reactions with different expression levels of *SOX2* are more apparent than ever. In summary, the potential applications of *SOX2* are extremely promising but precise targeting and expression in the right place and at the right dosage are crucial.

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Author Contributions

Writing—original draft preparation, HM Chuang; writing—review, MS Huang and YS Chen; conceptualization, HJ Harn.

Declaration of Conflicting Interests

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