Regulation of Normal Somatic Cell and Cancer Cell Reprogramming by p53

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Abstract

Reprogramming healthy somatic cells into disease-relevant cell types through cellular reprogramming has been intensively investigated. The discovery of reprogramming methods holds the promise of generating desired cells for disease modeling, drug screening studies and treatment of numerous diseases. Recently studies also focus on different disease cell reprogramming, including cancer cell reprogramming. Reprogramming and tumorigenesis share many similarities and the tumor suppressor p53 suppresses both processes. Understanding of the roles of p53 in somatic cell and cancer cell reprogramming could illuminate molecular mechanisms underlying the pathogenesis of human cancer and develop novel strategies for cell replacement therapy and cancer treatment.

Keywords

p53, Reprogramming, Cancer, iPS

Introduction

The ability to convert somatic cells into disease-relevant cell types through cellular reprogramming has opened new doors for basic research and regeneration medicine1. In the past, there were no reliable methods for converting somatic cells into another type of cells. Takahashi et al. demonstrated that defined factors could drive skin-derived fibroblasts to induced pluripotent stem (iPS) cells that could be further differentiated into the desired cell type [1].

Reprogramming healthy somatic cells into iPS cells with defined factors has been intensively investigated. However, reprogramming cancer cells has comparatively been lagging behind [2-4]. Direct reprogramming cancer cells into normal cells is an innovative strategy for cancer treatment. Recent reports have showed that defined transcription factors can induce reprogramming of cancer cells into iPS cells, supporting this notion [2-4]. The tumor suppressor p53 is considered today the most important tumor suppressor gene in humans [5,6] and its inactivation or mutations are the most common in cancers. Reprogramming and tumorigenesis are stepwise processes that share many similarities and p53 suppresses both reprogramming and tumorigenesis [2-4,7,8]. In this review, we discuss the roles of p53 in normal somatic cell and cancer cell reprogramming (Figure 1).

Figure 1: Schematic drawing of a proposed model for effects of p53 on somatic cell and cancer cell reprogramming.

p53 Regulates Pluripotency Reprogramming

Differentiated somatic cells have been reprogrammed to a pluripotent state by forced expression of a set of transcription factors [1], indicating that terminally differentiated cells can be induced to undergo cell fate change. p53 reduces cancer initiation by regulating apoptosis, DNA repair, cell cycle and senescence, contributing to its main role as the “guardian of the genome”. Recent studies show new roles of p53 in a wide range of processes, including cell self-renewal, differentiation, and cell fate decisions [9-11]. Several lines of evidence suggested that p53 governs the quantity and quality of various stem cells through regulation of self renewal ability and differentiation. One example is that the p53 protein can be phosphorylated and suppress the transcription of Nanog, leading to stem cells to lose self renewal potential and then differentiation [12]. Many groups reported that p53 has been shown to inhibit reprogramming of fibroblasts to iPS cells [7,8,13-17]. Yamanaka et al. found that c-Myc induces p53-dependent apoptosis in fibroblasts, leading to a reduced rate of reprogramming [16]. Zhao et al. showed that deletion of p53 combined with overexpression of Utf1 dramatically increased in iPS cell formation [17]. Other groups also provided strong evidence that p53 serves as a potent barrier to somatic cell reprogramming and dramatically reduces the efficiency of dedifferentiation [12-14].

Mechanistically, the overexpression of exogenous transcription factors is thought to activate p53, which might in turn lead to cell cycle arrest and apoptosis. In addition, during pluripotency induction,
cells presenting with DNA damage and chromosomal abnormalities will be excluded from becoming iPSCs. A report showed that p53-mediated DNA damage response limits reprogramming to ensure iPSC cell genomic integrity [8]. Another report showed that in the absence of p53, cells with a defective DNA repair pathway could undergo reprogramming, allowing the generation of iPSCs with genomic instability [19]. Hanna et al. showed that an additional role of p53 in reprogramming may be an indirect effect on cell proliferation [20]. Sarig et al. examined the role of p53 mutant in pluripotency induction and found that mutant p53 has a gain-of-function in reprogramming. p53 mutant mouse embryonic fibroblasts (MEFs) were reprogrammed more efficiently than MEFs derived from both wild-type and p53 knockout mice, indicating that mutant p53 actually increases reprogramming efficiency beyond that facilitated by the absence of p53 alone. Thus, these reports suggest that suppressing p53 regulates apoptosis, DNA repair, senescence, and cell cycle, thereby increasing reprogramming efficiency.

p53 Regulates Direct Lineage Reprogramming

The field of direct somatic lineage conversion bypass iPSCs has already attracted much attention. Wernig’s group demonstrated that a set of neural factors can directly convert fibroblasts into neurons [21,22]. Several laboratories have used various neural factors and microRNAs to generate fibroblast-neuron conversion [23-30]. Furthermore, recent studies have reduced the numbers of transcription factors required for neuronal trans-differentiation by including small molecules to promote neuronal phenotypes [31,32]. Direct fibroblast-neuron conversion provides an alternative, potentially complementary, tool to many of the proposed applications of iPSC technology for both disease modeling and development of cell-based therapies. This approach has a number of advantages, including: the time required to generate, expand and differentiate pluripotent cells is avoided, and the postmitotic state of induced cells has a much lower risk of cancer and teratoma formation.

Delineating the molecular mechanism behind somatic cell reprogramming will greatly aid further development of the method. In our recent studies, we have demonstrated that suppression of p53 or cellular senescence is a key step in the direct human somatic cell reprogramming [33,34]. We found that inhibition of p53 efficiently induces conversion of fibroblasts to neurons without introduction of any transcription factors. Knockout or knock-down of p53 efficiently reprogrammed fibroblasts into tri-neural cells by regulation of a set of neural transcription factors. The induction was specific to p53 depletion, because overexpression of wild-type p53 in cells expressing p53 shRNA essentially abolished fibroblast-neuron conversion. Furthermore, we found that mutant p53 lost the inhibitory function on reprogramming. Overexpression of a p53 mutant, R273H, in which Arg 273 in the DNA binding domain was replaced with His [35-37], did not affect the induction time course, indicating that this p53 mutant has lost the inhibitory function in reprogramming. In addition, we provided a new method to convert most fibroblasts to neurons within only a week of time. Moreover, the defined transcription factors failed in inducing fibroblast-neuron conversion in late passage fibroblasts, while depleting p53 is advantageous in inducing neuron in both early and late passage fibroblast cells.

p53 Regulates Cancer Cell Reprogramming

One critical question in cancer research is: are cancer cells capable of reprogramming from their malignant state? The iPSC reprogramming strategy might provide an opportunity that human cancer cells can be similarly reprogrammed and subsequently differentiated with loss of tumorigenicity. Although unidentified biological barriers may exist [38-40], reprogramming of both solid and liquid tumors to iPSC cells has been reported by different groups [39,41-50]. Loss of tumorigenicity by unknown mechanisms and induced de-differentiation to pluriotyposity seem to be common features of reprogrammed cells from different cancers [51-55]. Zhang et al. found that reprogramming with defined factors induces sarcoma cells to lose tumorigenicity, come back to a “normal state” and undergo differentiation into different terminal “normal” cells [41]. The epigenetic regulation of oncogene c-Myc might have a key role in the process of reprogramming sarcoma cells. These studies may, therefore, provide a novel strategy for cancer treatment via pluriotyposity induction.

Because most cancers have shown the suppression of the p53 signaling pathways, one would expect accelerated cancer cell reprogramming with p53 inactivation. Studies on the link between p53 and cancer cell differentiation were first published about 20 years ago [56-59]. Those reports showed that the de-differentiated phenotype of cancers correlates with p53 loss and increased tumorigenesis. Recently, Telerman et al. found that p53 plays a key role in cancer cell reprogramming through interaction with TPT1/TCTP encoding translationally controlled tumor protein [60,61]. They have previously established cancer reversion models and identified ~300 genes putatively implicated in reversion, including TPT1/TCTP [62]. TPT1/TCTP regulates the p53-MDM2-Numb axis in cancer cell programming. Another study showed that gain-of-function of mutant p53 enhances reprogramming efficiency and the Myc pathway cooperates with a p53 mutant protein to disrupt the efficiency of reprogramming [63]. Using a temperature sensitive mutant of the p53 gene, Levine and colleagues examined the impact of the temporal expression of wild type p53 in preventing stem cell induction from somatic and cancer cells [63]. Reactivation of p53 during the reprogramming process not only interrupted the formation of iPSCs, but also induced newly formed stem cells to differentiate. Moreover, p53 mutants showed differential effects on the stem cell reprogramming efficiency in a Myc dependent manner. Although both responded to the inhibition of reprogramming by the p53 protein, somatic cells and cancer cells are different from each other in several ways.

Possible Mechanisms of p53 in Reprogramming

Although current studies suggest that p53 regulates apoptosis, DNA repair, senescence, and cell cycle in reprogramming, the mechanisms by which the p53 protein inhibits normal and cancer cell reprogramming are largely unknown. To detect the p53 signaling pathway, Levine and colleagues found that p21 (Cdkn1a), but not Puma (Bbc3) played a partial role in iPSC cells formation probably by slowing cell division [63]. They also found that activation of p53 functions in iPS cells induced senescence and differentiation in stem cell populations. To examine epigenetic alterations of DNA, they found increases in DNA methylation at the IGFl2-H19 loci with high rate of birth defects in female offspring of p53 knockout mice, indicating that p53 knockout mice would display epigenetic alterations during embryonic development and thus resulted in birth defects. These results suggested that a portion of p53 ability to inhibit reprogramming may come from its influence on preventing epigenetic changes.

p21 is one of the most prominent targets of p53 involved in regulation of cell-cycle [64,65] and iPSC reprogramming [9,13,15]. To determine whether p53 depletion induced fibroblast-neuron conversion by inhibiting the p53-p21 signaling pathway, we inhibited p21 and did not observe neuronal morphology in shp21-expressing cells [34]. Furthermore, co-expression of shp21 or wild-type p21 with shp53 did not affect shp53-induced fibroblast-neuron conversion in IMR90 cells. These data indicate that p21 is not involved in shp53-induced fibroblast-neuron conversion.

Global gene expression analysis reveals that the gene profiling in induced cells is significantly changed, compared with that in the parental fibroblasts [33,34]. Moreover, p53 plays a key role through regulating a set of transcription factors during the reprogramming process, supporting the notion that p53 may act as a “master switch” in reprogramming. We found that p53 bound to the promoter DNA of neurogenic transcription factor Neurod2 and regulated its expression during fibroblast-neuron conversion [34]. Telerman et al. found that TPT1/TCTP and p53 are involved in a reciprocal negative-feedback loop in cancer cell reprogramming [60,61]. TPT1/TCTP inhibits...
MDM2 auto-ubiquitination and promotes p53 degradation by competing Numb for binding to p53-MDM2-containing complexes. In addition, p53 directly represses TPT1/TCTP transcription. Since TPT1/TCTP is a highly significant prognostic factor in breast cancer, targeting TPT1/TCTP in cancer cell reprogramming could open a new route to cancer treatment.

Lineage conversions between very distantly related cell types might involve two main steps: 1) reprogramming of prior donor cells into induced progenitors, which might not pass through a pluripotent state; 2) subsequent re-differentiation into a complete and functional lineage. Depletion of p53 enhanced reprogramming suggests that p53 may inhibit both steps (Figure 1). Consistent with this view, inhibition of p53 enhances both iPS cell reprogramming and neural conversion [33,34], implicating a general mechanism of reprogramming where inhibiting p53 may generate and develop complete and functional lineages. One hypothesis is that depletion of p53 prevents loss of proliferative potential in cell cycle-arrested cells and that it can therefore transform irreversible arrest into a reversible condition. When the cell-cycle is arrested by neural induction medium (for fibroblast-neuron conversion) or reprogramming factors (for iPS cell formation), in the absence of p53, the cells remain quiescent but not senescent because they retain the capacity to resume proliferation. Under defined conditions, the cells are reprogrammed into progenitors and sequentially re-differentiated into neural lineages or continue to reprogram into iPS cells.

Concluding remarks

Somatic cell conversion by introduction of defined factors might have significant implications for understanding critical processes for development, in vitro disease modeling and cell replacement therapies, although generally with low percentages and very slow time course of reprogramming [66-68]. Understanding the role of p53 in reprogramming may have wide-spread impact on our understanding and development of cell replacement therapy. Although the link between p53 and tumorigenicity makes using the induced cells lacking p53 impractical, treating cells during reprogramming with reversible compounds to promote immortalization transiently is feasible. In the future, testing compounds that block p53 downstream factors for cell reprogramming may be a novel approach for maximizing the generation of “safe” cells for clinical applications, including for old people who may suffer more disorders. We believe that the p53 pathway will be a key process to target in pursuit of finding more efficient strategies for generating desired cells.

Reprogramming and tumorigenesis are connected processes that share many similarities. Targeting the p53 pathways underlying the direct reprogramming of cancer cells opens new perspectives for understanding of mechanisms of cancer pathogenesis and development of an alternative approach in cancer treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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