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(REVIEW ARTICLE)

A review on the molecular basis of stemness of mesenchymal stem cells

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Abstract

Mesenchymal stem cells (MSCs) are increasingly becoming the focal point of research in regenerative medicine due to their potential to differentiate into several different lineages including those of non-mesodermal origin. With several reports of successful differentiation of MSCs into several different cell types such as osteocytes, chondrocytes, cardiocytes and even fully functional neurons, it is more important than ever to understand the stemness of these cells at a molecular level. While no stemness gene has been identified that is unique to MSCs, there have been speculations that the stemness of MSCs is maintained similarly to that of Embryonic Stem Cells (ESCs). This review takes a look at four genes, namely, NANOG, OCT4, SOX2 & KLF4, and their role in maintaining the stemness of MSCs. These genes are significant for the stemness of ESCs & therefore, could play a role in MSCs as well.

Keywords: Mesenchymal Stem Cells; NANOG; OCT4; SOX2; KLF4; Stemness; MSCs

1. Introduction

Mesenchymal stem cells are multipotent stem cells of mesodermal origin. They are characterized by three properties: i) cells should adhere to plastic; ii) cells should be able to differentiate into chondrocytes, osteocytes & adipocytes; iii) MSCs express cell surface markers like the cluster of differentiation CD29, CD44, CD73, CD90, CD105 and lack the expression of CD14, CD34, CD45 and HLA (human leukocyte antigen)-DR. MSCs were first discovered in the bone marrow and then several other sources such as adipose tissue, dental pulp and umbilical cord were also discovered. MSCs have been in clinical trials for treating several diseases as well as injuries such as bone restructuring, cardiovascular repair and nervous system repair. Although several studies have been conducted, very little is known about the molecular mechanism of MSC stemness. No unique transcription factor has thus far been identified that is unique to MSCs. This review will take a look at NANOG, OCT4, SOX2 & KLF4, four regulators of stemness in ESCs, and their role in MSCs [1]–[3].

2. Molecular Mechanism of Stemness

2.1. NANOG

NANOG is derived from "Tír na nÓg" of Celtic mythology which stands for "Land of the Ever Young" [4]. This is in reference to the role of the gene in maintaining the undifferentiated state of stem cells. The human NANOG gene is 305 amino acids long and contains three functional domains, namely, the homeodomain, the N-terminal domain and the C-terminal domain. NANOG is an essential transcription factor that is partly responsible for the maintenance of self-renewal and the undifferentiated state of stem cells. NANOG along with OCT4 & SOX2 is responsible for the upregulation of self-renewal and pluripotency-related genes and the downregulation of differentiation-related genes [5].

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The exact mechanism of NANOG-induced self-renewal and maintenance of an undifferentiated state is yet to be fully elucidated. There are major differences in the targets of NANOG between MSCs and ESCs which could be attributed to the difference in the epigenetics of the two cell types. NANOG regulates a unique set of genes in different cell types thus having a tissue-specific effect. This could suggest that NANOG is involved in more than just the maintenance of pluripotency and self-renewal [6].

A recent study established that the knockdown of NANOG resulted in reduced cell proliferation and induction of G0/G1 cell cycle arrest in MSCs. The paper found that NANOG knockdown resulted in the reduction in p53 expression which increased p21 & p27 levels resulting in cell cycle arrest. Also, NANOG resulted in the reduction in DNMT1 levels, which acts as a regulator of p21 & p27. NANOG knockdown also resulted in the reduction of PREF1 which is a marker for adipose mesenchymal stem cells [7].

Another study attempted to reverse the effects of ageing on MSCs by ectopic expression of NANOG. MSCs from older donors usually display reduced proliferative myogenic differentiation potential. NANOG when expressed ectopically upregulated genes of the cell cycle, DNA damage repair and replication. NANOG also significantly increased the proliferation capacity of the cells. This is especially helpful for elderly patients that suffer from diseases that can be treated with MSC therapy [8]. Ectopic expression of NANOG also improved the contractile function of senescent MSCs in three-dimensional microtissues. NANOG reversed the effects of stem cell senescence as well as restored the pluripotency and self-renewal capacity of senescent MSCs [9]. The molecular basis of restoring the self-renewal capacity of senescent MSCs was established by a study done in 2019. NANOG upregulates PBX1 which in turn increases the activity of the PI3K/AKT signalling pathway. The PI3K/AKT signalling pathway regulates cell proliferation, DNA repair & senescence [10].

2.2. OCT4

OCT4 is an octamer-binding transcription factor encoded in humans by the gene POU5F1. It consists of an octamer motif which binds for activating or deactivating the target genes [11].

There are conflicting reports about whether OCT4 is expressed in MSCs or not. Some studies suggest that it is expressed, albeit in low concentrations [12], [13], while others claim that it is not expressed [14], [15].

Regardless, ectopic expression of OCT4 results in the enhanced proliferation of MSCs as was seen in a study conducted in 2014. The cells saw an increase in the levels of cyclins that are responsible for cell proliferation [16]. Another study found that ectopic expression of OCT4 results in the upregulation of NANOG & SOX2. This could explain the molecular mechanism behind the increased proliferative & differentiation capacity of the cells [17].

Similar to NANOG which affects p21 through DNMT1, ectopically expressed OCT4 can also improve the self-renewal capacity of MSCs through DNMTs. OCT4 upregulated DNMTs which in turn methylated the promoters of p21 resulting in its inhibition. This resulted in the reversal of cell senescence and restored its self-renewal capacity [18]. β-catenin has been found to regulate OCT4. A 2022 paper established that β-catenin increased the survival of MSCs and promoted angiogenesis in the case of stem cell therapy for cardiac diseases. β-catenin positively regulates OCT4 which in turn regulates genes such as Ang1, bFGF, HGF, VEGF, Bcl2, and surviving which are responsible for the survival and angiogenesis of MSCs [19].

2.3. SOX2

SOX2 belongs to the highly conserved Sox family of transcription factors that play key roles in mammalian development. The Sox family was first discovered in 1990 during the discovery of the mammalian testis-determining factor 'Sry' [20]. SOX2 is the third element in the core transcription factors that govern the pluripotency of ESCs. SOX2 is activated by KLF4 (another transcription factor) as a result of the JAK-STAT signalling pathway [21]. SOX2 binds to several regions in the DNA using the HMG domain at the minor groove. This is in juxtaposition from OCT4 which binds to the major groove [22], [23].

Interestingly, the levels of SOX2 need to be tightly regulated as high or low levels of SOX2 can induce differentiation in the stem cells [22], [24]. Knockdown studies have established that reduced expression of SOX2 in hESCs results in loss of the undifferentiated state of the stem cell, changed cell morphology and cell marker expression as well as an increase in the expression of trophectoderm markers. It also, notably, results in a reduction in the expressions of NANOG & OCT4 thus again establishing the interconnected nature of the three master transcription factors [25]. SOX2 forms a heterodimer with OCT4 to bind to several sites in the DNA for the regulation of numerous genes [26]. Apart from

working in tandem with OCT4, some studies have established that SOX2, while being dispensable for the activation of the Oct-Sox heterodimer enhancer activation, is a key component in the regulation of OCT4 [27].

SOX2 expression in MSCs has been confirmed by several studies [28]. The overexpression of SOX2 in MSCs results in enhanced cell proliferation as a result of increased levels of cyclin D1 [16]. Interestingly, the expression of SOX2 is of great importance in the case of low-density MSC culturing & knockdown of the gene resulting in the inhibition of multipotentiality and cell proliferation [29]. One method of maintaining the self-renewal capacity of MSCs is through YAP1 which is directly regulated by SOX2 [30]. Also, SOX2 regulates DKK1 as well as c-Myc which determines the lineage differentiation of MSCs [31].

Apart from maintaining the pluripotency of the MSCs, SOX2 can also result in the differentiation of the MSCs into neuron-like cells [32]. A recent study used plant phenol Resveratrol on MSCs and established its capacity to maintain the pluripotency of the MSCs in vitro. On a molecular level, the compound acts as a SIRT1 activator which in turn maintains the levels of SOX2 required for pluripotency in the MSCs for extended culture durations. This could have great utility in MSC therapy for bone regeneration and other regenerative therapies that require extensive culturing [33]. Another interesting study established that apoptotic vesicles from ESCs could promote skin wound healing by transferring SOX2 to skin MSCs via the activation of the Hippo signalling pathway. This could open new avenues in regeneration therapy [34].

2.4. KLF4

KLF4 belongs to the family of SP1-like transcription factors [35], [36]. KLF4 is one of the four factors that was identified as part of the factors required for reprogramming fibroblasts into induced pluripotent stem cells (iPSCs) [37]. KLF4 is an important transcription factor in maintaining the pluripotency and differentiation capacities of ESCs and it does so through several pathways. One of these pathways is the activation of Lefty1 with OCT4 & SOX2 [38]. Also, the overexpression of KLF4 along with PBX1 upregulates NANOG in ESCs which results in the maintenance of the undifferentiated state of the cells [39].

The knockdown of KLF4 results in the commitment of ESCs to the endodermal lineage. This is because KLF4 is responsible for negatively regulating the endodermal marker expression [40]. The knockdown studies have been further validated by other studies. In a study conducted on the function of ERK1 & ERK2, the two molecules phosphorylated KLF4 which resulted in its inactivation which ultimately induced embryonic stem cell differentiation [41]. Loss of KLF4 in mice resulted in altered differentiation & proliferation as well as precancerous changes in the adult stomach [42]. This shows that the function of KLF4 isn't just restricted to stem cells.

The importance of KLF4 for the maintenance of the undifferentiated state of MSCs has been established by several studies [43]. Many recent studies focus on the repression of KLF4 in MSCs. A 2023 study established that CUL4B promotes mesenchymal stem cell commitment to osteogenic lineage by repressing KLF4 [44]. Another repressor of KLF4 a miRNA called miR-10a. This miRNA reduces with ageing in MSCs. Upregulation of miR-10a resulted in increased adipogenic, osteogenic and, chondrogenic differentiation & reduced senescence while downregulation resulted in increased cell senescence and decreased differentiation potential. This happens as a result of miR-10a binding and suppressing the expression of KLF4 [45]. These old MSCs with overexpressed miR-10a when implanted into infarcted mouse hearts displayed better stem cell survival, improved cardiac function & increased angiogenesis in comparison to the control [46]. MSCs-derived extracellular vesicles have been used to treat several diseases one of which is rheumatoid arthritis. MSC-derived EVs containing miR-21 targeting Tet1 which upregulates KLF4 was established to be a successful treatment for rheumatoid arthritis in mouse models [47].

3. Conclusion

Even though studies have shown that the ectopic expression of NANOG, SOX2, & OCT4 have maintained the pluripotency and the self-renewal capacity of MSCs in culture, and the repression of KLF4 has resulted in loss of pluripotency, the expression of these genes under normal conditions is highly debated. With conflicting reports proving their expression as well as inexpression, more robust studies need to be conducted to provide an irrefutable consensus. Also, further studies need to be conducted to elucidate the exact molecular mechanism of the maintenance of pluripotency and self-renewal of these cells.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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