



Editorial

Stemness of Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can self-renew and differentiate into a variety of cell types including chondrocytes, osteocytes and adipocytes. MSCs reside in bone marrow, adipose tissues, cord blood, peripheral blood, placenta, Wharton's jelly, fetal liver and lung among others. MSCs represent one of the most promising stem cells for regenerative medicine due to their multipotency, immunoprivileged properties and easy expansion in vitro. So far, MSCs are already in various phases of clinical application [1-4]. Their most immediate use is in the orthopedic context due to the clear demonstration of their ability to differentiate into bone and cartilage [5-8]. It has been 5 decades since Friedenstein et al described clonal and plastic adherent stromal cells from bone marrow in the 1960s [9,10]. Although there are a handful of genes suggesting possible MSC stemness markers, the molecular basis underlying MSC stemness, especially the key transcription factor to MSC stemness, is still poorly understood.

There are several reasons for poor understanding of MSC stemness. First of all, the heterogeneity of MSCs greatly hamper in-depth MSC study. Variations exist among MSCs from different sources and culture conditions, even fast and slow growing CFU-derived MSCs from the same patient also display differences [11,12]. So far, the factors that affect the heterogeneity of the MSC population is still largely unknown. Secondly, the limited lifespan of MSCs increases the difficulty to study MSCs, especially in case of large number of cells needed. Like other adult stem cells, MSCs undergo the replicative senescence after only a finite number of times in culture. At around passage 10, MSCs demonstrate morphological abnormalities, enlargement, attenuated expression of specific surface markers, and ultimately proliferation arrest [13,14]. In the meanwhile, MSCs reduce differentiation potential during prolonged in vitro culture [15]. The limited lifespan of MSCs also greatly compromises the therapeutic application of human MSCs due to limitation in cell number. Thirdly, little is known about MSC niche. Stem cell niche is a specific microenvironment, in which stem cells are able to self-renew and maintain the undifferentiated state. So far, the MSC niche remains poorly understood.

Stem cell niche provides a milieu that prolongs cellular lifespan and maintains the undifferentiated state of stem cells. Mimicking endogeneous niche of MSCs is able to delay the cell aging and maintain MSC stemness, including hypoxia [16-18], coating with extra-cellular matrix (ECM) [19,20], and 3D culture [21,22] among others. Hypoxic environment has been suggested as physiologic niche to maintain stemness of stem cells. MSCs resides in niche characterized by hypoxic condition. Culture of MSCs under hypoxia enhanced proliferation and preserved the expression of stemness-related genes [16,18]. Changed gene expression profile of MSCs by hypoxia included differentiation, extracellular matrix, intermediate filament, metabolic gene, antioxidant genes and striated muscle genes [17]. In addition, extracellular matrix (ECM) also plays important role in the stem cell niche. It was shown that hyaluronan (HA) prolonged the lifespan and prevented the cellular aging of murine adipose-derived MSCs [19]. MSCs expanded in flasks coating with ECM exhibited higher proliferation, formed more and larger sized cell colonies with smaller and more compactly arranged cells,

and had greater differentiation potential [20]. 3D culture of MSCs on under spheroids or nanoculture plates, closer to in vivo niche of MSCs, delayed replicative senescence and enhanced the differentiation potential [21,22]. In addition, expansion of MSCs in medium containing FGF-2 appeared to promote proliferation and inhibit cellular senescence through a PI3K/AKT-MDM2 pathway [23]. Even blockage of endogenous glucocorticoids using RU486 significantly increase the proliferation and osteogenic differentiation of human MSCs [24].

Although efforts have been made for past 5 decades, little is known about the molecular basis underlying MSC stemness. So far, no one key transcription factor to MSCs like pluripotent genes Oct4, Nanog and Sox2 to ES has been identified, deprivation of which leads to the complete loss of MSC identity. To decipher the signature genes of MSCs, by comparing genes expression profile before and after tri-lineage differentiation, a list of highly expressed genes in undifferentiated MSCs were identified, including 9 transcription factors. However, individual knockdown only partially decrease proliferation or differentiation of MSCs [25]. NRF2 was identified as potential marker by analyzing gene expression. Knockdown of NRF2 decreased osteogenesis whereas overexpression of NRF2 increased the proliferation and reduced the rate of apoptosis of MSCs [26]. In addition, overexpression of cell surface protein CD49f (integrin subunit $\alpha 6$) modulated the proliferation and differentiation potentials of MSCs through activating PI3K/AKT and suppressing p53 expression [27]. Epigenetic modification also controls MSC function. BCL-6 co-repressor (BCOR) interacts with BCL-6 to repress AP-2alpha, which is key factor that enhances osteo-dentinogenic capacity of MSCs. BCOR mutation results in abnormal activation of AP-2alpha, which leads to oculo-facio-cardio-dental (OFCD) syndrome characterized by canine teeth with extremely long roots, congenital cataracts, craniofacial defects and congenital heart disease. Further analysis showed that BCOR mutation activated silenced target genes by increasing histone H3K4 and H3K36 methylation in MSCs [28].

Although these studies expand our understanding towards MSCs, it is still not clear that these genes regulate MSC stemness or only differentiation. So far, the understanding towards MSC stemness is still the tip of the iceberg. To make better use of MSCs for regenerative medicine, more efforts are needed to decipher the molecular basis of MSCs, especially key transcription factors to MSCs.

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