

Review Article

Forced Expression of Transcription Factors in Human Mesenchymal Cells to Promote Proliferation and Osteogenic Differentiation

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Abstract Mesenchymal stromal cells (MSCs) derived from human bone marrow have capability to differentiate into cells of mesenchymal lineage [7]. Especially, the differentiation capability towards osteogenic cells is very well known. We have already used the patient's MSCs for the treatments of osteoarthritis [8], bone necrosis [2] and bone tumor cases [5]. In most cases, the MSCs were culture-expanded from patient's fresh bone marrow cells, and then combined with porous ceramics. The MSCs/ceramics composites were further cultured in a medium containing dexamethasone to promote osteogenic differentiation of the MSCs. In this culture condition, we could detect bone forming osteoblasts together with mineralized bone matrix on the ceramics [7]; therefore, we could fabricate cultured bone using patient's bone marrow and ceramics. However, the proliferation and differentiation capability of the MSCs are variable and many lose their capabilities after several passages. With the aim of conferring higher capability on human bone marrow MSCs, some of transcription factors could be introduced into the MSCs. This review paper demonstrates the importance of the transcription factors to promote the osteogenesis as well as proliferation capabilities of human MSCs.

Keywords mesenchymal stem cells (MSCs); osteogenesis; differentiation; transcription factor; cell culture; induced pluripotent stem cells (iPS cells)

1 Introduction

Embryonic stem (ES) cells are cultured cells derived from the inner cell mass of blastocysts. ES cells have pluripotency in that they can differentiate into cells of all lineages. Murine ES cells are commonly maintained on primary mouse embryonic fibroblast feeder cells in culture medium supplemented with bovine serum and leukaemia inhibitory factor (LIF). In the absence of LIF, murine ES cells differentiate spontaneously in serum containing culture medium [9]. In recent years, the mechanisms involved in maintaining the pluripotent state of human and mouse

embryonic stem cells have been shown to differ. Whilst mouse embryonic stem cells are dependent upon the LIF, human ES cells are dependent on basic fibroblast growth factor (bFGF) to maintain self renewal, pluripotency and prevent differentiation [3].

In addition to these factors, Oct4, Nanog and Sox2 are considered to form transcriptional regulatory circuitry for pluripotency and self-renewal of ES cells [4] (Figure 1). These observations demonstrate a possibility that forced expression of these transcription factors could render bone marrow mesenchymal stromal cells (MSCs) better growth and plasticity properties, because the MSCs have limited proliferation and differentiation capabilities (Figure 2). In this paper, I focused on transcription factors especially Sox2 and Nanog aiming to elucidate the role of human MSCs in bone tissue engineering strategy.

2 Expression of transcription factors in human MSCs

We used the construct in which IRES sequence was placed between the gene of interest and the Venus gene, a variant of GFP, so that expression of the construct was easily detectable during the cell culture. Sox2-expressing cells

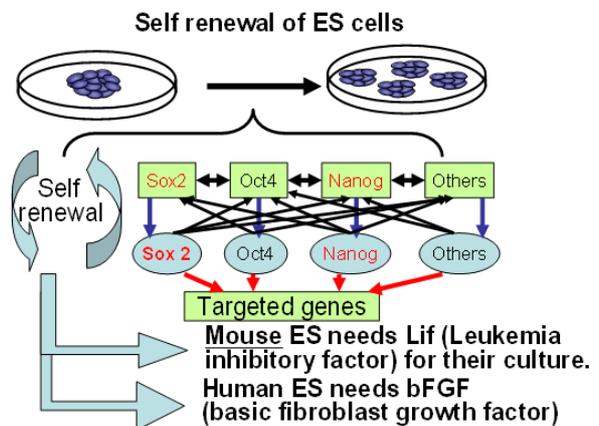


Figure 1: Transcription factors in ES cells.

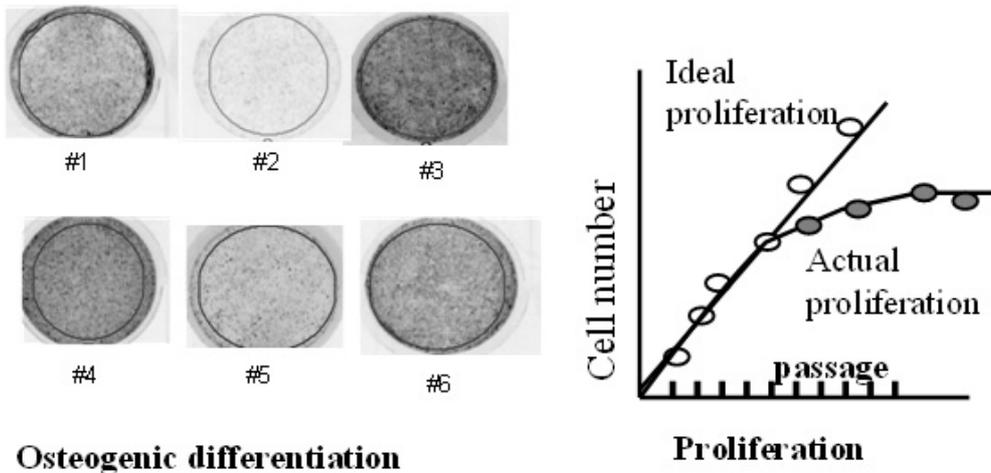


Figure 2: Proliferation and osteogenic differentiation capabilities among the patients.

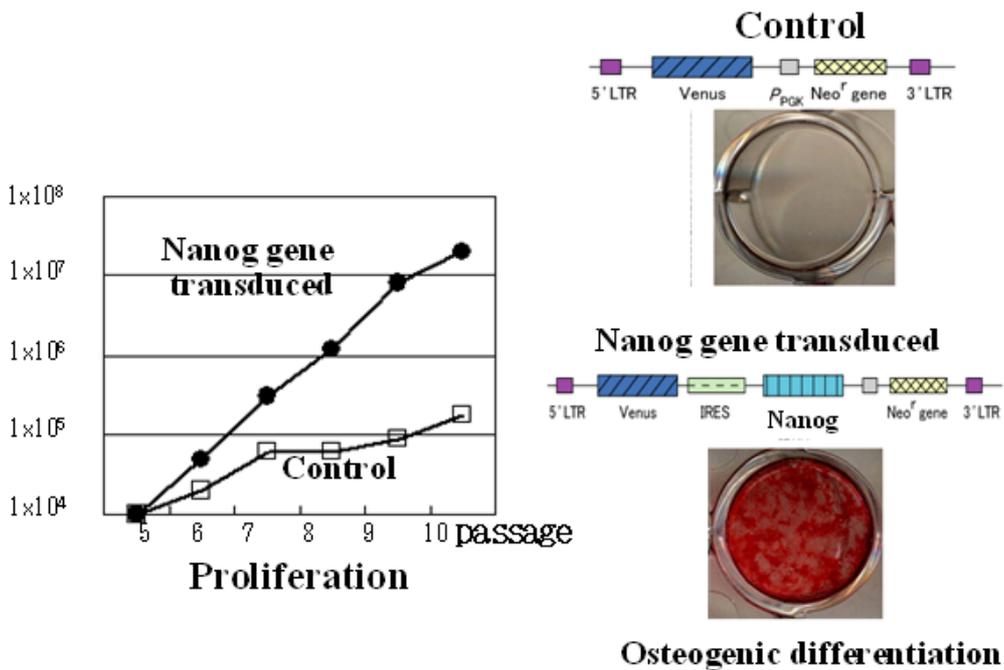


Figure 3: Nanog gene transduction into human mesenchymal cells.

showed distinct growth pattern in the presence of bFGF in culture media. In the presence of the bFGF protein in culture media, bone marrow MSCs show characteristic morphology changes, in which the cells become elongated in shape. In contrast, the Sox2-expressing MSCs responded to bFGF very differently, where the cells grew well as relatively round and small cells. The Sox2-expressing MSCs in the presence of bFGF had higher proliferation and osteogenic differentiation potential than control cells, in which only Venus was expressed [1].

We observed that Nanog-expressing MSCs were also relatively small and found that Nanog-expressing MSCs

showed significantly higher proliferation potential than control cells (Figure 3). We failed to observe significant effects of addition of bFGF in culture media in the case of Nanog-expressing cells in terms of both cell growth ability and cell morphology change. We also found that Nanog-expressing cells showed higher differentiation abilities for osteoblasts than control cells both in terms of both ALP activity and calcium deposition assayed by Alizarin Red staining (Figure 3) [1].

Recently Yamanaka et al. reported that pluripotent stem cells can be directly generated from mouse [11] and human fibroblasts [10] by the introduction of several defined

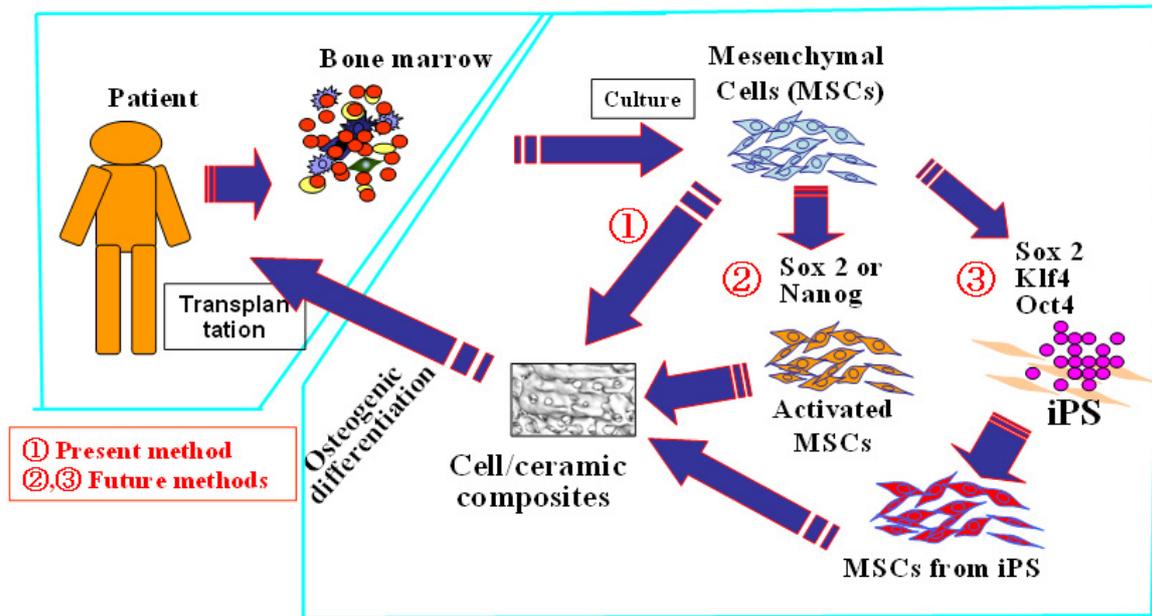


Figure 4: Current and future technologies for bone tissue engineering using MSCs.

genes, one of which was Sox2. Thomson et al. [12] also reported the generation of the induced pluripotent stem cells (iPS cells) by introduction of genes in which Nanog was included. These reports confirmed the importance of Sox2 and Nanog gene for the proliferation/differentiation capabilities of the stem cells. Though the single gene transduction is not sufficient to generate the iPS cells, the functional importance of Sox2 and Nanog for altering the cell status was clearly demonstrated.

3 Current and future technique for bone tissue engineering using MSCs

Our observations on the forced expression of Sox2 or Nanog in adult human MSCs are indeed consistent and succeeded to maintain the proliferation and osteogenic differentiation capabilities of otherwise senescent passaged cells by introducing single gene. We also experienced that these single gene expressing MSCs did not show teratoma formation, whereas the iPS cells have capability to show teratoma after their implantation. Based on our clinical experiences using patient's MSCs; serial passaged MSCs usually reduce their proliferation/differentiation capability (Figure 2). Therefore, our approach using single gene introduction could be an effective and realistic way of maintaining high quality of MSCs for regenerative medicine, especially for bone tissue regeneration. In addition, if we can solve the problem of teratoma formation after the iPS implantation, the iPS made from the patient cells could be available for bone tissue regeneration; especially in patients having severe bone diseases. Interestingly, we could generate the iPS cells from human MSCs and the iPS cells are indeed

pluripotent stem cells because they could differentiate into cell types of all three germ layers [6]. These strategies using single and multiple gene transduction could be available in a near future as seen in Figure 4.

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