Non-neuroectodermal/Mesodermal Lineage Commitment of Mouse Induced Pluripotent Stem Cells by Transcriptional Activation of Bone Morphogenetic Protein-4

論 文 内 容 要 旨

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Induced pluripotent stem cells (iPSCs) are an invaluable source for investigation of embryonic development and regenerative medicine. Genetic manipulation is useful in accessing the roles of genes of interest in directing iPSC differentiation toward a particular lineage. The *piggyBac* (PB) transposon gene delivery system presents several advantages, such as large packaging capacity and traceless excision, compared with conventional viral vectors. The bone morphogenetic protein-4 (BMP-4) performs various roles in regulating the embryonic development and differentiation of stem cells. However, the effects of BMP-4 on lineage commitment of iPSCs are poorly understood. The aim of this study was to investigate the effects of BMP-4 on iPSC lineage commitment by establishing a tet-regulated system for forced *BMP-4* expression. A PB-TAC-ERN/BMP-4 expression vector that contained reverse tet-transactivator/tet-operator sequences and a BMP-4-coding cDNA fragment was constructed using the Gateway® system and introduced with the pCAG-PB transposase expression vector by electroporation into mouse gingiva-derived iPSCs to generate iPSCs-*Tet/BMP-4*. The results showed that transcriptional activation of *BMP-4* strongly upregulated mRNAs for non-neural ectodermal and mesodermal lineage-related genes, while downregulated neural ectoderm- and endoderm-related genes. Based on these, we developed an efficient and simple approach for directly guiding iPSCs-*Tet/BMP-4* differentiation into chondrocyte capable of cartilage regeneration *in vivo* using a 3D shaking suspension culture system. The cartilaginous pellets derived from iPSCs-*Tet/BMP-4* showed an oval morphology and white smooth appearance, and highly express chondrogenic related markers *Sox9*, *Col2a1*, and *Aggrecan*. Histological analysis revealed that the cells presented typical round morphology and the extracellular matrix (ECM) stained intensively with safranin O and alcian blue and collagen II. In addition, the cartilaginous pellets totally repaired the joint osteochondral defects of immunosuppressed rat and completely integrated with the adjacent host cartilage. Moreover, after embryoid body (EB) formation of iPSCs-*Tet/BMP-4*, forced expression of BMP-4 resulted in formation of cystic structures, which strongly expressed oral epithelial markers, such as cytokeratin, Pitx1, and Pitx2. When the cell aggregates were subcutaneously transplanted into mice, formation of hair follicles was only observed in transplants with doxycycline. In the presence of TGF-β inhibitor (SB431542), the expression of mesodermal marker genes were downregulated and the non-neuroectodermal marker genes were significantly upregulated in iPSCs-*Tet/BMP-4*. When Wnt signaling activator (lithium chloride) and fibroblast growth factor 8b (FGF8b) were added to the SB431542-treated iPSCs-*Tet/BMP-4*, the cell aggregates enhanced expression of odontogenic-related marker genes. These findings represent an important development in genetically engineered self-organization of iPSCs, particularly for generation of mesodermal cartilage and non-neuroectodermal organs, such as hair and teeth.