Introduction:

Induced pluripotent stem cells (iPSC) have generated hope and excitement because of the potential they possess in regenerative medicine. Since the discovery by Yamanaka and colleagues that somatic cells can be reprogrammed to ESC like state, numerous reports have emerged focusing on methods to generate iPSC efficiently and safely for future clinical application (1, 2). Although more work remains to be done to get iPSC closer to clinical application, it is also critical to begin to understand factors that will direct iPSC to specific lineages for future clinical application. In the present work, we assessed effects of TGF-β family of proteins for enhancing generation of putative MSCs from iPSC and in vivo bone formation when seeded on HA/TCP and implanted into SCID mice.

Methods:

iPSC production: iPSCs were generated by reprogramming murine tail tip fibroblasts as reported previously (3).

Culture of iPSC: The iPSC generated from tail tip fibroblasts (TTF) were cultured on irradiated MEFs feeders in standard ES medium. After passage 1, the clones expanded rapidly and were trypsinized every 3–4 days. The reprogrammed fibroblasts were easily recognized by their morphological appearance.

Embryoid body formation and generation of MSCs-like cells: iPSC colonies were trypsinized and transferred to ultra-low attachment culture dishes to generate EBs. The EBs were maintained in ES medium without LIF for 3 days followed by incubation in a medium supplemented with 40 ng/ml retinoic acid (RA) for 2 days. EBs were then transferred to 0.1% gelatin-coated plates and cultured in ES medium and 5mg/ml ascorbic acid supplemented with RA and various growth factors (FGF, 10 ng/ml, TGF-β1, 10 ng/ml, TGF-β3, 10 ng/ml, BMP-2 100 ng/ml) for another two days. Cells were maintained in above culture conditions without growth factors. After two passages, the putative MSCs were subjected to FACS analysis and osteogenetic differentiation in vitro and in vivo.

FACS analysis: The iPSC-derived putative MSCs differentiated by retinoic acid and various growth factors treatment indicated above were assessed by FACS for expression of CD13, CD34, CD44, CD45, CD73, CD90, CD117 and CD105 using standard procedures.

Osteogenic and adipogenic differentiation: The putative MSCs indicated above, were plated in six-well plates, and cultured in an osteogenic or adipogenic medium for 28 days. The cells were then incubated in the Alizarin red or Oil Red O to assess osteogenic or adipogenic differentiation respectively.

Bone formation in vivo: the TGF-β1 derived putative MSCs were incubated in a medium supplemented with BMP2 (100 ng/ml) for two days; cells were trypsinized and loaded onto HA/TCP ceramic scaffolds at 5×10^6 cells/mL. Cell seeded Scaffolds were implanted subcutaneously into a thymic SCID mice. Five weeks after implantation; animals were sacrificed and the scaffolds were harvested, fixed in 4% paraformaldehyde, decalcified and embedded in paraffin for histological analysis.

Results: Putative iPSC derived MSCs by different growth factors expressed surface markers characteristic to MSCs. TGF-β family of proteins were however more effective in generating MSCs enriched in expression of putative surface markers e.g. CD73 (Fig. 1A). Morphological appearance of each cell population is also shown (Fig 1B).

Conclusion:

In this report, we have shown that TGF-β1 facilitates production of putative MSCs from iPSC that differentiate efficiently toward osteogenic lineage in vitro and bone formation in vivo. Pre-treatment with BMP-2 enhances in vivo bone formation. BMP-2 treatment is not effective in enhancing production of putative MSCs from iPSC but bone formation by TGF-b1 derived cells. The data suggest that iPSC may be a good source of MSCs for bone repair and regeneration.

Reference:


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