Early Prediction Of Osteogenic Potential Of Human Mesenchymal Stem Cells By Runx2/sox9 Ratio

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Introduction: Master genes regulating skeletal tissue formation have been identified, however the rules by which they regulate distinct tissue types are still unclear. The Runt-related transcription factor Runx2 was identified initially as a positive regulator of osteoblast differentiation. It is one of the most studied transcription factors expressed in human mesenchymal stem cells (hMSCs) upon their commitment toward an osteogenic differentiation (1). It is still hypothetical that Runx2 upregulation single-handedly induces direct ossification in vivo. The Sry-related transcription factor Sox9 is mainly described as the key regulator for chondrogenesis (2). It has been proposed that Sox9 downregulation is required to allow the onset of cartilage-bone transition, such as cartilage resorption and formation of bone marrow during development (3). The inhibitory effect of Sox9 on osteoblast maturation via Runx2 repression is an essential mechanism for osteo-chondroprogenitor fate determination in vitro (4).

However, little is known about the role of Sox9 in direct osteogenesis of hMSCs in vitro. We hypothesized that Sox9 is an early key-regulator during in vitro osteogenic differentiation of hMSCs. Moreover, we propose that the ratio between Runx2 and Sox9 gene expression can predict the osteogenic differentiation potential of hMSCs.

Methods: Human bone marrow was harvested from the iliac crest of eight patients after full ethical approval (mean age: 41 years; range: 20-66 years; male:female ratio: 4:4). hMSCs were isolated through Ficoll density gradient centrifugation and cultured in monolayers over a period of 28 days. During this period, hMSCs were cultured either in control medium (10 % fetal bovine serum and 1 % penicillin/streptomycin) or in osteogenic medium (additional 10^-7 M dexamethasone, 5 mM β-glycerol phosphate and 50 μg/ml ascorbic acid). DNA content, alkaline phosphatase (ALP) activity, alizarin-red S quantification, 45Ca incorporation and expression of osteogenic specific genes were assessed (n =3). To elucidate the roles of molecular determinants in bone forming cells, we analysed Sox9 and Runx2 expression patterns in relation to 45Ca incorporation. Sox9 expression was next silenced and hMSCs were maintained in control medium, osteogenic medium or osteo-permissive medium without dexamethasone to investigate if a knockdown of Sox9 alone can induce mineralization in vitro.

Results: hMSCs cultured in control medium had a significantly higher Sox9 mRNA expression compared to dexamethasone stimulated hMSCs on days 7 and 14 (p<0.05), whereas osteogenic medium led to a downregulation of Sox9 mRNA expression during early osteogenesis. However, Runx2 mRNA expression showed little changes without any significance. MSC donors with a high osteogenic potential (enhanced 45Ca incorporation) had a higher Runx2/Sox9 ratio on day 7 compared to donors with a low osteogenic potential. Following screening of eight donors, a positive correlation between Runx2/Sox9 on day 7 and 45Ca incorporation on day 28 was calculated (R² = 0.87) (Figure 1). However, on the basis of eight donors, a Runx2/Sox9 ratio of above 2 is required to reliably predict an increase in 45Ca incorporation associated with enhanced calcification (Figure 1). hMSCs treated with Sox9 siRNA cultured in osteogenic medium showed a significant increased 45Ca incorporation after 28 days compared to the control groups (no siRNA) in osteogenic medium (p<0.05). No significant difference in 45Ca incorporation could be observed between Sox9 siRNA treated cells cultured in osteogenic versus osteo-permissive medium without dexamethasone.

Discussion: The present study indicates that Sox9 downregulation is required for direct osteogenesis of hMSCs. Treatment of hMSCs with Sox9 siRNA enhanced mineralization in vitro; suggesting that downregulation of Sox9 is involved in direct osteogenesis. However, siRNA knockdown of Sox9 did not in itself induce osteogenesis in the absence of dexamethasone, implying that downregulation of Sox9 prior to osteogenic induction may be too early to take over the whole mineralization process. The impact of Sox9 downregulation in mineralization of hMSCs in vitro indicates a so far unprecedented role of Sox9 as a major regulator of direct osteogenesis. Rather than focusing on Runx2 as a crucial factor of direct osteogenesis, Sox9 seems to be key in better understanding in vitro osteogenesis of hMSCs. Our data imply that a decrease in Sox9 expression leads to a vastly increased Runx2/Sox9 ratio. In fact, we suggest that the ratio of Runx2 and Sox9 on day 7 can predict the osteogenic potential of each particular donor, assuming that the balance of both genes is the result of earlier regulatory events. In on-going studies, we are investigating if this screening method can be applied in evaluating freshly isolated hMSCs and biomaterials for their osteogenic potential.

Significance: These findings imply that the Runx2/Sox9 ratio is a promising early screening method for osteogenicity of hMSCs, which might be useful in evaluating freshly isolated hMSCs with regards to their osteogenic potential in vitro and in cell-based clinical applications.

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