

## Studying the effects of TGF- $\beta$ 2 and BMP-2 growth factors on differentiation of murine embryonic stem cells into cardiomyocytes

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### Abstract

**Introduction:** Stem cell therapy seems promising for the treatment of incurable diseases such as injured myocardium. Directed in vitro differentiation of embryonic stem cells (ES) to cardiomyocytes is invaluable. TGF $\beta$ -2 and BMP-2, two different growth factors from the transforming growth factor- $\beta$  superfamily (TGF- $\beta$ ), exhibit their effects on growth, immigration and differentiation and other cellular functions during embryonic development. In this study, the effects of these factors on differentiation of ES cells into cardiomyocytes were evaluated.

**Materials & Methods:** Murine embryonic stem cells (CCB derived from mouse strain 129) were used for cardiomyocyte differentiation. To increase the effects of the factors, ES cells were grown on inactivated fibroblasts for 24 hours in differentiating culture medium containing 8 ng/ml of TGF- $\beta$ 2 and 5 ng/ml of BMP-2 while decreasing serum concentration from 20% to 7.5%. Later, embryoid bodies (EB) were formed in 800-cells hanging drops and suspension from ES cells and eventually, EBs were cultured on gelatin-coated plates. After continuation of these steps, ES cells differentiated into beating cells. To determine the effects of the growth factors on ES cell differentiation into cardiomyocytes, expression of primary transcribing factors, MEF2, Nkx2.5, and specific cardiac genes including MHC, ANF, MLC-2v were assessed by semiquantitative RT-PCR and expression of desmin and  $\alpha$ -actinin in differentiated cardiomyocytes was studied by western blotting and immunostaining.

**Results:** After completion of the differentiation process, the EBs in the experimental groups exhibited beating compared with the control group. Considering the quantitative comparison of the electrophoretic bands of RT-PCR and PCR products of the plated EBs in different media, it was seen that TGF- $\beta$ 2 from BMP-2 and both factors together, compared to each one alone, is more effective on differentiation of embryonic stem cells into cardiomyocytes. In addition, in plated EBs, in a medium containing both factors, the expression of desmin and  $\alpha$ -actinin were determined by western blotting and the presence of cardiomyocytes, by marking desmin, by immunohistochemical staining.

**Conclusion:** The results showed that the synchronized effects of both factors, while decreasing serum concentration during ES and EB cell cultures, increases differentiation of ES cells into cardiomyocytes.

**Key Words:** Embryonic stem cells, Differentiation, Cardiomyocyte, TGF- $\beta$ 2, BMP-2.

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