Effect of Rhtgf-B1 on Osteogenic Differentiation of Sd Rat Mscs and Its Mechanism

Mengting He

Department of Orthodontics, West China School of Stomatology, Sichuan University, Chengdu, China

Keywords: Rhtgf-B1, Mscs Osteogenic Differentiation, Transforming Growth Factor

Abstract: in Order to Analyze the Effect of Rhtgf-B1 on the Osteogenic Differentiation of Sd Rat Mscs, the Study of, Sd Rat Mscs Were Isolated and Purified by the Whole Bone Marrow Adherence Method, and Different Rhtgf-B1 Were Applied by Mtt Method, and the Resulting Mscs Value-Added Activity Affects the Completion of the Test. According to the Alp (Alkaline Phosphatase) Activity and Alp Staining Positive Rate, the Optimal Concentration of Rhtgf-B1 on Osteogenic Differentiation of Sd Rat Mscs Was Performed, and the Mscs Osteogenic Differentiation Was Performed, According to the Osteogenic Induction Fluid That Was Added, Which is Divided into 4 Groups, Normal Group, Classical Group, Rhtgf-B1 Group, Rhtgf-B1+ Classic Group (n Group, C Group, t Group, t+C Group). the Ability of Alp, Osteocalcin Expression, and Calcified Nodules to Differentiate into Osteogenic Differentiation Was Evaluated, and the Results Showed That Rhtgf-B1 Was Used as the Optimal Concentration of Mscs in Sd Rats, and 5 Was the Optimal Concentration to Promote the Osteogenic Differentiation of Rat Mscs. in Addition to the Normal Three Groups, the Other Three Groups Can Promote the Osteogenic Differentiation of Mscs, and Stimulate Bmp-2 to Up-Regulate the Expression of Smad4 and Cbfal Mrna. Therefore, the Classical Group, Rhtgf-B1, Rhtgf-B1+ Classical Group Can Achieve Osteogenic Differentiation of Sd Rats in Sd Rats, and the Mechanism of Action is to Promote the Secretion of Bmp-2. the Tgf-B Superfamily/Smads Signaling Pathway is Regulated. That is to Achieve Bone Differentiation.

1. Introduction

MSCs (bone marrow mesenchymal stem cells), is a non-hematopoietic stem cell derived from mesoderm found in the bone marrow, which possess multipotential differentiation and self-replication potential. At present, the research community unanimously believes that TGF-β (transforming growth factor β), BMPs (bone morphogenetic protein), is the basic fibroblast growth factor, tumor necrosis factor and vascular endothelial growth factor, etc., all of these cytokines are on MSCs bone, because it has an impact on differentiation. Therefore, this study was conducted to investigate the effect and mechanism of rhtgf-β1 on osteogenic differentiation of SD rat MSCs.

2. Materials and Methods

2.1 Materials

A total of 5 female SD rats, about 3 months old, were selected and weighed (230 ± 10) g, which were provided by the Experimental Animal Center of Sun Yat-sen University. The test drugs and reagents used include: rhtgf-β1, L-DMEM, dexamethasone, β-glycerophosphate, vitamin C, ALP kit, rat type I collagen kit, BGP (osteocalcin) radioimmunoassay Box, ELISA kit.

2.2 Method

The rhtgf-β1 was prepared under aseptic conditions and stored at -20 °C for storage, as the classical osteogenic induction solution was prepared and stored at 4 °C, and the whole MSCs were isolated by whole bone marrow adherence method. After the inoculation for 24 hours, the first half was changed according to 3d/time, the frequency of liquid exchange was up to 80%~90% of the degree of cell fusion, and the stable isolation and culture system of rat MSCs was established

  DOI: 10.25236/ISMHI.2019.128

according to the ratio of 1:2. According to ALP (alkaline phosphatase) activity and ALP staining positive rate, the optimal concentration of rhtgf-β1 on osteogenic differentiation of SD rat MSCs includes 0, 5, 10, 20, 40, 80, 100 $\mu g / L$, respectively. The concentration was placed in a 5% CO2 incubator at 37 ° C for 72 h. Then, 5 g / L of MTT 20 $\mu L / well$ was added to each of the 6 replicate wells, and then placed in the same environment parameter incubator for 4 d, after the supernatant was discarded. Add 150 $\mu L / well$ DMSO, perform microwell shaking for 10 mi to fully dissolve the formazan standard, set the microplate reader wavelength to 490 nm, and perform bone differentiation intervention of MSCs. According to whether or not the osteogenic induction fluid was added, it was divided into 4 groups, normal group, classical group, rhtgf-β1 group, rhtgf-β1+ classic group. The ability of ALP, osteocalcin expression, and calcified nodules to differentiate into osteogenic differentiation was evaluated.

2.3 Statistical Methods

The data of this study were compared and analyzed by using SPSS18.0 software. The measurement data and technical data were expressed by ($\bar{x} \pm S$) and %, and the T value and X2 were tested. The significant difference was statistically significant at P<0.05.

3. The Result

3.1 Rhtgf-B1 Promotes the Optimal Concentration of Mscs Proliferation

The results showed that rhtgf-β10 $\mu g / L$ was the best value-enhancing concentration for promoting MSCs in SD rats. The value of 10 $\mu g / L$ TGF-β1 A was significantly higher than that of the control group (P<0.05), and others were not significantly different from the control group (P>0.05). figure 1.

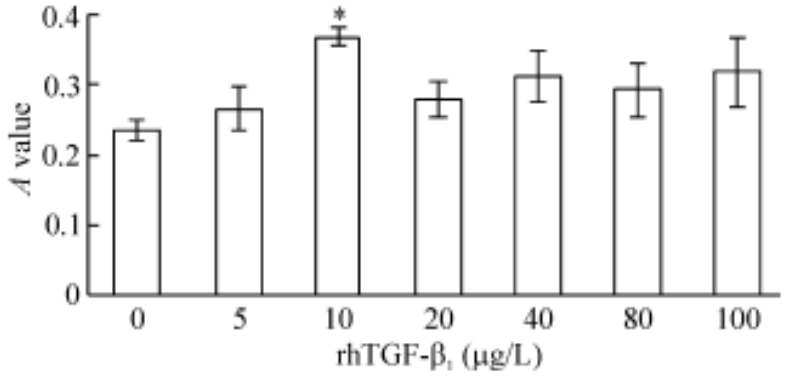

Fig.1 Different Concentrations Indicate That Rhtgf-B1 Affects Proliferation of Rat Mscs

3.2 Rhtgf-B1 Promotes the Optimal Concentration of Mscs for Osteogenic Differentiation

The results of ALP activity assay showed that 5 $\mu g / L$ was the best concentration to promote osteogenic differentiation of rat MSCs, $\mu g / L$ the ALP activity of 5rhtgf-β1 was significantly higher than that of the control group and other concentrations (P<0.05), but other concentrations were compared, and the difference in the control group was not significant (P>0.05), indicating that the optimal concentration of rhtgf-β1 to promote ALP secretion in MSCs was 5 $\mu g / L$ at the 7 concentrations.

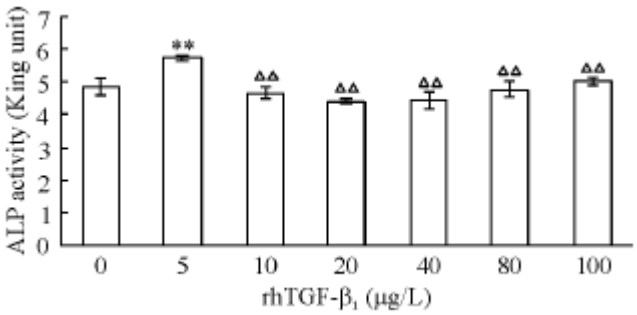

Fig.2 Effect of Different Concentrations of Rhtgf-B1 on Alp Secretion Activity

3.3 Effect of 2.3rhtgf-B1 on Bone Differentiation Index

According to the results of ALP secretion activity, the ALP value of group C, T group and T+C group was significantly higher than that of N group at 3d ($P<0.05$), and the ALP activity of group T was significantly higher than that of group N at 7d ($P< 0.05$), the C, T, C + T three groups at 10d were significantly higher than the N in ALP activity ($P <0.05$), thus indicating that rhtgf-β1 can promote the secretion of ALP as shown in Table 1.

Table 1 Effect of Rhtgf-B1 on Alp Secretion Activity

Group	3d	7d	10d	14d
N	6.367±0.269	6.916±0.142	6.812±0.165	5.676±0.294
C	7.580±0.597	0.883±0.372	7.204±0.148	6.071±0.354
T	7.511±0.369	7.513±0.344	8.431±0.195	6.361±0.389
T+C	7.648±0.656	7.065±0.299	7.741±0.234	6.287±0.496

Effect of 2.4rhtgf-β1 on BMP2 release

The results showed that the other three groups in the normal group could promote the osteogenic differentiation of MSCs and stimulate the expression of Smad4 and Cbfal mRNA in BMP-2.

4. Discussion

In the TGF-β superfamily, BMPs as a strong growth factor, the current research has unified BMP-2 of a strong bone formation growth factor, and has a special biological characteristics, to enhanced ALP activity, collagen and the secretory synthesis of BGP plays a role in cell differentiation. Studies have shown that BMP-2 expression is reduced locally in rats, which will reduce the proliferation and division of BMP-2-induced osteogenic stromal stem cells, so the gradual decrease of BMP-2 content in vivo is a crucial pathological process. Moreover, BMP-2 can also form osteogenic differentiation effects on MSCs, and it has been clinically proven to be an adjuvant treatment for improving bone healing ability. In this paper, the effect of rhtgf-β1 on osteogenic differentiation of SD rat MSCs and its mechanism were studied, and was found that osteogenic inducer can promote the expression of ALP, BGP and calcium nodules, and achieve a very significant effect of promoting ALP secretion, because it is significantly higher than the osteogenic inducer, but it does not play a significant role in BGP secretion and calcium nodule formation. However, in the rhtgf-β1+ osteogenic inducer group, it was found that it can effectively enhance ALP activity, and at 14d, it can promote better BGP secretion and calcium nodule formation than rhtgf-β1.

Summary, the classical group, rhtgf-β1, rhtgf-β1+ classical group can achieve osteogenic differentiation of SD rats in SD rats, and the mechanism of action is to promote the secretion of BMP-2, and the TGF-β superfamily is regulated, the /Smads signaling pathway achieves osteogenic differentiation.

References

[1] LI Xiaolong, ZHAO Houling, LIU Wenhua, et al. Effects of TGF-β1 on the proliferation and

differentiation of rat bone marrow mesenchymal stem cells induced by rhBMP-2[J]. Journal of Weifang Medical College, 2015(1): 42-44.

[2] Saiyin Qimuge, Hou Xianglin, Zhao Li. Effects of recombinant human transforming growth factor-β_1 on the differentiation of mesenchymal stem cells into osteoblasts [J]. China Comprehensive Clinical, 2005, 21(5): 413- 415.

[3] Lu Yuanting, Wei Lusheng, Wang Zhiyong, et al. Analysis of transforming growth factor-β3 promoting rat bone marrow mesenchymal stem cells to chondrocytes under hypoxic conditions[J]. Chinese Journal of Medicine, 2018, 98(27) :2198-2202.

[4] Sujuan Y, Ronghua Z, Xiaofeng Z, et al. Effects of rhTGF-β1 on proliferation and osteogenic differentiation of MSCs in SD rats [J]. Chinese Journal of Pathophysiology, 2014, 30(3): 460-466.

[5] YAN Cheng, XUE Gai, ZHANG Wei, et al. Effects of blood microenvironment on the differentiation of human umbilical cord MSCs into hepatocytes in rats with hepatic fibrosis[J]. Chinese Journal of Reparative and Reconstructive Surgery, 2016(30) :760.

[6] Zhao Xijiang, Wu Xiaotao, Wei Jinan, et al. Preparation of PLGA sustained-release scaffolds loaded with rhTGF-β1 and their effects on cell morphology of MSCs[J]. Journal of Southeast University(Natural Science), 2007(03):127- 131.