Rosiglitazone accentuates the adipogenesis of hemangioma-derived mesenchymal stem cells induced by adipogenic media

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Abstract: Hemangioma-derived mesenchymal stem cells (Hem-MSCs) expressed PPAR-γ, the key transcription factor in adipogenesis. We supposed that rosiglitazone, the agonist of PPAR-γ, may promote the adipogenesis of Hem-MSCs. In this study, MSCs were isolated from proliferating hemangioma. Four groups were set up, which were Group A (DMEM-LG/10% FBS), Group B (1 μM rosiglitazone + DMEM-LG/10% FBS), Group C (adipogenic media), and Group D (1 μM rosiglitazone + adipogenic media). Cells were cultured in the medium above. On the day 7 and 14, Oil Red “O” staining and Western blot were performed to detect the cytoplasmic lipid and perilipin A in the cells. The results showed that cytoplasmic lipid appeared in Group C and D, and no cytoplasmic lipid in Group A and B on the day 7 and 14. Analysis of Oil Red “O” staining showed the area of staining in Group D was significantly larger than that in Group C. Analysis of western blot showed no expression of perilipin A in Group A and B, and upregulated expression in Groups C and D, with the greater upregulation in Group D. In conclusion, our study demonstrated that rosiglitazone promoted the adipogenesis of Hem-MSCs initiated by adipogenic media via the activation of PPAR-γ pathway. The results may put forward the possibility of treating hemangioma via PPAR-γ pathway.

Keywords: Hemangioma, mesenchymal stem cells, adipogenesis, rosiglitazone, PPAR-γ

Introduction

Infantile hemangioma, a common benign tumor in children, grows quickly in the three to six months after birth, and regresses slowly into fibrofatty tissue in childhood. A unique character of hemangioma in involution is the accumulation of fibrofatty tissue, which indicates adipogenesis during this period [1, 2]. The mechanism of this phenomenon isn’t totally clear till now. Hemangioma-derived mesenchymal stem cells (Hem-MSCs) had the adipogenic potential in vitro [3, 4]. CD133 (+) multipotent stem cells (Hem-SCs) have the features of MSCs and recapitulate hemangioma’s evolution in an immunodeficient mouse model [5, 6]. MSCs are the cellular basis of the adipogenesis in hemangioma involution [1].

Studies showed the expression of PPAR-γ gene in Hem-MSCs [3, 4], which was a key nuclear transcription factor in controlling adipogenic differentiation. Thiazolidinediones (TZDs) are the agonists of PPAR-γ [7]. Rosiglitazone, one of the TZDs, can promote the adipogenic differentiation of preadipocytes and bone marrow-derived mesenchymal stem cells [8, 9]. In this study, we investigated the effects of rosiglitazone on the adipogenesis of Hem-MSCs. The results may put forward the possibility of treating hemangioma via PPAR-γ pathway.

Materials and methods

Isolation and culture of MSCs from hemangioma

Fresh IH samples in the proliferating phase were got from Children’s Hospital Nanjing, under a human subject’s protocol approved by the Committee on Clinical Investigation. Informed consent was provided according to
the Declaration of Helsinki. MSCs were isolated as described previously [3, 4]. In briefly, samples were rinsed in phosphate-buffered saline, minced, digested with 0.2% collagenase A (M9195, Sigma-Aldrich) at 37°C for one and a half hours, and then filtered through 70 μm cell strainers (BD Falcon™) to get a single-cell suspension. Cells were resuspended by DMEM-LG (Hyclone)/10% FBS (Hyclone) supplemented with 1×PG (100 U/ml penicillin, 100 μg/ml gentamycin) (briefly called DMEM-LG/10% FBS in the passage below), and plated on plastic culture dishes (Corning) at 1.0×10⁵/cm². On the second day, medium was changed to remove the floating cells. The attached cells were cultured in DMEM-LG/10% FBS.

**Induction of adipogenic differentiation of Hem-MSCs**

Based on the culture medium, four groups were set up to examine the effects of rosiglitazone on Hem-MSCs. They were Group A (DMEM-LG/10% FBS), Group B (1 μM rosiglitazone + DMEM-LG/10% FBS), Group C (adipogenic media) and Group D (1 μM rosiglitazone + adipogenic media). Adipogenic media included DMEM-LG/10% FBS, 5 μg/ml insulin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 60 μM indomethacin.

**Oil red O staining**

Hem-MSCs were plated on the culture coverslips in six-well dishes at the density of 1×10⁴ cells/well. When cells reached 80% confluence, the medium were changed by the medium above. On the day 7 and 14 after induction, three coverslips were taken out from each group, and Oil Red “O” staining was performed to observe the cytoplasmic lipid in cells. A bright-field microscope equipped with a digital camera (DS-L1-5M, Nikon, Japan) was used to observe the staining. And 10 pictures (200×) were taken randomly in each coverslip. The software “Image Proplus 6.0” was used to analyze the area of positive staining in each picture.

![Figure 1. Induction culture of Hem-MSCs. On the day 14, no lipid droplet occurred in the cells of Group A and B. Lipid droplets occurred in Group C and D, with more in Group D. Scale bar: 50 μm.](image)
Western blot

The culture, induction, and harvest of the cells in each group were conducted as above mentioned. The cells before induction were used as the normal control. Cells were lysed with RIPA Buffer (Keygen Biotech, Nanjing), containing protease and phosphatase inhibitors. Lysates were submitted to sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (SDS–PAGE) and transferred to a membrane. Membranes were incubated with goat anti-perilipin A antibody (1:1000, ab61682, Abcam) and mouse anti-β-actin antibody (1:500,
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sc-130301, Santa Cruze). Then membrane was incubated with rabbit anti-goat IgG H&L (HRP) (1:5000, ab6741, Abcam) and rabbit anti-mouse IgG H&L (HRP) (1:2000, ab6728, Abcam). Chemiluminescent sensitive film was used to detect antigen-antibody complexes. The software “Image J” was used to analyze the electrophorograms.

Statistics

Statistics was performed with Microsoft® Office Excel. Data were expressed as mean ± SD and analyzed by Student’s two-tailed t test where appropriate. Differences were considered significant at \( P < 0.05 \).

Results

Observation with inverted microscope

On the day 14, no lipid droplet was observed in the cells of Group A and B. Lipid droplets were observed in Group C and D, with more in Group D (Figure 1). The results preliminary suggested rosiglitazone couldn’t induce the adipogenic differentiation by itself, but might enhance the adipogenic differentiation of Hem-MSCs induced by the adipogenic media.

Oil red “O” staining

Oil red “O” staining was performed to detect the lipid droplets in the cells and staining area was analyzed by Image ProPlus 6.0 software. The results showed the staining area in Group D was obviously larger than that in Group C on the day 7 and 14, which further confirmed that rosiglitazone enhanced the adipogenic differentiation of Hem-MSCs induced by the adipogenic media (Figure 2).

Rosiglitazone upregulated the expression of perilipin a protein

Perilipin A was one of lipid-relating proteins, a marker protein in adipogenic differentiation. The results showed upregulated expression of perilipin A in Group C and D on the day 7 and 14, and no expression in Group A and B. Analysis showed the stronger expression in Group D than that in Group C both on the day 7 and on the day 14 (Figure 3).

Discussion

In this study, we investigated the effects of rosiglitazone on the adipogenic differentiation of Hem-MSCs. The results of induction culture, oil red “O” staining, and Western blot showed rosiglitazone accentuated the adipogenesis of Hem-MSCs induced by adipogenic media. Rosiglitazone couldn’t induce the adipogenic differentiation of Hem-MSCs by itself.

PPAR-γ is a member of the nuclear hormone receptor family of transcription factors, play a key role in adipogenesis and lipid storage [10, 11]. PPAR-γ exists as four isoforms, PPAR-γ1, PPAR-γ2, PPAR-γ3, and PPAR-γ4. Among them, PPAR-γ2 is mainly expressed in adipocytes [7]. PPAR-γ promotes the expression of genes related to adipogenesis via formation of a heterodi-
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In recent years, the role of PPAR-γ in tumor's development, growth, and metastasis attracted the attention [13, 14]. TZDs could inhibit the growth of cancer cells [15], promote the apoptosis [16], induce G1 cell cycle arrest [17], suppress the angiogenesis [18], and interfere energy metabolism [19]. TZDs activated PPAR-γ pathway and regulated the expression of genes, such as Bcl-2, Bcl-xl, Bax, p21, PARP, caspase 8, caspase 9, caspase 7, p53, p63 and p73 [15-17]. In the former study, we observed the expression of PPAR-γ in the endothelial cells in hemangioma tissue [4]. So, we suppose that TZDs may also activate PPAR-γ pathway of endothelial cells in hemangioma, inhibit cells proliferation, and promote the apoptosis.

Based on the studies above, we hypothesize that TZDs, such as rosiglitazone and pioglitazone, may become the new drug for hemangioma. Intralesional injection of the drug may inhibit the proliferation of endothelial cells, promote the apoptosis, and then led to the regression of microvessels. Simultaneously, MSCs in hemangioma are induced to differentiate into adipocytes. So, the involution of hemangioma may be accelerated. We will verify this hypothesis in the animal model of hemangioma. The animal model of hemangioma can be established as described previously [20]. At the proliferating phase of the transplanted tumor, we inject rosiglitazone, and take out the tumor regularly after drug injection to observe the pathological changes and the expression of genes and proteins related to adipogenic differentiation.

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Disclosure of conflict of interest

None.

References

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