Study on the expression of PPARγ and AQP7 in 3T3-L1 adipocyte differentiation and the promoting effect of emodin

Bai Xiao-Su, Liu Zhi-Ming, Li Zhi-Sen, Guo Dong

Endocrinology Department, People's Hospital of New District Longhua Shenzhen, Shenzhen, Guangdong Province, 518000

ARTICLE INFO

Article history:
Received 1 May 2016
Received in revised form 13 May 2016
Accepted 5 May 2016
Available online 28 May 2016

Objective: To study the expression of PPARγ and AQP7 in 3T3-L1 adipocyte differentiation and the promoting effect of emodin. Methods: In the process of adipocyte differentiation induced by traditional adipocyte differentiation inducer, different concentration of emodin was added for co-incubation, and the changes in the expression of adipose differentiation-related marker genes FAS, AP2, GR, LPL and Pref-1 as well as the PPARγ and AQP7 protein expression were detected. Results: The study confirmed that during 3T3-L1 adipocyte differentiation process, differentiation marker proteins FAS, AP2, GR, LPL and Pref-1 expression significantly increased, AQP7 and PPARγ expression increased, and emodin had dose-dependent promoting effect on cell differentiation. Conclusion: emodin has promoting effect on the process of 3T3-L1 adipocyte differentiation, and this process may be related to PPARγ and AQP7 expression regulated by it.

1. Introduction

With the change of the diet and lifestyle in modern society, the incidence of obesity and type 2 diabetes has increased year by year. Existing evidence shows that abnormal lipid precipitation and insulin resistance are the important factors of the pathogenesis[1]. Adipocyte hypertrophy and an increased number of adipocytes are the important causes of lipid accumulation and metabolic disorder in the body[2,3]. Adipose tissue expresses aquaporin 7 (AQP7)[4], the expression of AQP7 drops in adipose tissue of the obese, and it may be related to the formation of obesity, insulin resistance and type 2 diabetes[5].

peroxisome proliferator-activated receptor (PPARγ) is an important transcription factor in cells that regulates the expression of a variety of nuclear genes, and is associated with a variety of abnormal fat metabolism processes[6,7]. Emodin belongs to free anthraquinone derivatives, is the main effective monomer composition of Chinese traditional medicine rhubarb, and has the role of improving the glucolipid metabolism[8]. In this research, the effect of emodin on PPARγ and AQP7 expression in 3T3-L1 adipocyte differentiation and the relationship were explored.

2. Materials and methods

2.1. Cells and reagents

Mice adipose cell line 3T3-L1 was bought from American type culture collection (ATCC); DMEM high-glucose medium and fetal bovine serum were bought from Gibco Company in the United States; trypsin was bought from Sigma Aldrich Company; 3-Isobutyl-1-methylxanthine (IBMX), insulin (Ins) and dexamethasone (DEX) were purchased from Sigma Aldrich Company in the United States; RNA extraction and reverse transcription kits were bought from TaKaRa Company; cell culture plate was bought from Corning Costar Company in the United States; microplate reader was bought from US Thermo Fisher Company. The rest reagents were commercially available analytically pure.

2.2. Grouping and detecting items

3T3-L1 adipocytes were cultured to good growth status under...
the condition of 37 °C, 5% CO₂ and saturated humidity, and then induced to differentiate in accordance with the previously reported method[9] (10 µg/mL insulin, 1 µmol/L dexamethasone and 0.5 mmol/L IBMX). At the same time, different concentration of emodin (low dose 1 µmol/L, medium dose 5 µmol/L, and high dose 25 µmol/L) was added in the process of induction for co-culture and processing. The changes of adipose differentiation-related marker genes FAS, AP2, GR, LPL and Pref-1 expression as well as PPARγ and AQP7 protein expression were detected.

2.3. Fluorescence quantitative PCR

mRNA extraction and reverse transcription process were carried out in accordance with the specifications. Fluorescence quantitative PCR reaction procedures: 95°C 10 s, 94°C 30 s, 60°C 30 s, 72°C 40 s, amplification for 30 cycles.

2.4. Enzyme–linked immunosorbent assay

PPARγ detection kits were bought from Wuhan Boster Biological Engineering Co., Ltd.; AQP7 protein detection kits were purchased from R&D Company; all operations were carried out in accordance with the kit instructions.

2.5. Statistical analysis

SPSS 18.0 software was used to analyze data, measurement data was in terms of average ± standard deviation (Mean ± SD) and comparison between groups was by single factor analysis of variance. P<0.05 indicated statistical significance in differences.

3. Results

3.1. FAS, AP2 and GR expression in 3T3-L1 adipocyte differentiation process and the promoting effect of emodin

Research confirmed that cell differentiation marker proteins FAS, AP2 and GR expression significantly increased in 3T3-L1 adipocyte differentiation process, and emodin had dose-dependent promoting effect on cell differentiation (P<0.05), shown in Table 1.

Table 1. FAS, AP2 and GR expression in 3T3-L1 adipocyte differentiation process and the promoting effect of emodin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FAS/GAPDH</th>
<th>AP2/GAPDH</th>
<th>GR/GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal induction</td>
<td>0.26±0.08</td>
<td>0.29±0.07</td>
<td>0.36±0.09</td>
</tr>
<tr>
<td>Normal induction + emodin</td>
<td>0.34±0.05</td>
<td>0.34±0.05</td>
<td>0.36±0.11</td>
</tr>
<tr>
<td>Medium dose</td>
<td>0.39±0.09</td>
<td>0.39±0.09</td>
<td>0.40±0.14</td>
</tr>
<tr>
<td>High dose</td>
<td>0.49±0.12</td>
<td>0.49±0.12</td>
<td>0.53±0.13</td>
</tr>
<tr>
<td>P value</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

3.2. LPL and Pref-1 expression in 3T3-L1 adipocyte differentiation process and the promoting effect of emodin

Research confirmed that cell differentiation marker proteins LPL and Pref-1 expression significantly increased in 3T3-L1 adipocyte differentiation process, and emodin had dose-dependent promoting effect on cell differentiation (P<0.05), shown in Table 2.

Table 2. LPL and Pref-1 expression in 3T3-L1 adipocyte differentiation process and the promoting effect of emodin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPL/GAPDH</th>
<th>Pref-1/GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal induction</td>
<td>0.33±0.06</td>
<td>0.20±0.05</td>
</tr>
<tr>
<td>Normal induction + emodin</td>
<td>0.35±0.09</td>
<td>0.31±0.08</td>
</tr>
<tr>
<td>Medium dose</td>
<td>0.42±0.12</td>
<td>0.36±0.07</td>
</tr>
<tr>
<td>High dose</td>
<td>0.48±0.13</td>
<td>0.43±0.11</td>
</tr>
<tr>
<td>P value</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

3.3. PPARγ and AQP7 expression in 3T3–L1 adipocyte differentiation process and the promoting effect of emodin

Research confirmed that AQP7 and PPARγ expression significantly increased in 3T3-L1 adipocyte differentiation process, and emodin had dose-dependent promoting effect on PPARγ and AQP7 expression (P<0.05), shown in Table 3.

Table 3. PPARγ and AQP7 expression in 3T3-L1 adipocyte differentiation process and the promoting effect of emodin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PPARγ/GAPDH</th>
<th>AQP7/GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal induction</td>
<td>0.34±0.08</td>
<td>0.50±0.11</td>
</tr>
<tr>
<td>Normal induction + emodin</td>
<td>0.35±0.07</td>
<td>0.57±0.09</td>
</tr>
<tr>
<td>Medium dose</td>
<td>0.48±0.10</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td>High dose</td>
<td>0.61±0.12</td>
<td>0.62±0.12</td>
</tr>
<tr>
<td>P value</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

4. Discussion

Emodin belongs to free anthraquinone derivatives, is chemically called 1,3,8-trihydroxy-6-methylanthraquinone, is the main effective monomer composition of Chinese traditional medicine rhubarb, exists in rhubarb, polygonum cuspidatum, cassia seed, polygonum multiflorum, aloe and other traditional Chinese medicines containing anthraquinone constituents, and has function of improving lipid metabolic disorder, resisting inflammation, regulating immunity and so on[9].

In the process of 3T3-L1 adipocyte differentiation, differentiation-related marker proteins FAS, LPL, etc have different degree of change. In this experiment, by adjusting different emodin intervention concentration, the expression of adipocyte differentiation marker proteins FAS, AP2, GR as well as LPL and Pref-1 were higher than those of normal induction group, and increased with increasing concentration of emodin, confirming that the emodin could promote adipocyte differentiation. At the same time of promoting adipocyte differentiation, high doses of emodin can inhibit the proliferation of adipocytes, reduce adipocyte formation, reduce lipid accumulation...
and improve glucolipid metabolism[10].

Emodin is PPARγ ligand and has a high affinity with PPAR-γ[11], and this study also confirmed that with the increase of the concentration of emodin, the expression of PPARγ also gradually increased, and emodin has dose-effect relationship with PPARγ expression. PPARγ regulates the activity of many lipid-specific genes at transcriptional level, and is closely related to a variety of lipid metabolism processes[6,7]. Previous studies have also found that emodin can activate AMPK and PPARγ to influence glucose uptake of 3T3-L1 adipocyte[12].

This study also found that AQP7 was also involved in the process of adipocyte differentiation. Aquaporin AQP7 is only expressed in fat tissue, participates in the glycerinum transportation of adipocyte differentiation. Aquaporin AQP7 is only expressed in adipocyte differentiation process, the detected content of AQP7 increases after differentiation maturation, and the glycerinum accumulation in adipocytes significantly reduces, so it is believed that the increase of AQP7 decreases insulin resistance, improves lipid homeostasis and reduces visceral fat in high-risk subjects: Prediabex study rct. Moreover, this study also confirmed that with the increase of the expression of aquaglyceroporin AQP7, the extracellular discharge of glycerinum in adipocytes, and reduces the glycerinum accumulation in adipocytes[13]. AQP7 knockout mice show obesity and insulin resistance, and blood FFA, insulin, blood glucose and Leptin increase while adiponectin reduces[14]. Further study shows that the obesity is because that AQP7 is short, extracellular transport of glycerinum decreases, glycerol kinase is activated and triglyceride synthesis increases, finally causing fat cell size increase. The research team[15] connect aP2 gene enhancer and promoter with AQP7cDNA to build pEGFP-aP2-AQP7 3T3 adipose cell line, the detected content of AQP7 increases after differentiation and maturation, and the glycerinum (oil red staining) of cell lines significantly reduces, so it is believed that the increase of AQP7 makes glycerinum content decrease in adipocytes.

It was observed in this study that emodin had dose-dependent relationship with PPAR-γ and AQP, which might be because that emodin up-regulated PPAR-γ expression and it formed dimers with tretinoin X receptor to be combined with cis-acting element of AQP7 gene promoter, thereby leading to up-regulated adipocyte AQP7mRNA expression and positively regulating the expression of AQP7 at the transcription level[16].

In conclusion, it is confirmed in this study that AQP7 and PPARγ expression increase in 3T3-L1 adipocyte differentiation process, and emodin has dose-dependent regulation effect on AQP7 and PPARγ expression, showing that both also participate in adipocyte differentiation promotion by emodin. Therefore, the promoting effect of emodin in 3T3-L1 adipocyte differentiation is probably related to its regulation of PPARγ-AQP7 expression.

References