Gadd45a Enhances Fibroblasts to Leydig cells Transdifferentiation

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Abstract

Gadd45a (Growth arrest and DNA damage gene a) is a family member of Gadd45 and Gadd45a plays an essential role in gene-specific active DNA demethylation during adult stem cell differentiation. Therefore, Gadd45a is closely related to cell transdifferentiation. In this study, transcriptome analysis of cells obtained by fibroblasts(MEF) to leydig cells (LC) transdifferentiation by DGN (Dmrt1/Gata4/Nr5a1) transcription factors revealed significant up- regulation of Gadd45a and then interference with Gadd45a , the Gadd45a down - regulation. The results of western blotting, RT-qPCR, methylation level and radioim-munoassay show that Gadd45a is involved in the transdifferentiation of MEF into LC. Gadd45a and can be used as a target for research MEF to LC transdifferentiation, The above study provide us further understanding of transdifferentiation.

Keywords

Gadd45a, transdifferentiation, transcriptome analysis, methylation.

1. Introduction

Growth arrest and DNA damage gene a(Gadd45a) belongs to the family of Gadd45, which is closely related to biological life. Research has shown that GADD45A protein plays an essential role in gene-specific active DNA demethylation[1-3]. Studies show a significant up-regulation of Gadd45a expression during transdifferentiation [4]. The methylation inhibitor inhibits the methylation process and improve ransdifferentiation efficiency[5]. It means that Gadd45a can be used as a target for studying transdifferentiation can be used as an accelerating agent for promoting transdifferentiation.

2. Experimental Detail

2.1 Materials

DMEM medium, fetal bovine serum was purchased from Life Technologies. GADDD45A antibody was purchased from affinity. RNA Extraction Kit from magen. RT-qRNA enzyme purchased from Vazyme. Radioimmunoassay kit purchased from Beijing North Institute of Biotechnology.

2.2 Reagent Formulation

Table 1.PBS buffer

Chemicals	Amount
Nacl	8 g
Kcl	0.2 g
KH2PO4	0.24 g
Na2HPO4•12H20	3.63 g
Ultra-pure water volume to	1 L

If the solid medium is added agar powder 15 g, 121°Cautoclave 20min, stored at 4°C.

Table 2.Electrophoresis buffer

Chemicals	Amount
Tris-Base	3.03 g
SDS	1.0 g
Glycine	14.4 g
Ultra-pure water volume to	1 L

Table 3.Transfer membrane buffer

Chemicals	Amount
Glycine	5.8 g
Tris-Base	0.37 g
SDS	2.9 g
Methanol	200 mL
Ultra-pure water volume to	1 L

2.3 Experimental Procedure

A: RT-qPCR detection of mRNA expression

Total RNA was extracted from the reprogramming cells and then reverse transcribed to cDNA by TaKaRa (6210A).The product was used for RT-qPCR reaction The primer sequence and RT-qPCR reaction procedure as follow

Table 4.Primer sequence:

Gene	Sense	Anti-sense
Gapdh	CCTTCCGTGTTCCTACCC	CCCAAGATGCCCTTCAGT
Gadd45a	TGCTGCTACTGGAGAACGAC	TCCATGTAGCGACTTTCCCG

Table 5.Reaction system:

Chemical	Volume
SYBR	10 μL
Primer F(10 μM)	0.4 μL
Primer R(10 μM)	0.4 μL
RNase-Free Water	7.2 μL
cDNA	2 μL
Total Volum	20 μL

Reaction procedure:

95℃ 3:00min；95℃ 10s；60℃ 30s； 40 cycles；65℃~95℃ 5s

B: Radioimmunoassay detecting the secretion of testosterone

50 μL of the standard product and the sample to be tested are added to the radioimmuno tube;

Add 100 μL rabbit anti-T antibody mix, and incubate at 4°C overnight;

adding 100 μL of 125I-T, 37°C water bath for 45 min;

Add 500 μL donkey anti-rabbit immunoseparator, and stand for 15 min under room temperature conditions;

Centrifuge at 3540 rpm for 17 min, aspirate the supernatant and perform radioactivity counting on a gamma counter;

Analysis of results

C: Western blotting detection of protein expression

Collect cells

Lysing cells, measuring protein concentration, making samples

Protein electrophoresis and transfer

Closed, incubate primary antibody, washing membrane

Incubate secondary antibody, washing membrane

Detecte

2.4 Statistical Analysis

All data were presented as mean±SD and statistically significant was determined by one-way ANOVA. P value less than 0.05 was considered statistically significant

3. Results and Discussion

Transcriptome analysis of transdifferentiated cells revealed a significant up-regulation of Gadd45a expression and we used RT-qPCR to detect the levels of Gadd45a expression in different days. The results showed that Gadd45a showed significant up-regulation on different days after transfer to DGN transcription factor. as shown in Figure 1.

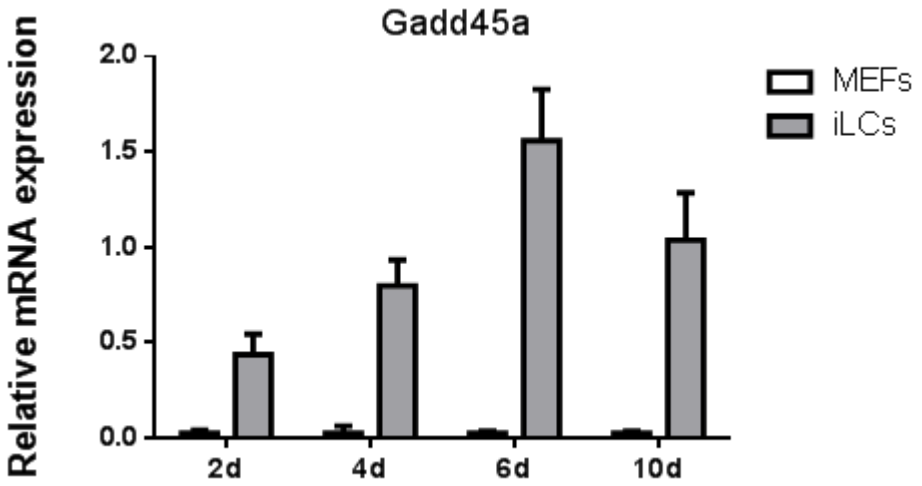

Figure 1. The Gadd45a expression on different days after transfer of DGN transcription factor

We used western blot to detect the level of GADD45A protein and RT-qPCR to detect the level of Gadd45a gene after interference with Gadd45a. The results showed that Gadd45a has a significant down-regulation in gene and protein levels. As show in Figure 2.

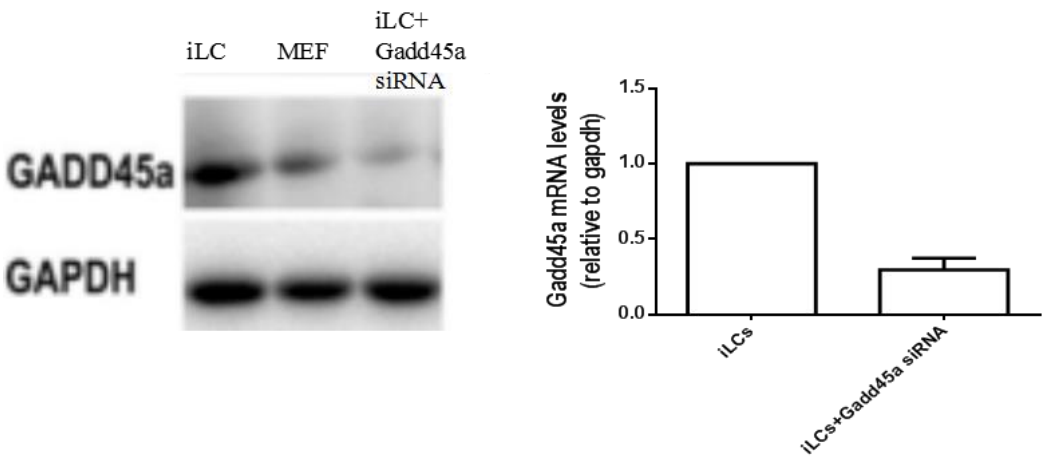

Figure 2. The Gadd45a expression in gene and protein level after interference with Gadd45a

After successfully interfering with Gadd45a, we used RT-qPCR to detect the related genes for iLC synthesis of the testosterone pathway. The results showed that after interference with Gadd45a the related genes in the testosterone synthesis pathway were significant down-regulation. It suggesting that Gadd45a is an important gene for iLCs obtained by transdifferentiation, as shown in Figure 3.

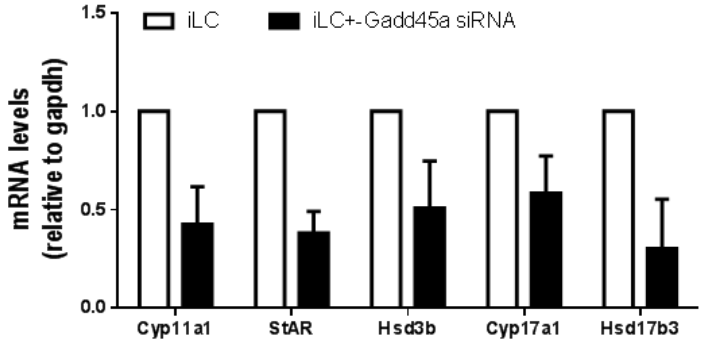

Figure 3. The expression of testosterone synthesis related genes in iLC after Gadd45a interference

We used radioimmunoassay to detect the levels of testosterone after interfered with Gadd45a to study the effect on testosterone levels. The results showed that there was a significant down-regulation of testosterone levels after interference with Gadd45a. as shown in Figure 4.

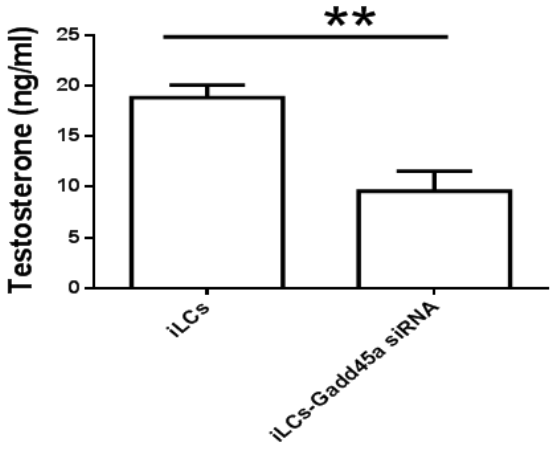

Figure 4. Testosterone levels after interfered with Gadd45a

Methylation and transdifferentiation are closely related, we detect the changes in methylation after interference with Gadd45a in iLCs. The results showed that there was a significant up-regulation of methylation levels after interference with Gadd45a. as shown in Figure 5.

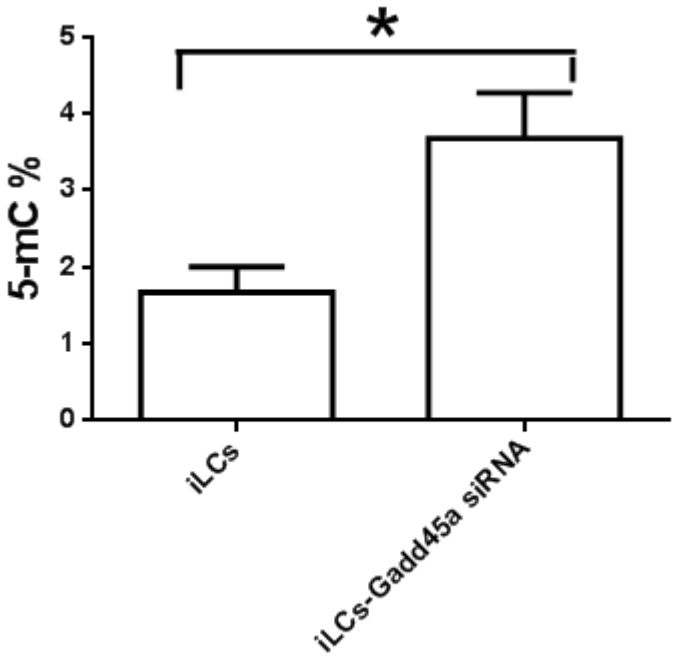

Figure 5. Methylation levels after interference with Gadd45a in iLCs

4. Conclusion

In this study, we found that the transfer of DGN transcription factor in MEFs can obtain iLCs and significantly up-regulate Gadd45a gene and to promote the production of testosterone and decrease the methylation. We also found that after interference with the Gadd45a the key genes in the testosterone synthesis pathway were significant down-regulation and methylation level was significant up-regulation during transdifferentiation. So Gadd45a and can be used as a target for research MEFs to LCs transdifferentiation.

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