BF-1461
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RESEARCH ARTICLE Indian Journal of Animal Research

Chip Detection of Rabbit Peripheral Blood Mononuclear Cells and 293T Cells Induced by Chicken Egg White Extract Shows Significant Differences in the Signaling Pathways that Regulate the Pluripotency of Stem Cells

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10.18805/IJAR.BF-1461

ABSTRACT

Background: Stem cell protein chips can detect stem cell-associated proteins expressed in cells.

Methods: We isolated and cultured rabbit peripheral blood mononuclear cells (R-PBMCs) in two bottles, one containing medium and one containing medium with 40% chicken egg white extract. Similarly, 293T cells were also divided into two vials: one vial contained medium and one vial contained medium with 40% chicken egg white extract. After 3 days of culture, proteins were extracted from the cells and used for the stem cell protein chip.

Result: Protein chip detection showed that 10 stem cell-associated proteins were increased in the rabbit peripheral blood mononuclear cells induced by chicken egg white extract. There were also 4 stem cell-associated proteins increased in the 293T cells induced by chicken egg white extract. The results of KEGG enrichment analysis indicated that peripheral blood mononuclear cells of rabbits were involved in the regulation of stem cell pluripotency before and after induction. Moreover, 293T cells were involved in the regulation of stem cell pluripotency before and after induction.

Key words: 293T cells, Chicken egg white extract, Induction, Rabbit peripheral blood mononuclear cells, Stem cell protein chip.

INTRODUCTION

To date, many studies have reported the composition and function of chicken egg white extract (Fang, *et al.* 2012; Kodama, *et al.* 2012; Abeyrathne, *et al.* 2014). However, few studies have detected whether chicken egg white extract can induce reprogramming of somatic cells (Mizutani, *et al.* 2012; Qiu, *et al.* 2013; Yang, *et al.* 2013; Ruan, *et al.* 2014; Ruan, *et al.* 2017). Previous studies have shown that human cells can be directly reprogrammed into an induced pluripotent stem cell (iPSC) state by expressing low levels of transcription factors (Takahashi, *et al.* 2007; Yu, *et al.* 2007). There are also reports that animal egg cell extracts can reprogram somatic cells, including pig egg extract (Miyamoto, *et al.* 2009) [11] and toad egg (Hansis, *et al.* 2004; Miyamoto, *et al.* 2007) extracts can reprogram somatic cell nuclei. In this study, we used 40% chicken egg white extract to induce rabbit peripheral blood mononuclear cells and 293T cells, cocultured them for 3 days, assessed the uninduced and induced cells with a stem cell protein chip and found that in the rabbit peripheral blood mononuclear cells treated with chicken egg white extract, 10 stem cell-related proteins were significantly increased. After 293T cells were induced with chicken egg white extract, 4 stem cell-related proteins were also significantly increased. The results of KEGG enrichment analysis showed that before and after induction of rabbit peripheral blood mononuclear cells, signaling pathways are involved in regulating the pluripotency of stem cells. Before and after the induction of 293T cells, signaling pathways are involved in regulating the pluripotency of stem cells. This finding shows that the cell has undergone reprogramming and it is reported as follows. We used advanced chip technology to detect the effect of chicken egg white extract to reprogram somatic cells and confirmed its effect. This research is very innovative.

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How to cite this article: Ruan, G.P., Yao, X., Mo, P., Wang, K., He, J., Yang, Z.L., Pang, R.Q., Zhu, X.Q. and Pan, X.H. (2022). Chip Detection of Rabbit Peripheral Blood Mononuclear Cells and 293T Cells Induced by Chicken Egg White Extract Shows Significant Differences in the Signling Pathways That Regulate the Pluripotency of Stem Cells Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1461.

Submitted: 31-10-2021 **Accepted:** 07-02-2022 **Online:** 11-03-2022

MATERIALS AND METHODS

Preparation of chicken egg white extract

Approximately 20 ml of egg white was aspirated from the egg, 20 ml of PBS was added, mixed well, frozen and thawed 3 times and centrifuged and the supernatant was collected for later use.

This work was carried out at The Basic Medical Laboratory of 920th Hospital of Joint Logistics Support Force of PLA in 2021.

Isolation and culture of rabbit peripheral blood mononuclear cells (R-PBMCs)

First, 25 ml of blood was collected from the rabbit ear venous vein, 12.5 ml of lymphocyte separation liquid was added to a centrifuge tube, blood was carefully added on top of the separation liquid and the sample was centrifuge at 2000 rpm for 20 min. Then, the buffy coat layer was aspired and the cells were washed twice. Two culture bottles were used: one had ordinary culture medium and the other had ordinary culture medium containing 40% chicken egg white extract. The ordinary culture medium was DMEM-F12 medium supplied with 10% fetal bovine serum. Three days after induction, the cells were collected to generate the stem cell protein chip. The 293T cell strain was purchased from the cell bank of the Kunming Institute of Zoology, Chinese Academy of Sciences. Two bottles of 293T cells were also cultured. One bottle had normal medium and the other had normal medium containing 40% chicken egg white extract. After 3 days of induction, the cells were collected. The stem cell protein chips were used to detect Stem Cell Associated Proteins in Cells. After the cells were collected and centrifuged, the supernatant was discarded and the pellet was quickly frozen in a refrigerator at -80°C.

Sample preparation

The lysate (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS) was added to the cell pellet and mix repeatedly and centrifuged at 14000 rpm for 10 min in a refrigerated centrifuge; the supernatant was collected.

Protein concentration determination and dilution scheme

After the cells were lysed, the protein concentration was determined (BCA method, Pierce Company, catalog number: 23227). A BCA kit was used to detect the protein concentration of the tissue lysates. The measurement results and sample preparation are shown in Table 1.

Table 1: Protein concentration results

Sample ID	μg/mL
293T (control)	9173.333
293T (induction)	7985.833
R-PBMC (control)	7048.333
R-PBMC (induction)	8348.333

Determination

Complete drying of the slide chip

The slide chip was taken out of the box (RayBio® Human Stem Cell Array G-Series 1). After equilibration at room temperature for 20-30 minutes, the packaging bag was opened, the sealing strip was uncovered and then, the chip was placed in a vacuum desiccator or dried at room temperature for 1-2 hours.

Blocking and incubation

1. First, 100 μL of 1× blocking solution (1% BSA in TBST) was added to each chip hole and incubate for 1 hour on a shaker at room temperature to avoid air bubbles.
2. The blocking solution was removed, 100 μL of sample was added to each well and one sample per array was incubated overnight at 4°C with shaking (load at 500 μg/ml).
3. A Thermo Scientific Wellwash Versa chip washer was used to clean the slides in two steps. First, the cells were washed with 1× Washing Solution I; 250 mL of 1× Washing Solution I was added per well and 10 washes were performed, with shaking for 10 s each time at a high intensity, followed by dilution with 20× solution I with deionized water. Then, 1×Washing Solution II was added to the channel for cleaning. A total of 250 μL of 1×Washing Solution II per well was used for 6 washes, with shaking for 10 s each time. The shaking intensity was high and 20×Washing Solution II was diluted with deionized water.
4. Biotin-labeled antibody was prepared, the biotin-labeled antibody tube was rapidly centrifuged and 300 μL of 1× blocking solution was added to each tube and mixed well. Then, 70 μL of biotin-labeled antibody was added to each well and incubated for 2 h at room temperature.
5. Cleaning was performed, as described in step 3.
6. Then, 70 μL of 1500 times diluted HiLyte Plus™ 555 streptavidin-Fluor was added to each well (after rapid centrifugation, 1.5 mL of blocking solution was added to the fluorescent agent-streptavidin tube) and the glass slide was sealed with a sealing strip. Then, the slide was wrapped with aluminum foil and incubated for 1-2 hours at room temperature with shaking at room temperature in the dark.
7. Cleaning was performed, as described in step 3.

Fluorescence detection was performed

1) The frame of the slide was removed, taking care not to touch the side of the slide with printed antibodies.
2) A laser scanner such as InnoScan 300 was used to scan the signal using the Cy3 or green channel (excitation frequency = 532 nm).
(i) Instrument model: InnoScan 300 Microarray Scanner.
(ii) Manufacturer: Innopsys.
(iii) Place of origin: Parc d'Activités Activestre; 31 390 Carbonne - France.
(iv) Scanning parameters: wavelength: 532 nm; resolution: 10 μm.

Chip analysis software was used to extract data and AAH-SC-G1 data analysis software was used for data analysis.

The original data obtained from the chip scanning were processed by Raybiotech software for chip background removal, normalization between chips and differential protein screening.

RESULTS AND DISCUSSION

1. After rabbit peripheral blood mononuclear cells were induced with chicken egg white extract, 10 stem cell-related proteins were found to be significantly increased (Table 2).
2. After 293T cells were induced with chicken egg white extract, there were also 4 stem cell-related proteins that were significantly increased (Table 3).
3. The results of KEGG enrichment analysis showed that before and after induction of rabbit peripheral blood mononuclear cells, signaling pathways were involved in regulating stem cell pluripotency (Fig 1).
4. The results of KEGG enrichment analysis showed that before and after induction of 293T cells, signaling pathways were involved in regulating stem cell pluripotency (Fig 2).

AFP, BMPR-1A, CD38, E-cadherin, HCG-beta, NANOG, NESTIN, Brachyury, GATA4, SOX2, SOX17 and VEGFR2 are proteins expressed in stem cells. When the cells are transformed into pluripotent stem cells, the expression of these proteins significantly increases (Teshigawara, *et al.* 2017; Wang, *et al.* 2017). Thus, when the expression of these proteins is significantly increased in the cell, the cell may be transformed into a pluripotent stem cell.

We found that the expression of 10 stem cell-related proteins in rabbit peripheral blood mononuclear cells cocultured with 40% chicken egg white extract for 3 days was significantly increased. KEGG enrichment analysis of the rabbit peripheral blood mononuclear cells was performed before and after induction. The signaling pathway was related to regulation of the pluripotency of stem cells. In 293T cells cocultured with 40% chicken egg white extract for 3 days, the expression of 4 stem cell-related proteins was significantly increased. The results of KEGG enrichment analysis showed that before and after induction of 293T cells, signaling pathways were involved in regulating stem cell pluripotency. This finding further proves that the chicken egg white extract promotes somatic cell reprogramming.

Compared with pig egg extract and toad egg extract, chicken egg white extract is more convenient to obtain, the amount obtained is greater and the extraction process should be sterile (Monika 2021). This extract can be directly used for cell culture without filtration and sterilization and has greater application value (Lyayla 2021). In our previous research, we found that chicken egg white extract can promote cell proliferation and increase the expression of the pluripotent factor NANOG. In this research, we further used a stem cell protein chip to detect the proteins expressed by a variety of stem cells (Bujun 2021). The results showed that in the rabbit periphery, the expression of 10 stem cell-related proteins (Jyotsana 2021) in blood mononuclear cells was significantly increased and the expression of 4 stem cell-related proteins in 293T cells was significantly increased, indicating that the chicken egg white extract contains components that promote somatic cell reprogramming. We speculate the components of chicken egg white extract may have contributed to the regulation of pluripotency of stem cell are proteins with molecular weight less than 10KDa. The proteins effect or the function of those proteins may be promote somatic cell reprogramming. Further research on chicken eggs and the active ingredients in the clear extract is needed. This extract will have a wide range of applications in cell biology (Liyun 2021). The increase of stem cell protein expression in cells after induction with 40% final concentration

Table 2: The expression of 10 stem cell-related proteins was significantly increased after rabbit peripheral blood mononuclear cells were induced with chicken egg white extract.

Entrez ID	Protein ID	Log2 FC	R-PBMC control	R-PBMC 40% egg white
174	AFP	-1.35943	200.3553	515.6454
657	BMPR-IA	-0.26859	148.1619	178.6845
952	CD38	-1.33959	164.4372	417.6863
999	E-Cadherin	-1.10169	335.0479	720.1801
1082	HCG-beta	-0.5826	439.9959	659.4093
79923	NANOG	-1.64911	93.16239	294.3306
10763	NESTIN	-0.06754	482.9291	506.1216
6657	SOX2	-0.68387	155.4577	250.3397
64321	SOX17	-4.28815	6.73463	150.1131
3791	VEGFR2	-0.30538	143.6721	177.7775

Table 3: After 293T cells were induced with chicken egg white extract, the expression of four stem cell-related proteins was significantly increased.

Entrez ID	Protein ID	Log2FC	293T control	293T 40% egg white
174	AFP	-1.04504	185	382.7967
6862	Brachyury	-0.36933	241.25	311.9269
2626	GATA4	-0.24703	3942	4678.405
6657	SOX2	-0.46259	348.5	480.617

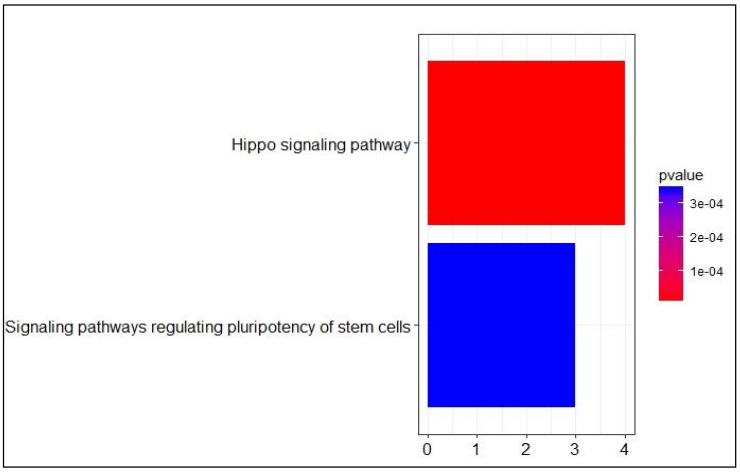

Fig 1: KEGG enrichment analysis results before and after induction of rabbit peripheral blood mononuclear cells. Blue represents the signaling pathway that regulates the pluripotency of stem cells.

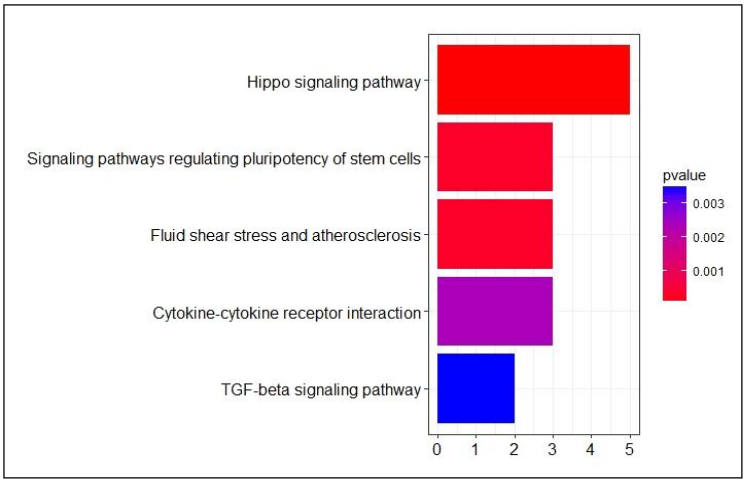

Fig 2: KEGG enrichment analysis results before and after induction of 293T cells. From top to bottom, the second box represents the signaling pathway that regulates stem cell pluripotency.

of chicken egg white extract indicates the transformation of somatic cells to stem cells, indicating that there are small protein molecules in the chicken egg white extract that can induce reprogramming, which will help the research in the field of cell biology.

CONCLUSION

Cocultivation with 40% chicken egg white extract can induce reverse differentiation of cells into stem cells and the expression of stem cell-related proteins is significantly increased.

ACKNOWLEDGMENT

We thank American Journal Experts for assisting with the English preparation of this manuscript.

Author contribution

Jie He, Ping Mo, Guang-ping Ruan and Xiang Yao made substantial contributions to the study conception and design, data acquisition and data analysis and interpretation. Jie He, Guang-ping Ruan, Zai-ling Yang, Kai Wang and Xiang Yao conducted the experiments. Xiang Yao and Jie He agree to be accountable for all aspects of the work and ensure that questions related to the accuracy or integrity of any part of the study will be appropriately investigated and resolved. Xing-hua Pan and Guang-ping Ruan provided final approval of this version of the manuscript for publication. Guang-ping Ruan, Xing-hua Pan, Jie He, Xiang-qing Zhu and Rong-qing Pang were involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript.

Funding statement

This work was supported by grants from the Yunnan Science and Technology Plan Project Major Science and Technology Project (2018ZF007), the Yunnan Province Applied Basic Research Program Key Project (2018FA041, 202101AS070039), the General Project of Yunnan Applied Basic Research Program (202101AT070212), the National Natural Science Foundation (31970515) and the 920th Hospital of

the PLA Joint Logistics Support Force In-hospital Technology Plan (2019YGB17, 2019YGA05).

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