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Ginsenoside Rg1 promotes neural differentiation of mouse adipose-derived stem cells via the miRNA-124 signaling pathway*

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We have explored the role of ginsenoside Rg1 in promoting the differentiation of mouse adipose-derived stem cells (mADSC) towards the neuronal lineage. The central nervous system has long been regarded as incapable of self-repair; therefore neuronal differentiation from stem cells is of great interest. However, the use of embryonic stem cells is limited due to their inaccessibility and for ethical reasons, so the search is on for alternative pluripotent cells capable of differentiating into neuronal cells. Adipose-derived stem cells (ADSC) can differentiate into different cell types, including neuronal cells; their accessibility, low risk, and capacity for long-term growth and self-renewal have made them the preferred stem cell type for clinical applications. Several methods have been indicated for promoting the neuronal differentiation of ADSC, but the mechanism of this process has not been clearly identified. As our previous study showed that microRNA-124 (miRNA-124) plays a positive role in promoting the neural differentiation of ADSC, we wanted to find reagents

that can upregulate miRNA-124 expression during neural differentiation.

Recent studies have indicated that ginsenoside Rg1, one of the most active and abundant components of ginseng, plays an important role in neural cell proliferation and differentiation (Attele et al., 1999). Lu et al. (2009) found that it has a neuroprotective role, and that it can regulate the proliferation of neural progenitor cells. Rg1 also plays an important role in cell differentiation: for example, it stimulates odontogenic/osteogenic differentiation of human dental pulp stem cells (Wang et al., 2014) and facilitates neural differentiation of mouse embryonic stem cells (Wu et al., 2013). To understand the role of ginsenoside Rg1 in neural differentiation, we investigated its effects on the neural differentiation of mouse ADSC. Our findings suggest that it promotes ADSC neural differentiation through the miRNA-124 signaling pathway.

First we demonstrated that ginsenoside Rg1 promotes cell proliferation during ADSC neural differentiation. As shown in Fig. 1a, the isolated mouse ADSC at passage 3 had strong11 tr Ginsenoside Rg1-treated group 1 higher proliferation rate after 24 h compared with the control group (Fig. 1c), suggesting that ginsenoside Rg1 has a positive effect on cell proliferation during neural differentiation.

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Second we found that ginsenoside Rg1 can pro-

Unsurprisingly, ginsenoside Rg1 treatment significantly decreased anti-neural factor SCP1 expression (Fig. 2d), suggesting that ginsenoside Rg1 could promote ADSC neural differentiation through down-regulation of anti-neural factor SCP1 expression.

In conclusion, our study demonstrated that ginsenoside Rg1 promotes cell proliferation and neural differentiation of mouse ADSC *in vitro*, but further work is needed to identify the molecular mechanisms involved, particularly in ginsenoside Rg1-mediated miRNA-124 expression.

We found that ginsenoside Rg1 promoted mouse ADSC proliferation and neural differentiation in a dose-dependent manner. A high concentration of ginsenoside Rg1 (100 $\mu\text{g/ml}$) enhanced the expression of neural specific markers when mouse ADSC were maintained in a neural inductive medium for 24 h (Fig. 2a). Despite the fact that nestin and β -tubulin III represent just one marker of ADSC neural differentiation, these findings, together with previous reports

demonstrate the potential use of Rg1 in nerve regeneration, and highlight the potential application of ginsenoside Rg1 to promote cell proliferation and differentiation. More studies are needed to elucidate the mechanisms of Rg1-induced neural differentiation.

During the process of neural differentiation, ginsenoside Rg1 increased the level of miRNA-124 in a dose-dependent manner. miRNA-124 plays an important role in CNS development, and is expressed abundantly in neural cells. miRNA-124 over-expression induces neural differentiation of mouse neuroblastoma cell lines Neuro2a and CAD and embryonal cell line P19 (Makeyev

The process of neurogenesis is accompanied by down-regulation of nonneuronal genes and up-regulation of neuronal genes. Repressor element 1 (RE1)-silencing transcription factor (REST), also known as neuron-restrictive silencer factor (NRSF), acts as a transcriptional repressor. Down-regulation of REST increases neuronal genes expression (Ballas et al., 2005). Like REST, SCP1 is recruited to RE1-containing genes by REST and exerts an anti-neural role in nonneuronal tissues (Yeo et al., 2005). In our results, during the process of neural differentiation, ginsenoside Rg1 facilitated neural markers expression through miRNA-124-mediated SCP1 degradation. However, our results do not rule out the possibility that ginsenoside Rg1 may exert its role through other miRNAs or proteins.

Compliance with ethics guidelines

Juan DONG, Guo ZHU, Tian-cheng WANG, and Fu-shan SHI declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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| | Rg1 | miRNA-124 | |
|-------------------|------|-----------|-------------|
| | Rg1 | | |
| | Rg1 | miRNA-124 | |
| BALB/c | | | |
| PBS | 3- | -1- | IBMX |
| IBMX+Rg1 | | IBMX+Rg1 | |
| IBMX+Rg1 | | | |
| | | qPCR | |
| miRNA-124 | | | |
| nestin | III- | | III-tubulin |
| | 1 | SCP1 | |
| | IBMX | | |
| Rg1 | | | |
| Ig19HEF##10cpB0p@ | | | |