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

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 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 -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 *et al*

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.

References

Attele, A.S., Wu, J.A., Yuan, C.S., 1999. Ginseng pharmacology: multiple constituents and multiple actions. *Biochem. Pharmacol.*, **58**(11):1685-1693. http://dx.doi.org/10.1016/S0006-2952(99)00212-9

Ballas, N., Grunseich, C., Lu, D.D., *et al.*, 2005. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell*, **121**(4):645-657. http://dx.doi.org/10.1016/j.cell.2005.03.013

Krichevsky, A.M., Sonntag, K.C., Isacson, O., *et al.*, 2006. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells*, **24**(4):857-864. http://dx.doi.org/10.1634/stemcells.2005-0441

Lagos-Quintana, M., Rauhut, R., Yalcin, A., *et al.*, 2002. Identification of tissue-specific microRNAs from mouse. *Curr. Biol.*, **12**(9):735-739. http://dx.doi.org/10.1016/S0960-9822(02)00809-6

Lu, M.C., Lai, T.Y., Hwang, J.M., *et al.*, 2009. Proliferation- and migration-enhancing effects of ginseng and ginsenoside Rg1 through IGF-I- and FGF-2-signaling pathways on RSC96 Schwann cells. *Cell Biochem. Funct.*, **27**(4): 186-192. http://dx.doi.org/10.1002/cbf.1554

Makeyev, E.V., Zhang, J., Carrasco, M.A., *et al.*, 2007. The microRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol. Cell*, **27**(3):435-448. http://dx.doi.org/10.1016/j.molcel.2007.07.015

Ning, H., Lin, G., Lue, T.F., *et al.*, 2006. Neuron-like differentiation of adipose tissue-derived stromal cells and vascular smooth muscle cells. *Differentiation*, **74**(9-10): 510-518. http://dx.doi.org/10.1111/j.1432-0436.2006.00081.x

Ning, H., Lin, G., Fandel, T., *et al.*, 2008. Insulin growth factor signaling mediates neuron-like differentiation of adipose tissue-derived stem cells. *Differentiation*, **76**(5):488-494. http://dx.doi.org/10.1111/j.1432-0436.2007.00240.x

Shi, F., Yang, Y., Wang, T., *et al.*, 2016. Cellular prion protein promotes neuronal differentiation of adipose-derived stem cells by upregulating miRNA-124. *J. Mol. Neurosci.*, **59**(1):48-55. http://dx.doi.org/10.1007/s12031-016-0733-8

Wang, P., Wei, X., Zhang, F., *et al.*, 2014. Ginsenoside Rg1 of *Panax ginseng* stimulates the proliferation, odontogenic/osteogenic differentiation and gene expression profiles of human dental pulp stem cells. *Phytomedicine*, **21**(2): 177-183. http://dx.doi.org/10.1016/j.phymed.2013.08.021

Wu, J., Pan, Z., Cheng, M., *et al.*, 2013. Ginsenoside Rg1 facilitates neural differentiation of mouse embryonic stem cells via GR-dependent signaling pathway. *Neurochem. Int.*, **62**(1):92-102. http://dx.doi.org/10.1016/j.neuint.2012.09.016

Yeo, M., Lee, S.K., Lee, B., *et al.*, 2005. Small CTD phosphatases function in silencing neuronal gene expression. *Science*, **307**(5709):596-600. http://dx.doi.org/10.1126/science.1100801

Rg1 miRNA-124

Rg1

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BALB/c

PBS 3- -1- IBMX
IBMX+Rg1 IBMX+Rg1
IBMX+Rg1

qPCR
miRNA-124
nestin III- III-tubulin
1 SCP1
IBMX

Rg1

IBMX [illegible]