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Contributed Mini Review

Role of gangliosides in the differentiation of human mesenchymal-derived stem cells into osteoblasts and neuronal cells

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Gangliosides are complex glycosphingolipids that are the major component of cytoplasmic cell membranes, and play a role in the control of biological processes. Human mesenchymal stem cells (hMSCs) have received considerable attention as alternative sources of adult stem cells because of their potential to differentiate into multiple cell lineages. In this study, we focus on various functional roles of gangliosides in the differentiation of hMSCs into osteoblasts or neuronal cells. A relationship between gangliosides and epidermal growth factor receptor (EGFR) activation during osteoblastic differentiation of hMSCs was observed, and the gangliosides may play a major role in the regulation of the differentiation. The roles of gangliosides in osteoblast differentiation are dependent on the origin of hMSCs. The reduction of ganglioside biosynthesis inhibited the neuronal differentiation of hMSCs during an early stage of the differentiation process, and the ganglioside expression can be used as a marker for the identification of neuronal differentiation from hMSCs. [BMB Reports 2013; 46(11): 527-532]

INTRODUCTION

Gangliosides are sialic acid-containing glycosphingolipids (GSLs) ubiquitously distributed in tissues and body fluids, and are abundantly expressed in the nervous system (1). The biological role of gangliosides in cellular regulation is wellrecognized (2-6). Gangliosides are known to function in cell proliferation, adhesion, migration, apoptosis, and cell-cell and cell-substratum interactions. They can also act as receptors for bacterial toxins (7-9). Numerous studies have confirmed that various gangliosides and their expression levels are developmentally controlled, and are specific for cell types (10-12). Recently, it has also been suggested that gangliosides initiate the aggregation of amyloid-β peptide and contribute to the onset of Alzheimer's disease (13).

Stem cells can be used for the study of developmental processes and offer tremendous potential for clinical applications as an unlimited source for transplantation and tissue regeneration therapies (14). Generally, there are 2 types of stem cells used in clinical applications: mouse embryonic stem cells (mESCs) and mesenchymal stem cells (MSCs). The mESCs are pluripotent cells, which are generated from the inner cell mass of blastocysts (15). In recent years, MSCs have received considerable attention as a potential source of cell-based therapies, and as a cell type that supports the engraftment of hematopoietic stem cells (HSC) (16, 17). MSCs can be easily obtained, typically from bone marrow, but also from other sources, such as umbilical cord blood, adipose tissue, and the placenta (18-20). In previous studies, multipotent neural cells have been generated from MSCs cultured in neural stem cell (NSC) culture conditions, and these cells could be further differentiated into astrocytes, neurons, and oligodendrocytes (21-23).

Osteoblasts are mononucleated cells that are responsible for bone formation. When osteoprogenitors start to differentiate into osteoblasts, they begin to express a range of genetic markers, including alkaline phosphatase (ALP), osteocalsin, collagen I, and osterix (24, 25).

Cell differentiation is a highly regulated process that depends on many extracellular and intracellular factors for its modulation. Several studies have reported that gangliosides are important for neuronal (26) and osteoblast differentiation (27) of mESCs and MSCs. In the present study, we show different functions of gangliosides in the differentiation of human MSCs (hMSCs) into osteoblasts (Table 1 and 3) and neuronal cells (Table 1 and 2).

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FUNCTIONS OF GANGLIOSIDES IN THE DIFFERENTIATION OF hMSCs INTO OSTEOBLASTS

Several studies reported different functions of gangliosides in the differentiation of hMSCs into osteoblasts. Gangliosides are known to functionally regulate several growth factor receptors and fibroblast growth factor receptors (28). Epidermal growth factor receptor (EGFR) is a 170 kDa transmembrane glycoprotein that signals various processes, including proliferation, and differentiation, in a wide variety of cell types (29). Several studies have shown that EGFR, extracellular signal-regulated kinases 1/2 (ERK1/2), and mitogen-activated protein (MAP) kinase are involved in the regulation of osteoblastic differentiation (30-33). In addition, differentiation of hMSCs into osteoblasts is regulated by EGFR activation (34). Therefore, we investigated the relationship between gangliosides and EGFR activation during the differentiation of hMSCs into osteoblasts. In a previous study, the effects of gangliosides on osteoblastogenesis were observed (34). However, only GM3, GM2, and GD1a were observed in the hMSCs. In addition, high-performance thin-layer chromatography (HPTLC) showed that ganglioside GM3 expression was decreased, whereas ganglioside GD1a expression was increased during the differentiation of hMSCs into osteoblasts.

In previous studies examining the expression patterns of

Table 1. Gangliosides expression in the differentiation of hMSCs, hADSCs and hDPSCs into neuronal cells and osteoblasts

Cells	Gangliosides expression on neuronal cells differentiation	Cells	Gangliosides expression on osteoblasts differentiation
hDPSCs	GM3, GM2, GD1a	hADSCs	GM3, GM2, GD1a
20% FBS + 1 mM BME	GM3, GD1a	hADSCs-derived osteoblasts	GM3, GM2, GD1a
SPM + 2 mM BME	GM3, GD1a	hDPSCs	GM3, GM2, GD1a
Differentiation on 1 week	GM3, GD3, GD1a	hDPSCs-derived osteoblasts	GM3, GM2, GD1a
Differentiation on 2 weeks	GM3, GD3, GD1a		

Table 2. Roles of gangliosides in the differentiation of hMSCs into neuronal cells

Gangliosides	Roles	References
GD2	Deficiency leads to down-regulation of genes	Takamiya *et al.*, 1996 (51)
	Marker for neuronal differentiation	Kwak *et al.*, 2006 (46)
	Promoter the differentiation of neuronal cells	Todeschini *et al.*, 2008 (42)
GM1	Protection from apoptosis	Ferrari *et al.*, 1995 (52)
	Regulatory role during neurogenesis and regeneration	Cavallini *et al.*, 1999 (53)
		Stojiljkovic *et al.*, 1996 (54)
GT1b	It enhances actin-rich dendrite generation	Higashi and Chen, 2004 (44)
	Inhibitory effect on neuritis out growth	Vinson *et al.*, 2001 (55)
	Induction of differentiation of (mESs and) MSCs into neuronal cells	Kwak *et al.*, 2006 (46)
	Up-regulation in synapses in brain	Kotani *et al.*, 1993 (43)
GD3 & GD1a	Induction of early neuronal differentiation	Ryu *et al.*, 2009 (40)
	Brain development	Jennemann *et al.*, 2005 (56)
	Maturation of neuronal cells	Yamashita *et al.*, 1999 (57)
GM3 & GD3	Regulation cell differentiation and proliferation	Kwak *et al.*, 2006 (46)
GT1b & GM1	Induction of differentiation of (mESCs and) MSCs into neuronal cell	Kwak *et al.*, 2006 (46)
	Biomarker to neuronal differentiation	Kwak *et al.*, 2011 (58)

Table 3. Roles of gangliosides in the differentiation of hMSCs into osteoblasts

Gangliosides	Roles	References
GD1a	It enhances EGF-induce EGFR phosphorylation, which promotes osteoblast differentiation	Jaiswal *et al.*, 2000 (30)
		Liu *et al.*, 2004 (35)
	It improves osteoblast ERK signaling through EGFR phosphorylation	Kim *et al.*, 2008 (34)
GM3	It reduces EGFR phosphorylation	Kim *et al.*, 2008 (34)
GD1a & GM3	They regulate the initiation step of osteoblast differentiation	Kim *et al.*, 2008 (34)
	They are important for beta-glycophosphate-, ascorbic acid-, and dexamethasone-induced osteoblastogenesis	

gangliosides in the differentiation of human adipose and dental pulp-derived MSCs into osteoblasts, the expression of GD1a was significantly increased (27, 34). Additionally, it was reported that the addition of gangliosides to culture media enhanced the phosphorylation of EGFR during differentiation of hMSCs into osteoblasts, and that the expression levels of ganglioside GD1a in the differentiated osteoblasts increased compared to that in hMSCs. According to Kim *et al*. (2008), a reduction in AG1478-stimulated EGFR phosphorylation was recovered by GD1a (34). However, treatment with GM3 reduced EGF and AG1478-stimulated EGFR phosphorylation. This interpretation represents a novel effect of gangliosides on cell signaling, in which stochastic increases in the proximity of these receptors to one another leads to enhanced efficiency of binding and signaling after stimulation by a growth factor. Indeed, GM3 seems to act as a physiological competitor for EGFR dimerization by binding directly to the extracellular domain of EGFR, consequently inhibiting EGFR autophosphorylation (28). In contrast to GM3, GD1a increases the effective amount of high-affinity EGFR without total receptor protein and facilitates receptor-receptor interactions, which triggers increased EGFR dimerization, eventually enhancing EGFR-mediated signaling (35).

It has been revealed that ganglioside GD1a expression was significantly elevated in the differentiation of osteoblasts from hMSCs. Therefore, the specific role of ganglioside GD1a was investigated because its role in osteogenic celldifferentiation was not fully understood. Previous studies (36, 37) suggest that ganglioside GD1a plays a major role in regulating the differentiation of hMSCs into osteoblasts. The suppression of ganglioside GD1a synthesis by the knockdown of ST3Gal II mRNA, which is a rate-limiting enzyme for ganglioside GD1a synthesis, possibly disturbs the osteoblast differentiation of hMSCs. Yang *et al*. (2011) reported that osteoblasts that had been differentiated from hMSCs by ST3Gal II mRNA knockdown showed a significant decrease in ALP activity and ganglioside GD1a expression (37). The decrease in ganglioside GD1a expression in osteoblasts showed accordance with a dramatic reduction in ST3Gal II mRNA expression in hMSCs, indicating that ST3Gal II shRNA-inserted lentiviral infection in hMSCs successfully suppressed the expression of ST3Gal II mRNA, thereby resulting in inhibition of ganglioside GD1a biosynthesis. These results possibly indicate that suppression of ganglioside GD1a expression disturbed the differentiation into osteoblasts.

Several studies have also reported that MSCs are found in various tissues, such as bone marrow, umbilical cord blood, adipose tissue (38), and dental pulp (20, 25). Therefore, the roles of gangliosides in osteoblast differentiation depend on the origin of the hMSCs. Lee *et al*. (2010) have compared ganglioside expression for the differentiation of human adipose-derived stem cells (hADSCs) and human dental pulp-derived stem cells (hDPSCs) into osteoblasts (27). Gangliosides GM3, GM2, and GD1a were detected in hADSCs and hDPSCs (Table 1). In addition, only GD1a expression was increased during osteoblast differentiation in hADSCS, whereas in hDPSCs, GM3, GM2, and GD1a were mostly increased. ALP activity was also increased in differentiated osteoblasts when compared to hADSCs and hDPSCs. Interestingly, there was more increase in the ALP activity of differentiated osteoblasts from hDPSCs than hADSCs-derived osteoblasts. These results suggest that gangliosides might play a role in the differentiation of hADSCs and hDPSCs into osteoblasts, and that the role is more important in regulating the osteoblast-differentiation of hDPSCs compared to hADSCs.

FUNCTIONS OF GANGLIOSIDES IN THE DIFFERENTIATION OF hMSCS INTO NEURONAL CELLS

Accumulating evidence has suggested cellular roles of gangliosides in the regulation of cell differentiation and proliferation (39, 40). Previous studies have suggested that gangliosides are important factors for neuronal differentiation of hMSCs (7, 26). There have been a number of fruitful approaches in determining the role of gangliosides in neuronal differentiation. One of the earliest and most direct was the study of correlative changes in ganglioside composition that accompany normal development *in vivo* and *in vitro* (41). For example, the monosialoganglioside GM1 has been shown to promote the differentiation of various neuronal cells in culture (42). Ganglioside GT1b is expressed in the synapses of the brain (43). Higashi and Chen (2004) found that the exposure of neurons to ganglioside GT1b for 3 days drastically enhanced actin-rich dendrite generation (44).

Another study showed that when hMSCs were cultured under neuronal differentiation conditions, neuronal cell marker genes, such as Nestin, MAP-2, and NeuN, were expressed (40). Moreover, immunostaining and HPTLC analysis showed that an increase in ganglioside biosynthesis was associated with neural differentiation of hMSCs. Specifically, a significant increase in GD3 and GD1a expression was observed during neural differentiation. Table 1 shows ganglioside expression during neuronal differentiation of hMSCs. To evaluate the importance of gangliosides in the neural differentiation of hMSCs, UCGC gene expression was knocked down using viral shRNA to block the biosynthesis of gangliosides. The results suggested that gangliosides play a role in the neural differentiation process of hMSCs. Next, it was demonstrated that expression of GD3 increased, along with early neuronal differentiation of embryonic stem cells (ESCs), and that the expression of GD1a was only detected when ESCs further differentiated into neuronal cells (36). Therefore, the ganglioside expression patterns during neuronal differentiation of hMSCs are similar to those of ESCs.

Numerous studies have suggested a close relationship between the regulation of ganglioside levels through exogenous drug analogues and the induction of neuronal differentiation. In a study by Osanai *et al*. (2003), levels and types of gangliosides were observed to change during neuronal differentiation,

and GD3, GT1b, and GQ1b were enhanced when neural differentiation of embryonic carcinoma cells was induced by retinoic acid (RA) (45). Kwak *et al*. (2006) have suggested that ganglioside GT1b is necessary for the differentiation of mESCs and MSCs into neuronal cells (46). There is accumulating evidence that ganglioside GT1b may regulate neuronal cell differentiation. Previous studies reported that ganglioside GD2 may also be involved in cell-context-specific cellular functions (10, 11). Gangliosides are ubiquitously expressed in many tissues, including the central nervous system, where GD2 plays a modulatory role in balancing the expression of both simple and complex gangliosides on the cell surface (47). Another study showed that ganglioside GD2 expression is closely associated with neuronal differentiation of human umbilical cord blood-derived mesenchymal stem cells (48). It has also been suggested that the expression of ganglioside is closely related to neuronal differentiation of embryonic stem cells *in vitro* (36). According to one study, ganglioside expression can be used as a marker for identification of neuronal differentiation from embryonic bodies (EBs) and MSCs (46). Some researchers have also found that GD2 is useful as a marker molecule for isolating mesenchymal stem cells, multipotent stromal cells that can differentiate into cells of the mesodermal lineage, such as myocytes, osteocytes, adipocytes, and chondrocytes, from human bone marrow (49) and umbilical cord blood (50). Table 2 summarizes the different roles of gangliosides in the differentiation of hMSCs into neuronal cells, and Table 3 indicates the various roles of gangliosides in the differentiation of hMSCs into osteoblasts. As described above, hMSCs have the potential to differentiate into osteoblasts or neuronal cells. This study also suggests that various gangliosides have important roles regarding osteoblast or neuronal differentiation of hMSCs, and those roles depend on the origin of the hMSCs. This study reveals that more gangliosides are involved in neuronal differentiation than in osteoblast differentiation. Such information will undoubtedly stimulate progress in the understanding of stem cell-based therapeutic strategies for a variety of tissue damage conditions and degenerative diseases. Further identification of gangliosides in stem cells and thorough characterization of the expression of marker gangliosides will contribute to progress in basic research and clinical applications in stem cell therapy.

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REFERENCES

1. Yu, R. K., Nakatani, Y. and Yanagisawa, M. (2009). The role of glycosphingolipid metabolism in the developing brain. *J. Lipid Res.* **50 (Suppl)**, S440-445.
2. Allende, M. L. and Proia, R. L. (2002). Lubricating cell signaling pathways with gangliosides. *Curr. Opin. Struct. Biol.* **12**, 587-592.
3. Spiegel, S. and Fishman, P. H. (1987). Gangliosides as bimodal regulators of cell growth. *Proc. Nat. Acad. Sci. U. S. A.* **84**, 141-145.
4. Hannun, Y. A. and Bell, R. M. (1989). Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. *Science* **243**, 500-507.
5. Hakomori, S. and Igarashi, Y. (1993). Gangliosides and glycosphingolipids as modulators of cell growth, adhesion, and transmembrane signaling. *Adv. Lipid Res.* **25**, 147-162.
6. Oliver, S. P., Lewis, M. J., Gillespie, B. E. and Dowlen, H. H. (1992). Influence of prepartumantibiotic therapy on intramammary infections in primigravid heifers during early lactation. *J. Dairy Sci.* **75**, 406-414.
7. Hakomori, S. (1990). Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. *J. Biol. Chem.* **265**, 18713-18716.
8. Martini, F., Riondino, S., Pignatelli, P., Gazzaniga, P. P., Ferroni, P. and Lenti, L. (2002). Involvement of GD3 in platelet activation. A novel association with Fcgamma receptor. *Biochim. Biophys. Acta* **1583**, 297-304.
9. Wang, X. Q., Sun, P. and Paller, A. S. (2002). Ganglioside modulation regulates epithelial cell adhesion and spreading via ganglioside-specific effects on signaling. *J. Biol. Chem.* **277**, 40410-40419.
10. Yu, R. K., Macala, L. J., Taki, T., Weinfield, H. M. and Yu, F. S. (1988). Developmental changes in ganglioside composition and synthesis in embryonic rat brain. *J. Neurochem.* **50**, 1825-1829.
11. Yu, R. K. (1994). Development regulation of ganglioside metabolism. *Prog. Brain Res.* **101**, 31-44.
12. Yamamoto, A., Haraguchi, M., Yamashiro, S., Fukumoto, S., Furukawa, K., Takamiya, K., Atsuta, M., Shiku, H. and Furukawa, K. (1996). Heterogeneity in the expression pattern of two ganglioside synthase genes during mouse brain development. *J. Neurochem.* **66**, 26-34.
13. Matsuzaki, K. (2010). Ganglioside cluster-mediated aggregation and cytotoxicity of amyloid beta-peptide: molecular mechanism and inhibition. *Yakugaku Zasshi* **130**, 511-515.
14. Fortier, L. A. (2005). Stem cells: classifications, controversies, and clinical applications. *Vet. Surg.* **34**, 415-423.
15. Evans, M. J. and Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154-156.
16. Kulterer, B., Friedl, G., Jandrositz, A., Sanchez-Cabo, F., Prokesch, A., Paar, C., Scheideler, M., Windhager, R., Preisegger, K. H. and Trajanoski, Z. (2007). Gene expression profiling of human mesenchymal stem cells derived from bone marrow during expansion and osteoblast differentiation. *BMC Genomics* **8**, 70.
17. Bron, D., De Bruyn, C., Balasse, H., Ley, P., De Hemptinne, D., von Lennep, E., Homans, C., Marckowicz, E., Mathieu, P., Deleuse, M. D., Francotte, J., Thomas, d., dorval, C., Dejeneffe, M., Andrien, M. and Delforge, A. (2008). Cord blood: from bench to bedside.

Bull Cancer **95**, 314-319.
18. Kern, S., Eichler, H., Stoeve, J., Kluter, H. and Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* **24**, 1294-1301.
19. Fukuchi, Y., Nakajima, H., Sugiyama, D., Hirose, I., Kitamura, T. and Tsuji, K. (2004). Human placenta-derived cells have mesenchymal stem/progenitor cell potential. *Stem Cells* **22**, 649-658.
20. Gronthos, S., Mankani, M., Brahim, J., Robey, P. G. and Shi, S. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc. Nat. Acad. Sci. U. S. A.* **97**, 13625-13630.
21. Fu, L., Zhu, L., Huang, Y., Lee, T. D., Forman, S. J. and Shih, C. C. (2008). Derivation of neural stem cells from mesenchymalstemcells: evidence for a bipotential stem cell population. *Stem Cells Dev.* **17**, 1109-1121.
22. Deng, J., Petersen, B. E., Steindler, D. A., Jorgensen, M. L. and Laywell, E. D. (2006). Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells* **24**, 1054-1064.
23. Yang, Q., Mu, J., Li, Q., Li, A., Zeng, Z., Yang, J., Zhang, X., Tang, J. and Xie, P. (2008). A simple and efficient method for deriving neurospheres from bone marrow stromal cells. *Biochem. Biophys. Res. Commun.* **372**, 520-524.
24. Gerstenfeld, L. C., Barnes, G. L., Shea, C. M. and Einhorn, T. A. (2003). Osteogenic differentiation is selectively promoted by morphogenetic signals from chondrocytes and synergized by a nutrient rich growth environment. *Connect. Tissue Res.* **44(Suppl 1)**, 85-91.
25. Ikeda, E., Hirose, M., Kotobuki, N., Shimaoka, H., Tadokoro, M., Maeda, M., Hayashi, Y., Kirita, T. and Ohgushi, H. (2006). Osteogenic differentiation of human dental papilla mesenchymal cells. *Biochem. Bbiophys. Res. Commun.* **342**, 1257-1262.
26. Ledeen, R. W. and Yu, R. K. (1982). Gangliosides: structure, isolation, and analysis. *Methods Enzymol.* **83**, 139-191.
27. Lee, S. H., Ryu, J. S., Lee, J. W., Kwak, D. H., Ko, K. and Choo, Y. K. (2010). Comparison of ganglioside expression between human adipose- and dental pulp-derived stem cell differentiation into osteoblasts. *Arch. Pharm. Res.* **33**, 585-591.
28. Prinetti, A., Iwabuchi, K. and Hakomori, S. (1999). Glycosphingolipid-enriched signaling domain in mouse neuroblastoma Neuro2a cells. Mechanism of ganglioside- dependent neuritogenesis. *J. Biol. Chem.* **274**, 20916-20924.
29. Frey, M. R., Golovin, A. and Polk, D. B. (2004). Epidermal growth factor-stimulated intestinal epithelial cell migration requires Src family kinase-dependent p38 MAPK signaling. *J. Biol. Chem.* **279**, 44513-44521.
30. Jaiswal, R. K., Jaiswal, N., Bruder, S. P., Mbalaviele, G., Marshak, D. R. and Pittenger, M. F. (2000). Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. *J. Biol. Chem.* **275**, 9645-9652.
31. Mirkin, B. L., Clark, S. H. and Zhang, C. (2002). Inhibition of human neuroblastoma cell proliferation and EGF receptor phosphorylation by gangliosides GM1, GM3, GD1A and GT1B. *Cell Prolif.* **35**, 105-115.
32. Radio, N. M., Doctor, J. S. and Witt-Enderby, P. A. (2006). Melatonin enhances alkaline phosphatase activity in differentiating human adult mesenchymal stem cells grown in osteogenic medium via MT2 melatonin receptors and the MEK/ERK (1/2) signaling cascade. *J. Pineal Res.* **40**, 332-342.
33. Tanikawa, R., Tanikawa, T., Okada, Y., Nakano, K., Hirashima, M., Yamauchi, A., Hosokawa, R. and Tanaka, Y. (2008). Interaction of galectin-9 with lipid rafts induces osteoblast proliferation through the c-Src/ERK signaling pathway. *J. Bone Miner. Res.* **23**, 278-286.
34. Kim, S. M., Jung, J. U., Ryu, J. S., Jin, J. W., Yang, H. J., Ko, K., You, H. K., Jung, K. Y. and Choo, Y. K. (2008). Effects of gangliosides on the differentiation of human mesenchymal stem cells into osteoblasts by modulating epidermal growth factor receptors. *Biochem. Biophys. Res. Commun.* **371**, 866-871.
35. Liu, Y., Li, R. and Ladisch, S. (2004). Exogenous ganglioside GD1a enhances epidermal growth factor receptor binding and dimerization. *J. Biol. Chem.* **279**, 36481-36489.
36. Lee, D. H., Koo, D. B., Ko, K., Ko, K., Kim, S. M., Jung, J. U., Ryu, J. S., Jin, J. W., Yang, H. J., Do, S. I., Jung, K. Y. and Choo, Y. K. (2007). Effects of daunorubicin on ganglioside expression and neuronal differentiation of mouse embryonic stem cells. *Biochem. Biophys. Res. Commun.* **362**, 313-318.
37. Yang, H. J., Jung, K. Y., Kwak, D. H., Lee, S. H., Ryu, J. S., Kim, J. S., Chang, K. T., Lee, J. W. and Choo, Y. K. (2011). Inhibition of ganglioside GD1a synthesis suppresses the differentiation of human mesenchymal stem cells into osteoblasts. *Dev. Growth Differ.* **53**, 323-332.
38. Liang, L., Ma, T., Chen, W., Hu, J., Bai, X., Li, J. and Liang, T. (2009). Therapeutic potential and related signal pathway of adipose-derived stem cell transplantation for rat liver injury. *Hepatol. Res.* **39**, 822-832.
39. Zuberbier, T., Guhl, S., Hantke, T., Hantke, C., Welker, P., Grabbe, J. and Henz, B. M. (1999). Alterations in ganglioside expression during the differentiation of human mast cells. *Exp. Dermatol.* **8**, 380-387.
40. Ryu, J. S., Ko, K., Lee, J. W., Park, S. B., Byun, S. J., Jeong, E. J., Ko, K. and Choo, Y. K. (2009). Gangliosides are involved in neural differentiation of human dental pulp-derived stem cells. *Biochem. Biophys. Res. Commun.* **387**, 266-271.
41. Ledeen, R. W., Wu, G., Lu, Z. H., Kozireski-Chuback, D. and Fang, Y. (1998). The role of GM1 and other gangliosides in neuronal differentiation. Overview and new finding. *Ann. N. Y. Acad. Sci.* **845**, 161-175.
42. Todeschini, A. R., Dos Santos, J. N., Handa, K. and Hakomori, S. I. (2008). Ganglioside GM2/GM3 complex affixed on silica nanospheres strongly inhibits cell motility through CD82/cMet-mediated pathway. *Proc. Nat. Acad. Sci. U. S. A.* **105**, 1925-1930.
43. Kotani, M., Kawashima, I., Ozawa, H., Terashima, T. and Tai, T. (1993). Differential distribution of major gangliosides in rat central nervous system detected by specific monoclonal antibodies. *Glycobiology* **3**, 137-146.
44. Higashi, H. and Chen, N. H. (2004). Ganglioside/protein kinase signals triggering cytoskeletal actin reorganization. *Glycoconj. J.* **20**, 49-58.

45. Osanai, T., Kotani, M., Yuen, C. T., Kato, H., Sanai, Y. and Takeda, S. (2003). Immunohistochemical and biochemical analyses of GD3, GT1b, and GQ1b gangliosides during neural differentiation of P19 EC cells. *FEBS Letters* **537**, 73-78.
46. Kwak, D. H., Yu, K., Kim, S. M., Lee, D. H., Kim, S. M., Jung, J. U., Seo, J. W., Kim, N., Lee, S., Jung, K. Y., You, H. K., Kim, H. A. and Choo, Y. K. (2006). Dynamic changes of gangliosides expression during the differentiation of embryonic and mesenchymal stem cells into neural cells. *Exp. Mol. Med* **38**, 668-676.
47. Giraudo, C. G., Rosales Fritz, V. M. and Maccioni, H. J. (1999). GA2/GM2/GD2 synthase localizes to the trans-golgi network of CHO-K1 cells. *Biochem. J.* **342 Pt 3**, 633-640.
48. Jin, H. J., Park, S. K., Oh, W., Yang, Y. S., Kim, S. W. and Choi, S. J. (2009). Down-regulation of CD105 is associated with multi-lineage differentiation in human umbilical cord blood-derived mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* **381**, 676-681.
49. Martinez, C., Hofmann, T. J., Marino, R., Dominici, M. and Horwitz, E. M. (2007). Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. *Blood* **109**, 4245-4248.
50. Xu, J., Liao, W., Gu, D., Liang, L., Liu, M., Du, W., Liu, P., Zhang, L., Lu, S., Dong, C., Zhou, B. and Han, Z. (2009). Neural ganglioside GD2 identifies a subpopulation of mesenchymal stem cells in umbilical cord. *Cell. Physiol. Biochem.* **23**, 415-424.
51. Takamiya, K., Yamamoto, A., Furukawa, K., Yamashiro, S., Shin, M., Okada, M., Fukumoto, S., Haraguchi, M., Takeda, N., Fujimura, K., Sakae, M., Kishikawa, K., Shiku, H., Furukawa, K. and Aizawa, S. (1996). Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proc. Nat. Acad. Sci. U. S. A.* **93**, 10662-10667.
52. Ferrari, G., Anderson, B. L., Stephens, R. M., Kaplan, D. R. and Greene, L. A. (1995). Prevention of apoptotic neuronal death by GM1 ganglioside.Involvement of Trkneurotrophin receptors. *J. Biol. Chem.* **270**, 3074-3080.
53. Cavallini, L., Venerando, R., Miotto, G. and Alexandre, A. (1999). Ganglioside GM1 protection from apoptosis of rat heart fibroblasts. *Arch. Biochem. Bioph.* **370**, 156-162.
54. Stojiljkovic, M., Blagojevic, T., Vukosavic, S., Zvezdina, N. D., Pekovic, S., Nikezic, G. and Rakic, L. (1996). Ganglioside GM1 and GM3 in early human brain development: an immunocytochemical study. *Int. J. Dev. Neurosci.* **14**, 35-44.
55. Vinson, M., Strijbos, P. J., Rowles, A., Facci, L., Moore, S. E., Simmons, D. L. and Walsh, F. S. (2001). Myelin-associated glycoprotein interacts with ganglioside GT1b. A mechanism for neurite outgrowth inhibition. *J. Biol. Chem.* **276**, 20280-20285.
56. Jennemann, R., Sandhoff, R., Wang, S., Kiss, E., Gretz, N., Zuliani, C., Martin-Villalba, A., Jager, R., Schorle, H., Kenzelmann, M., Bonrouhi, M., Wiegandt, H. and Grone, H. J. (2005). Cell-specific deletion of glucosylceramide synthase in brain leads to severe neural defects after birth. *Proc. Nat. Acad. Sci. U. S. A.* **102**, 12459-12464.
57. Yamashita, T., Yamauchi, A., Miyai, A., Taniguchi, M., Yoshimine, T. and Tohyama, M. (1999). Differential regulation of adenine nucleotide translocators by hypertonicity in the brain. *J. Neurochem.* **72**, 1259-1265.
58. Kwak, D. H., Seo, B. B., Chang, K. T. and Choo, Y. K. (2011). Roles of gangliosides in mouse embryogenesis and embryonic stem cell differentiation. *Exp. Mol. Med.* **43**, 379-388.