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GD3 synthase gene found expressed in dental epithelium and shown to regulate cell proliferation

Aya Yamada[a], Emiko Fukumoto[b], Yoko Kamasaki[a], Hiroko Ida-Yonemochi[c], Takashi Saku[c], Taku Fujiwara[a], Satoshi Fukumoto[a,d,*]

[a]*Division of Pediatric Dentistry, Department of Developmental and Reconstructive Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan*
[b]*Division of Oral Health Services Research, Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan*
[c]*Division of Oral Pathology, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences 2-5274 Gakkocho-dori, Niigata 951-8514, Japan*
[d]*Section of Pediatric Dentistry, Division of Oral Health, Growth, Development, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan*

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Summary GD3 synthase is one of the key enzymes involved with ganglioside synthesis, and its activity regulates the main profile of ganglioside expression. We analyzed the expression of the GD3 synthase gene in laser-dissected teeth germs using RT-PCR. The GD3 synthase gene was found expressed in brain, thymus, and tooth germ tissues, however, not in liver or skin specimens. Further, it was highly expressed during the early stage of tooth germ development (embryonic day 14.5), especially in dental epithelia, which gradually reduced in the molar site until postnatal day 7, whereas it was not in dental mesenchyme tissues. In addition, dental epithelial cells transiently transfected with the GD3 synthase gene showed enhanced proliferation. These results indicate that the GD3 synthase gene may be involved in early tooth development, particularly in the proliferation of dental epithelium.
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Abbreviations: GD3, disialoganglioside 3; E, embryonic day; P, postnatal day; PBS, phosphate-buffered saline; RT-PCR, reverse transcriptase-polymerase chain reaction; AMBN, ameloblastin; AMEL, amelogenin; DSPP, dentin sialophosphoprotein; G3PDH, glyceraldehyde 3-phosphate dehydrogenase

* Corresponding author. Tel.: +81 92 642 6402; fax: +81 92 642 6468.
E-mail address: fukumoto@dent.kyushu-u.ac.jp (S. Fukumoto).

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Introduction

Glycolipids are likely present in all animal cell plasma membranes, where they generally constitute approximately 5% of the lipid molecules in the outer monolayer. The most complex of these are the gangliosides, which contain oligosaccharides with one or more sialic acid residues that give them a net negative charge. Gangliosides are most abundant in the plasma membrane of nerve cells and constitute 5–10% of the total lipid mass, though they are also found in much smaller quantities in most other types of cells. Changes in ganglioside expression have been studied extensively in regard to cell differentiation and development. Further, all gangliosides have been shown to have a common Cer–Glc–Gal backbone structure, though they differ in the further branches composed of NeuAc and GalNAc.

GD3 is a disialylhematoside (Cer–Glc–Gal–NeuAc–NeuAc) expressed mainly in retina and fetal brain tissues. The GD3 synthase gene, which transfers NeuAc to GM3 (Cer–Glc–Gal–NeuAc), has been isolated in an α2,8 linkage[1–3] and its expression analyzed in mouse tissues. GD3 synthase is also characteristically expressed in the early developmental stages of the brain and has been found to be strongly expressed in the neural tube on embryonic day (E) 9.[4] GD3 synthase gene expression is low after birth and scarcely detectable in adult brains, with the exception of the choroid plexus. Taken together, these data suggest that GD3 is involved in neuronal proliferation and subsequent differentiation, such as with neurite extension and synapse formation.

GD3 has also been identified as a melanoma-specific antigen,[5–7] as established melanoma cell lines and all primary melanoma tissues have been shown to contain high levels of GD3 as the major ganglioside component. In contrast, human melanocytes, the normal counterpart of melanoma cells, express minimal or no GD3. These findings have led to the speculation that GD3 might play an important role in the maintenance of the malignant characteristics of melanoma cells.

A number of studies have shown that gangliosides modulate the functions of receptors for various cytokines and growth factors,[8] such as nerve growth factor (NGF),[9,10] platelet-derived growth factor (PDGF),[11,12] epidermal growth factor (EGF),[13,14] insulin,[15] and insulin-like growth factor (IGF).[16] In the majority of those studies, gangliosides suppressed growth factor receptor functions by reducing receptor phosphorylation. Recently, we showed that a rat pheochromocytoma cell line (PC12) transfected with the ganglioside GD3 synthase gene exhibited marked changes in ganglioside profile and enhanced proliferation.[10] Together, these results suggest regulatory roles for gangliosides in cell proliferation during physiological and malignant processes.

In the present study, we analyzed the expression of the GD3 synthase gene in tooth germ cells using RT-PCR. Further, in order to analyze the effects on cell proliferation, we attempted to modulate the ganglioside composition of cells derived from dental epithelium and mesenchymal tissues by introducing glycosyltransferase genes.

Materials and methods

Animals

Time-mated pregnant BALB/c mice were purchased from Japan SLC (Shizuoka, Japan). The day a vaginal plug appeared was designated as embryonic day 0.5 (E0). On E13.5, E14.5, or E17.5 the animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). All experimental procedures were approved in advance by the Animal Ethics Committee of our institution.

Cell cultures

The mandibular arch of individual E14.5 embryos was removed and the first molar tooth forming region, visible as a shallow depression, was excised and placed into Dulbecco's modified Eagle's medium (D-MEM). For analysis of the expression of the tooth marker gene, ameloblastin, amelogenin, and dentinsialophosphoprotein (DSPP), first molars were dissected on postnatal day (P) 3. To separate the dental epithelium from mesenchymal tissues, the samples were incubated for 5 min in 0.25% trypsin/1% collagenase, and then transferred to keratinocyte-SFM supplemented with EGF and pituitary gland extract (PGE), according to the manufacturer's instructions. The tissues were then microsurgically separated, after which they were treated with 0.25% trypsin/1% collagenase for 10 min at 37 °C. Dental epithelium specimens were plated in keratinocyte-SFM with PGE, 2% fetal bovine serum (FBS), or EGF, as well as 100 units/ml of penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Dental mesenchymal cells were plated in D-MEM with 10% FBS as previously described.[17] For gene transfection and proliferation assays, these cells were seeded in the above growth medium at 1×10^5 cells per well in type I collagen-coated 48-well culture plates (Falcon, Lincoln Park, NJ).

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