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研究題目：Effects of Oral Malodorous Compound on Hepatic Differentiation from Human Dental Pulp

目　的：

Hydrogen sulfide（H_2S), oral malodorous compound, involves cytotoxic and pro-apoptotic effects of H_2S have been studied, but many studies focus on the non-apoptotic effects of H_2S and its potential role in several physiological processes and disease states. H_2S is normally produced in human tissues. There are three main types of stem cells：embryonic stem cells（ESC）derived from the inner blastocyst mass, induced pluripotent stem（iPS）cells produced by reprogramming the nuclei of differentiated adult cells, and postnatal or adult stem cells（ASC）found amongst other differentiated cells in the tissues of mature organisms. Dental pulp is a valuable and accessible resource for acquiring ASC. We recently reported the moderating effect of H_2S on the differentiation of progenitor cells in osseous tissues. Also we developed a method to encourage *in vitro* differentiation of deciduous tooth-pulp stem cells（DPSC）into hepatocyte-like cells. The objective of this study is to determine the effect of H_2S at physiological levels on hepatogenic differentiation of dental pulp stem cells.

対象および方法：

Deciduous

teeth were collected from healthy patients undergoing routine tooth extraction and were processed within 2 h of collection. Informed consent was obtained from the subjects or their parents, and they agreed to participate voluntarily in this study. Pulp tissue was extracted through the resorbed tooth root canal by a sterile barbed broach and subjected to enzymatic dissociation in 4 mg ml_{-1} Dispase II and 4 mg ml_{-1} Collagenase I（Sigma, St Louis, USA）for 24 h at 4°C. Cell suspensions were seeded into 25 cm^2 flasks（Iwaki, Tokyo, Japan）with Dulbecco's modified Eagle's medium（DMEM, Invitrogen, Eugene, USA）supplemented with 10% FBS（HyClone, Road Logan, USA）and 100 Uml_{-1} penicillin, 100 μg ml_{-1} streptomycin and 0.25 μg ml_{-1} amphotericin（Invitrogen, Eugene, USA), and then incubated at 37°C in 5% CO_2. Cell cultures were grown until they reached 85%-90% confluency, and then were passaged in 3：1 ratio.The CD117 positive cell fraction was obtained from deciduous HTPC using magnetic sell sorting. After 3-4 passages, cells were grown in Dulbecco's modified Eagle's medium supplemented with insulin-transferrin-selenium-x（ITS-x）, embryotrophic factor（ETF）and hepatocyte growth factor（HGF）for hepatic commitment（five days). For hepatic differentiation the cells were cultured in Iscove's modified Dulbecco's medium supplemented with ITS-x, ETF, oncostatin, HGF and dexamethasone for 15 days in air

containing 5% CO_2, with or without H_2S at 0.05 ng ml_{-1}. Cells were assayed for the expression of hepatic markers α-fetoprotein, albumin or carbamoyl phosphate synthetase, and urea concentrations and glycogen synthesis were also determined.

結果および考察：

We characterized the cells for the following stem cell markers：STRO-1, CD133 and c-met, and found them positive for all of them. No differences in morphology or cell number were observed in any test group after incubation with the differentiatingmedia. This result is consistent with the results of our previous hepatocyte differentiation study. The expression of the hepatic-specific markers albumin, α-fetoprotein and CPS-1 was found after 3, 6 and 9 days of incubation with 0.1 ng ml_{-1} H_2S. Each test group had its respective control group, differentiated for the same time but without the presence of H_2S. Glycogen production and storage form one of the distinguishing processes taking place in hepatic cells. The analysis of PAS-stained glycogen showed increased accumulations in the test-group cells' cytoplasm after 3, 6 and 9 days；respectively 6.18% versus 2.53%, 8.86% versus 2.11% and 6.38% versus 2.96%. Urea and glycogen production were also increased. We concluded that H_2S at physiological concentrations increased the ability of HTPC to undergo hepatogenic differentiation.

成果発表：（予定を含めて口頭発表，学術雑誌など）

1. Yaegaki K, Ishkitiev N, Calenic B, Imai T.Nakahara T, Ishikawa H.
Hydrogen Sulfide Increases Hepatic Differentiation of Tooth Pulp Stem Cells
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2. Ishkitiev N, Yaegaki K, Calenic B, Imai T, Nakahara T & Ishikawa H. Hydrogen sulfide increases hepatic differentiation of tooth pulp stem cells, The 9th International Society of Breath Odor Research, Salvador, Brazil, May 25th-28th, 2011　口頭発表
3. Ishkitiev N, Calenic B, Aoyama I, Ii H, Yaegaki K, Imai T. Hydrogen sulfide increases hepatic differentiation in tooth-pulp stem cells. J. Breath Res. 6：017103, 2012 学術雑誌