

## R-spondin 2 promotes osteoblastic differentiation of immature human periodontal ligament cells through the Wnt/ $\beta$ -catenin signaling pathway

有馬, 麻衣

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氏 名	有馬 麻衣		
論 文 名	R-spondin 2 promotes osteoblastic differentiation of immature human periodontal ligament cells through the Wnt/ $\beta$ -catenin signaling pathway (R-spondin2 は Wnt/ $\beta$ -catenin シグナル伝達経路を介して未分化なヒト歯根膜細胞の骨芽細胞様分化を促進する)		
論文調査委員	主 査	九州大学	教授 久木田 敏夫
	副 査	九州大学	教授 西村 英紀
	副 査	九州大学	教授 清島 保

### 論 文 審 査 の 結 果 の 要 旨

Author measured the expression of R-spondin 2 (RSP02) in periodontal ligament (PDL) tissue and cells. Author examined the effects of RSP02 on osteoblastic differentiation of immature human PDL cells (HPDLCs). R-spondin (RSP0) family proteins are secreted glycoproteins that play important roles in embryonic development and tissue homeostasis through activation of the Wnt/ $\beta$ -catenin signaling pathway. RSP02, a member of the RSP0 family, has been reported to enhance osteogenesis in mice. However, little is known regarding the roles of RSP02 in PDL tissues.

Expression of RSP02 in rat PDL tissue and primary HPDLCs was examined by immunohistochemical and immunofluorescence staining, as well as by semi-quantitative RT-PCR. The effects of stretch loading on the expression of RSP02 and Dickkopf-related protein 1 (DKK1) were assessed by quantitative RT-PCR. Expression of receptors for RSP0s, such as *Leucine-rich repeat-containing G-protein coupled receptors (LGRs) 4, 5, and 6* in immature human PDL cells (cell line 2-14, or 2-14 cells) was investigated by semi-quantitative RT-PCR. Mineralized nodule formation in 2-14 cells treated with RSP02 under osteoblastic inductive condition was examined by Alizarin Red S and von Kossa staining. Nuclear translocation of  $\beta$ -catenin and expression of active  $\beta$ -catenin in 2-14 cells treated with RSP02 were assessed by immunofluorescence staining and western blotting analysis, respectively. In addition, the effect of Dickkopf-related protein 1 (DKK1), an inhibitor of Wnt/ $\beta$ -catenin signaling, was also examined. Rat PDL tissue and HPDLCs expressed RSP02, and HPDLCs also expressed *RSP02*, while little was found in 2-14 cells. Expression of *RSP02* as well as *DKK1* in HPDLCs was significantly up-regulated by exposure to stretch loading. *LGR4* was predominantly expressed in 2-14 cells, which expressed low levels of *LGR5* and *LGR6*. RSP02 enhanced the Alizarin Red S and von Kossa-positive reactions in 2-14 cells. In addition, DKK1 suppressed nuclear translocation of  $\beta$ -catenin, activation of  $\beta$ -catenin, and increases of Alizarin Red S and von Kossa positive reactions in 2-14 cells, all of which were induced by RSP02 treatment.

RSP02, which is expressed in PDL tissue and cells, might play an important role in regulating the osteoblastic differentiation of immature human PDL cells through the Wnt/ $\beta$ -catenin signaling pathway. This paper contains novel findings and was deemed worthy as a thesis paper (Doctor of Dental Science).