Inhibition of sonic hedgehog signaling pathway in ameloblastoma cell line

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（エナメル上皮腫細胞株におけるソニックヘッジホッグシグナル経路の抑制）

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論 文 内 容 の 要 旨

Ameloblastoma is a benign odontogenic tumor characterized by locally invasive growth into the jaw and often recurs, the quality of life is reduced by resection of the jaw as a radical treatment. Therefore, development of new treatment is eagerly expected.

Sonic Hedgehog (SHH) is a secreted protein that activates a membrane-receptor complex formed by patched (PTCH) and smoothened (SMO). Hedgehog signaling pathway has crucial roles in growth and patterning during organogenesis and tumorigenesis. In the present research, we demonstrated the necessity of the SHH signaling pathway in the growth of ameloblastoma cell lines, and the suppression of the proliferation by using SHH signaling pathway inhibitors.

Several Hedgehog signaling pathway inhibitors were used to examine. Cyclopamine, GDC-0449 and itraconazole were used as SMO inhibitors, meanwhile GANT-61 and JQ1 as GLI inhibitors. The human ameloblastoma cell line AM-1, was established from human plexiform type ameloblastoma tissue and immortalized by the transfection of human papillomavirus type 16 DNA. We examined the effects of each SHH signaling pathway inhibitors on growth in AM-1 cells by WST-8 cell proliferation assay. We also examined the apoptotic cells by TUNEL assay and Annexin V assay.

RT-PCR analysis revealed that SHH, PTCH, SMO, GLI1, GLI2, and GLI3 were expressed in AM-1 cells and gene products were also detected by immunocytochemistry. The addition of cyclopamine, GDC-0449 and itraconazole suppressed proliferation of AM-1 cells. GLI inhibitors, Gant-61 and JQ1, also suppressed the proliferation of AM-1 cells. The TUNEL positivity in the cells treated by SHH signaling inhibitors was significantly higher than those in control. Furthermore, BCL2 expression was decreased and BAX expression was increased in AM-1 cells by immunocytochemistry. Our study suggested that the inhibition of SHH signaling pathway is a strong candidate of the novel treatment of ameloblastoma by growth inhibition and apoptosis induction.