Studies on growth mechanisms and their suppression in canine squamous cell carcinoma cells

Abstract

Ryo Miyamoto

Graduate School of Veterinary Medicine and Life Science

Nippon Veterinary and Life Science University

Treatment of unresectable canine squamous cell carcinoma (SCC) remains challenging and new therapeutic strategies is needed. Because previous studies have been demonstrated that survivin and EGFR are overexpressed in canine SCC tissues, these molecules are considered to be closely associated with growth of canine SCC. In this study, to establish new therapeutic strategies for canine SCC, sensitivities of seven SCC cell lines to YM155, afatinib, and osimertinib were examined. Moreover, by focusing to YM155 and afatinib, growth inhibitory mechanisms in canine SCC cell lines were investigated. Furthermore, anti-tumor effect of afatinib against canine SCC cell line *in vivo* was tested. YM155 and afatinib potently and selectively inhibited growth of HAPPY and SQ4 cells and POCO and CSCC-R1 cells, respectively. In contrast, osimertinib did not show such growth inhibitory effects against SCC cell lines. Both YM155-sensitive cell lines HAPPY and SQ4 cells highly expressed survivin, while suppression mechanisms of survivin by YM155 were differed between HAPPY and SQ4 cells, in which YM155 inhibited survivin expression by suppression of *survivin* in HAPPY cells, while it inhibited survivin expression via post-transcriptional

mechanism in SQ4 cells. Although YM155 induced autophagy and subsequent PARP-dependent apoptosis in both cell lines, HAPPY cells primarily underwent cell death via PARP-dependent apoptosis, while there were two different cell death mechanisms including PARP-dependent apoptosis and probably autophagic cell death in SQ4 cells. In afatinib-sensitive POCO and CSCC-R1 cell lines, no aberrant of known afatinib target molecules was identified. In a comprehensive analysis of phosphorylated proteins using POCO cells, it was found that afatinib mainly suppressed activation of MAPK pathway. Furthermore, afatinib showed a remarkable anti-tumor effect against POCO cells xenograft mouse. In conclusion, it was suggested that the expression of survivin or phosphorylation of the MAPK pathway plays a crucial role in growth/survival of certain canine SCCs. YM155 and afatinib may be promising as new therapeutic strategies for such canine SCCs.