**TITLE**
Notch signaling is required for the in vivo growth of mucoepidermoid carcinoma likely through regulating cancer stem/progenitor-like cells

**HYPOTHESIS:**
Notch signaling regulates and sustains MEC cancer stem/progenitor-like cells.

**BACKGROUND/AIMS:**
Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy and no targeted therapy is currently available. MEC is characterized by a unique chromosomal translocation t(11;19)(q14-21;p12-13) that encodes the CRTC1-MAML2 fusion. CRTC1-MAML2 is a major driver for MEC and has potential diagnostic and therapeutic values. Evidence supports that MEC is a disease of stem/progenitor cells. However, the molecular properties and regulation of MEC stem/progenitor cells are poorly characterized. Notch signaling is a highly conserved signaling pathway and critically regulates many normal and cancerous stem cells. However, whether Notch signaling has a role in regulating human MEC cancer stem/progenitor cells had not been studied. Therefore, this study was aimed to assess the functional role of Notch signaling in human MEC.

**METHODS:**
Western blotting and quantitative RT-PCR were used to assess gene expression.
For *in vitro* study, we assessed the effects of Notch signaling inhibition on cell proliferation and oncosphere formation. Two approaches were used to inhibit endogenous Notch signaling in MEC cells, including dnMAML1, a pan-Notch inhibitor that blocks the formation of the Notch transcriptional activation complex, and gamma secretase inhibitor (GSI) that interferes with Notch receptor processing.
For *in vivo* study, we determined the effects of the GSI DBZ and the EGFR inhibitor Erlotinib treatments, either alone or in combination, on the growth of human MEC xenografts.

**RESULTS & CONCLUSIONS**
First, Notch signaling was active in MEC cells. Second, the inhibition of Notch signaling had no effect on the proliferation of bulk MEC cells, but significantly reduced the oncosphere forming capacity in vitro, suggesting that Notch signaling contributes to the maintenance of MEC cancer stem/progenitor cells. Third, GSI treatment significantly reduced the growth of human MEC xenografts, indicating that Notch signaling is essential for the in vivo MEC growth. Finally, combinatorial treatment of Notch and EGFR signaling inhibition via the GSI DBZ and the EGFR inhibitor Erlotinib synergistically inhibited colony formation and oncosphere formation capacity of human MEC cells in vitro and the growth of MEC xenografts in vivo. Collectively, our data implicate Notch signaling as a critical regulator of the maintenance of cancer stem/progenitor cells and revealed that the combined therapy of targeting Notch and EGFR signaling is a potential effective approach for blocking MEC.