Movie S1. Inhibition of muscarinic signaling promotes a ductal phenotype, related to Figure 2. Increased ductal development under inhibition of muscarinic signaling with 4-DAMP. Time-lapse imaging shows E13 SMG cultured without (left panel) or with (right panel) 10μM 4-DAMP for 24h. Images were captured every 20 minutes (Zeiss Axiovert 40c with Infinity CCD camera).

Movie S2. Microlumens are present in developing SMG, related to Figure 3 and 5. Rotation of 3D projection of presumptive ducts showing ZO1(+) microlumens that will fuse to form a single lumen. Samples were imaged using a Leica Sp5 LSM confocal microscope. Image is a 30μm stack of 0.8μm section.

Supplemental Materials and Methods

Materials

FGFR2b-Fc chimera was from R&D systems (Minneapolis, MN, US). The pan-caspase inhibitor Z-VAD-FMK was from Tocris (Minneapolis, MN, US). Antibodies: goat anti-VIPR1 (N17, 1:100; Santa Cruz Biotech Ltd) and anti-CFTR (M3A7, 1:50; Chemicon (Doucet et al., 2003).

Mouse lines

Strain used on this study: $Fgf10^{tm1Wss}$ was provided by Dr. Ophir Klein, UCSF.CA (Min et al., 1998). Salivary glands of heterozygous embryos were analyzed at E17.

RNA interference

RNA interference was performed using 2 siRNAs for Vipr1 (J-065007-05-0005 and J-065007-06-0005), a non-silencing scrambled siRNA (Dharmacon, CA) and RNAiFect reagent (Qiagen, CA). Transfection and analysis of knockdown by qPCR and quantitative immunofluorescent analysis have been previously described (Rebustini et al., 2007).

Gene	Primers for Sybr Green qPCR
Vip	sense 5'- AACAGAGCAGAACAGATAATCAGT-3', antisense 5'-GCAAAGCCTAATGGG AGAGAT-3'
Vipr1	sense 5'- AAGTCATTGTAGAGGCAGAT-3', antisense 5'-AATATGTCAAGACGGAATCAG-3'
Chat	sense 5' - GTGGAAGAATCGTCATCTCATCA antisense 5' - GAA CTC AAG GAA GAC TGT GCT AT
Ret	sense 5' - ACA CGG TGG TAG CCA CTG AC antisense 5' - GCG CCT CTT GTT TAC TGC AC
Syn2	sense 5' - TAG ACT GCT GTG GAG GTG AA antisense 5' - GCT CTG AAA GGT AAA GGT AAC TG
Vacht	sense 5' – GAGTGGGAGATGGGCATGGTTTGG antisense 5' – GCAGGC AGGTACGACGCAAGA G
Rsp29	sense 5'- GGAGTCACCCACGGAAGTTCGG-3', antisense 5' – GGAAGCACTGGCGGCACATG-3'
Chrm1	sense 5' - TCCCAAGGCTCACCCAGATGTC antisense 5'- GCTCTGTGTGCTTTATTCTGTTGTTTCC
Etv4	sense 5' - GGTCCTGTGTTCTTGGTGCTGTG antisense 5'- GGTCCTGTGTTCTTGGTGCTGTG
Etv5	sense 5' - AAGCCCTTCAAAGTGATAGCGGAGAC antisense 5'- GTGTCCACAAACTTCCTCTTTCTGTCAATC
Fgf10	sense 5' - TCTTCCTCCTCCTCGTCCTTCTCCTCTCCTTCC antisense 5'- CCGCTGACCTTGCCGTTCTTCTCAATCG
Nrtn	sense 5' - CGCTACCACACGCTGCAAGAG antisense 5'- TCCCACACTTATGTGAAGTCAGTTCTC
Tubb3	sense 5' - CCAGAGCCATCTAGCTACTGACACTG antisense 5'- AGAGCCAAGTGGACTCACATGGAG
Cdh1	sense 5' - GACTGGAGTGCCACCACCAAAGAC antisense 5'- CGCCTGTGTACCCTCACCATCGG

Primer efficiencies were performed prior to use and were in the range of 90-100%

Supplemental References

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