Online Data Supplement

Breeding Strategies:

K14rtTA$^{+/0}$/TREcre$^{+/0}$/DE3$^{+/+}$: The Breeding design for the mice involved in this study are as follows: A Keratin 14-promoter rtTA (K14rtTA) mouse is crossed with a mouse containing the tet-response element driving the expression of cre-recombinase (TREcre). The K14rtTA and the TREcre transgenes were identified by PCR analysis of genomic DNA from the tails of the progeny of this breeding. The following primer sequences were used to assay for these transgenes: K14rtTA 5'-AGCTTGGTGTAGAGCAGCCTACACTGTATT-3' and 5'-GCTCCATTGCGATGACTTAGTAAAGCACAT-3'. TREcre: 5'-GCATTACCGGTCGATGCAACGAGTGATGAG-3' and 5'-GAGTGAACGAACCTGGTCGAAATCAGTGCG-3'. First generation progeny of this cross containing both the K14rtTA and TREcre transgenes are then bred with mice that have homozygous floxed alleles at Delta exon 3 (DE3) of the beta-catenin gene. Progeny from this cross will contain K14rtTA, TREcre and one floxed allele of DE3. The Progeny that contain all of these transgenes will then breed together providing a 25% chance of producing progeny having both alleles at DE3 being floxed. To identify the mice that have the homozygous floxed DE3 alleles, PCR analysis is done using the following Primer sequences. DE3: 5'-GACACCGCTGCGTGGACAATG-3' and 5'-GTGGCTGACAGCAGCTTTTCT-3'. The mice that have the K14rtTA, TREcre, and both floxed DE3 alleles will then breed with each other producing 100% of progeny with both DE3 alleles being floxed as well as being hemizygous K14rtTA and TREcre transgenes.

K14rtTA$^{+/0}$/TREcre$^{+/0}$/DE3$^{+/-}$/RS$^{+/0}$: The strain was created by breeding a mouse containing the K14rtTA, TREcre, and homozygous floxed DE3 alleles transgenes (as mentioned from the strain above) with a mouse containing the ROSA26-floxed recombination substrate transgene (Hong et al, 2004). Mice containing the recombination substrate were identified by assaying for the beta-galactosidase(LacZ) reporter using the following primer sequence 5'-GTTGCAGTGCACGGCAGATACACTTGCTGA-3' and 5'-GCCACTGGTGTGGGCCATAATTCAATTCGC-3'. The tail DNA of the progeny from this cross was assayed for the K14rtTA, TREcre, and recombination substrate transgenes by PCR using the primer sequences mentioned above to determine the mice of interest for experiment. This breeding also produced 100% of the progeny containing one floxed DE3 alleles and one wild type allele.

K14rtTA$^{+/0}$/TREcre$^{+/0}$/DE3$^{+/-}$/TOPGal$^{+/0}$: The strain was created by breeding a mouse containing the K14rtTA, TREcre, and homozygous floxed DE3 alleles transgenes with a mouse containing the TOPGal reporter transgene on the C57BL/6 background. The top gal reporter gene is identified by assaying for the beta-galactosidase(LacZ) reporter using the same primer sequence used to assay for the recombination substrate The tail DNA of the progeny from this cross was assayed for the K14rtTA, TREcre, and top gal reporter transgenes to determine the mice of interest for experiment. This breeding also produced 100% of the progeny containing one floxed DE3 alleles and one wild type allele.

Supra-optimal antibody dilution analysis.

In order to determine the supraoptimal antibody dilutions for detection of the two different phenotypes of Clara-like and Clara cells, a antibody dilution series was evaluated. The primary antibody (goat-αCCSP) was diluted to 1:2000, 1:4000, 1:8000, 1:10000, 1:20000, 1:40000, 1:80000, and 1:160000. These dilutions were used to stain both trachea and lung sections under standard conditions. The supraoptimal dilution for each cell type was defined as the lowest dilution at which signal was detectable. In contrast with studies done at the supraoptimal dilution, all quantitative analysis was done on sections that maximized the number of CCSP+ cells detected. (1, 2).

Supplemental Results:

We tested the hypothesis that CCSP protein concentration is less in WT compared to BiTg using the supra-optimal antibody dilution method (McBride et al. (1990) Am J Respir Cell Mol Biol 3:587-593; Roncalli et al., 1993. Am J Respir Cell Mol Biol 9:467-474). The goat-anti-CCSP antibody was titered from 1/250 to 1/160,000 using serial 2-fold dilutions. The dilution step that resulted in a transition from negative (no difference from the no-primary control) to positive was defined as the supra-optimal antibody dilution. This value was determined for tracheal and lung tissue from all 4 genotypes. The supra-optimal dilution for the goat-αCCSP antibody on WT and rtTA+ tracheal tissue was 1 in 4,000, and for WT and rtTA+ lung tissue was 1 in 10,000. In contrast, the supra-optimal dilution for cre+ and BiTg tracheal tissue was 1 in 80,000, and for cre+ and BiTg lung tissue was 1 in 40,000. Using this method, we conclude that CCSP+ cells from WT and rtTA genotypes contain a lower concentration of CCSP protein than the brightest cells in cre+ and BiTg. These data are presented in SFig 1.

We used pixel intensity analysis to determine if the antibody dilution used for quantification of Vv/Sv detected all CCSP+ cells. This analysis indicated that the antibody dilution used in the Vv/Sv analysis, 1/500, was greater than the maximum value of the linear range. Thus, the 1/500 dilution detected all CCSP+ cells including both the normal CCSP+ cells (found in all genotypes) and the abnormal CCSP+ cells found in the cre+ and BiTg mice.

Finally, we converted the Vv/Sv analysis into planar terms. For the WT vs. BiTg analysis, the Vv/Sv difference is 8-fold and Sv does not vary by genotype. Converting from volume to a linear measurement, where r_1= the radius of a WT cell and r_2= the radius of a BiTg cell, then

$$4/3\pi r_2^3 / 4/3\pi r_1^3 = 8$$
$$r_2^3 = 8r_1^3$$
$$r_2 = 2r_1$$

So the average radius of a WT CCSP+ cell is half that of a CCSP-bright BiTg cell.

Supplemental References

1. McBride JT, Springall DR, Winter RJ, Polak JM. Quantitative immunocytochemistry shows calcitonin gene-related peptide-like immunoreactivity in lung neuroendocrine cells is increased by chronic hypoxia in the rat. *Am J Respir Cell Mol Biol* 1990;3(6):587-593.

2. Roncalli M, Springall DR, Maggioni M, Moradoghli-Haftvani A, Winter RJ, Zhao L, Coggi G, Polak JM. Early changes in the calcitonin gene-related peptide (cgrp) content of pulmonary endocrine cells concomitant with vascular remodeling in the hypoxic rat. *Am J Respir Cell Mol Biol* 1993;9(5):467-474.