Conditional Clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells

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Reynolds, Susan D., Kyung U. Hong, Adam Giangreco, Gregory W. Mango, Charanjeet Guron, Yasuo Morimoto, and Barry R. Stripp. Conditional Clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am J Physiol Lung Cell Mol Physiol 278: L1256–L1263, 2000.—The neuroepithelial body (NEB) is a highly dynamic structure that responds to chronic airway injury through hyperplasia of associated pulmonary neuroendocrine (PNE) cells. Although NEB dysplasia is correlated with preneoplastic conditions and PNE cells are thought to serve as a precursor for development of small cell lung carcinoma, mechanisms regulating expansion of the PNE cell population are not well understood. Based on studies performed in animal models, it has been suggested that NEB-associated progenitor cells that are phenotypically distinct from PNE cells contribute to PNE cell hyperplasia. We have previously used a Clara cell-specific toxicant, naphthalene, to induce airway injury in mice and have demonstrated that naphthalene-resistant Clara cells, characterized by their expression of Clara cell secretory protein (CCSP), and PNE cells contribute to airway repair and associated hyperplasia of NEBs. This study was conducted to define the contribution of NEB-associated CCSP-expressing progenitor cells to PNE cell hyperplasia after Clara cell ablation. Transgenic (CCtk) mice were generated in which herpes simplex virus thymidine kinase was expressed within all CCSP-expressing cells of the conducting airway epithelium through the use of transcriptional regulatory elements from the mouse CCSP promoter. Chronic administration of ganciclovir (GCV) to CCtk transgenic mice resulted in selective ablation of CCSP-expressing cells within conducting airways. Proliferation and hyperplasia of PNE cells occurred in the absence of detectable proliferation among any other residual airway epithelial cell populations. These results demonstrate that PNE cell function as a self-renewing progenitor population and that NEB-associated Clara cells are not necessary for PNE cell hyperplasia.

lungs; airway; stem cell; herpes simplex virus thymidine kinase; transgenic mice

Regeneration of the chronically injured airway epithelium is associated with alterations in the number and cellularity of neuroepithelial bodies (NEBs) and an enrichment of nascent epithelial cell populations within the NEB (1, 8, 15, 29, 31, 34). Although NEB hyperplasia characterized by increases in the number of pulmonary neuroendocrine (PNE) cells per NEB and in the number of NEBs has been described, mechanisms regulating this process are unclear. Cell differentiation leading to enhanced detectability of PNE cell markers contributes to the perception of PNE hyperplasia in animals exposed to normobaric hypoxia (21, 24, 30) and to the early phase of NEB hyperplasia after coexposure to diethylnitrosamine (DEN) and hyperoxia (36). However, cell proliferation has also been shown to contribute to the maintenance of PNE cells in the normal lung and hyperplasia of this population in various disease states. PNE cells are known to act as a progenitor cell for the establishment of NEB hyperplasia in the hypoxia- (23) and DEN plus hyperoxia-exposed lung (41) and represent one of two proliferative populations within hyperplastic NEBs of the naphthalene-injured lung (29). Participation of non-PNE progenitor cells in this process has also been demonstrated (12, 17) and may contribute to the intermediate phase of NEB hyperplasia in the DEN-O2 model (36, 41). These data suggest that multiple cell types contribute to maintenance and expansion of the NEB-associated PNE population and that progenitor selection may be a dynamic feature of NEB hyperplasia.

NEB-associated epithelial cells share many morphological and biochemical characteristics with cells distributed throughout the airway. All PNE cells, whether isolated or clustered within NEBs, are characterized by basally located dense core granules that contain one or more bioactive amines and/or neuropeptides (reviewed in Ref. 38). However, NEB-associated PNE cells are distinguished from isolated PNE cells by their innervation and by a distinct pattern of gene expression (38). Selective entry of NEB-associated PNE cells into the cell cycle after carcinogen exposure (17) and their representation as a major proliferative population in the naphthalene-injured lung (29) further distinguish the two populations of PNE cells. These observations suggest that sequestration within the NEB microenvironment results in the establishment of a functional dichotomy between NEB-associated and isolated PNE cells. Clara cells exhibit a similarly distinct ultrastructural and functional phenotype when associated with the NEB microenvironment. Clara cells are morphologically diverse but are commonly recognized as nonciliated cells with abundant smooth endoplasmic reticulum and apically enriched secretory granules (25). Functional attributes of these cells include the ability to synthesize and secrete proteins into the extracellular...
mice and to metabolize lipophilic pollutants through cytochrome P-450 (CYP)-mediated metabolism (26). These cells also serve as a multipotential progenitor cell for renewal of airway epithelium after oxidant injury (6). Before entry into the cell cycle, Clara cells undergo a phenotypical transformation involving loss of secretory granules and smooth endoplasmic reticulum (7). The predicted reduction in secretory and pollutant metabolizing functions may be a necessary adaptation for protection of the mitotic population during chronic pollutant exposure. In contrast with the majority of Clara cells, those associated with NEBs exhibit distinct ultrastructural characteristics, express lower levels of Clara cell secretory protein (CCSP), and are deficient in the phase I xenobiotic enzyme CYP2F2 (9,13,14,29). Proliferation of this variant Clara cell population is selectively stimulated by specific environmental pollutants and is associated with NEB hyperplasia and hypertrophy (29).

This study was designed to test the hypothesis that CCSP-expressing cells are required for development of NEB hyperplasia. To this end, we have developed transgenic (CCtk) mice expressing herpes simplex virus thymidine kinase (HSVtk) (3,10) under the transcriptional control of the mouse CCSP promoter (33). We demonstrate that temporally controlled ablation of all CCSP-expressing cells is achieved through exposure of CCtk mice to the prodrug HSVtk substrate ganciclovir (GCV) and that NEB hyperplasia occurs independently of Clara cell involvement.

METHODS

Generation of CCtk mice. The CCtk transgene was generated by ligation of a 2.1-kb Hind III-Xho I fragment of the mouse CCSP promoter (–2,100 to +35 bp) (33) to a 2.0-kb Bgl II-Xba I fragment of the HSVtk gene (22) flanked by a bovine growth hormone polyadenylation signal. An internal 200-bp Xho I-Bgl II fragment containing an upstream translation initiation site was deleted, and the resulting plasmid, pCCTK (Fig. 1A), was linearized with Hind III-Xba I and microinjected into the male pronucleus of FVB/n zygotes through the use of standard techniques (11). Founders were identified by PCR analysis of tail DNA (11) with the transgene-specific primers 5'-GCCGTTGTGTTGTTGTTGATG-3' and 5'-GT-TGGCAAGTCTACAGTTGC-3' (Fig. 1A). The transgene was maintained in the FVB/n background and propagated through the female germ line. All recombinant DNA techniques used in generation and analysis of the CCtk mice conformed to guidelines established by the National Institutes of Health.

Animals and treatments. Male wild-type and transgenic CCtk littermates (2–4 mo) used in this study were maintained as a specific pathogen-free in-house colony. They were allowed food and water ad libitum and maintained on a 12:12-h light-dark cycle. Representative animals from the colony were screened quarterly for the absence of pathogens with a comprehensive 16-agent serologic panel (Microbiological Associates, Rockville, MD). GCV (375 mg/ml in saline; Hoffman-La Roche, Nutley, NJ) was continuously administered at 4.5 mg/day with an Alzet miniosmotic pump (Alza Scientific, Palo Alto, CA). Control mice were implanted with pumps charged with saline alone. All animal treatments were approved by the University of Rochester (Rochester, NY) University Committee for Animal Research.

Fig. 1. Cellular distribution of herpes simplex virus thymidine kinase (HSVtk) protein in CCtk transgenic mice. A: schematic showing CCtk transgene construct. mCCSP, murine Clara cell secretory protein; pA, bovine growth hormone polyadenylation signal. Arrowhead, transcription start site; solid bars, positions of primers used for genotype analysis. B: dual-immunofluorescence and laser scanning confocal microscopic analysis of CCSP (Texas Red detection, red) and HSVtk (Cy 2 detection, green) in wild-type (TG−) and CCtk transgenic lung tissue (CCtk 171 and CCtk 30). TG− and CCtk 171 are summation of multiple optical sections. CCtk 30 represents a single optical section. Perinuclear localization of HSVtk protein is illustrated by orthogonal section analysis of a single cell (xz and yz). Final magnification, ×1,000.

Tissue collection and immunohistochemistry. Mice were killed by intraperitoneal injection of 100 mg/kg of pentobarbital sodium followed by exsanguination. Right lung lobes were fixed by inflation at 10 cmH2O with neutral-buffered Formalin instilled through a tracheal cannula. The lobes were embedded in paraffin, and adjacent serial 5-μm sections were cut from the caudal lobe to reveal the major axial pathway, three to five minor daughter airways, and numerous terminal bronchioles. The Clara cell-specific marker CCSP (5,37,42), the PNE cell-specific marker calcitonin gene-related peptide (CGRP) (36,41), and the ciliated cell marker acetylated tubulin (ACT) (16) were detected by standard immunohistochemical techniques as previously described (28). Rabbit
anti-CCSP antibody was obtained from Dr. G. Singh (University of Pittsburgh, Pittsburgh, PA) and used at a dilution of 1:12,000. Rabbit anti-CGRP and mouse monoclonal anti-ACT antibodies were purchased from Sigma (St. Louis, MO) and used at dilutions of 1:5,000 and 1:8,000, respectively. Tissue analyzed for proliferating cell nuclear antigen (PCNA) (40) was pretreated by the citrate-microwave antigen retrieval method according to the manufacturer's directions (Vector Laboratories, Burlingame, CA) followed by standard immuno- histochemical methods (28). Mouse monoclonal anti-PCNA antibody was purchased from Sigma and used at a dilution of 1:2,000. Antigen-antibody complexes were detected with diaminobenzidine (DAB), and the tissue was counterstained with hematoxylin. Analysis was carried out on an Olympus Provis AX70 microscope equipped with a Sony 9000 charge-coupled device video capture system linked to a Pentium processor PC running ImagePro Plus (Media Cybernetics, Silver Spring, MD). Tissue from at least three animals was analyzed for each treatment group.

The specificities of the rabbit anti-CCSP antibody and CGRP were previously verified (5, 29, 37). The mouse anti-ACT antibody (16) detects an antigen localized to the cilia of cells lining the conducting airway. Reactivity was not observed on nonciliated airway epithelial cells or alveolar epithelial, endothelial, or mesenchymal cells. Specificity of the mouse anti-PCNA antibody is indicated by detection of adjacent positive and negative nuclei within the conducting airway epithelium (see Fig. 4B). As predicted by the low labeling index of the normal lung, PCNA-positive nuclei are rare in untreated tissue (data not shown). Immunoreactivity was not observed in the absence of primary antibody.

Confocal microscopy. Tissue sections were stained by the double-immunofluorescence method with a combination of goat anti-CCSP and rabbit anti-thymidine kinase (HSVtk) antibodies as previously described (29). Goat anti-CCSP antibody was supplied by Dr. G. Singh and used at a dilution of 1:3,000. Rabbit anti-HSVtk antibody was supplied by W. Summers (Yale University, New Haven, CT) and used at a dilution of 1:1,000. Antigen-antibody complexes were detected with Texas Red-conjugated mouse anti-goat Ig and Cy2-conjugated donkey anti-rabbit Ig (Jackson ImmunoResearch Laboratories, West Grove, PA). Specificity of the anti-CCSP antibody has been previously verified (28). HSVtk antibody did not stain wild-type tissue (Fig. 1B, TG−), indicating that there were no antigens within nontransgenic tissue that reacted with this antibody. Immunoreactivity was not detected in the absence of primary antibody. Images were acquired with a Leica TCS SP confocal microscope and overlaid in Adobe Photoshop.

Morphometry. One section from each of 5 CCTk animals treated with either saline or GCV for 12 days was stained for CGRP and counterstained with hematoxylin. The total length of basement membrane (BM) along the major axial pathway was determined using the measurement function of Image-Pro Plus (Media Cybernetics) at a final magnification of ×100. The length of CGRP-immunoreactive regions subtending the BM was similarly determined except that the final magnification was ×400. All CGRP-immunoreactive cells containing a nuclear profile were counted for determination of the number of PNE cells per millimeter of BM. NEBs were defined as clusters of more than two PNE cells. The means ± SE for four control and five GCV-treated CCTk mice are reported. Differences between means were considered significant when P values were <0.05 as determined by Student's t-test.

ACT staining intensity was determined with the line profile and reference function of ImagePro Plus (Media Cybernetics). A continuous line was traced along the apical portion of the epithelium intersecting the immunopositive apical ciliary tufts. Each pixel encountered by the line was split into its red, green, and blue components and the intensity per pixel for each color was determined. The summation of red intensity for each line was divided by the length of BM. Six nonoverlapping low-power fields from the most proximal region of a section from each of four control mice or five GCV-treated CCTk mice were analyzed, and the average ± SE was determined for each treatment group. Results are reported as intensity per micrometer of BM. Differences between means were considered significant when P values were <0.05 as determined by Student's t-test. Nuclear density in these fields was determined by counting all nuclear profiles and dividing by the length of BM. The average ± SE is reported for each treatment group. Differences between means were considered significant when P values were <0.05 as determined by Student's t-test.

RESULTS

Generation of CCTk transgenic mice. Twenty-two founder mice harboring the CCTk transgene were identified by PCR and backcrossed to transgene-negative mates for perpetuation of lines. Three lines of HSVtk mRNA-expressing transgenic animals were identified. Each of these directed lung-specific expression of HSVtk mRNA (data not shown), which is consistent with earlier reporter transgene studies using transcriptional control elements from the murine CCSP gene (27, 33). The cell type specificity of HSVtk expression in the CCTk-171 and -30 lines was determined by immunofluorescent colocalization of HSVtk protein with the Clara cell marker CCSP (Fig. 1). Transgene-negative littermates express CCSP but not HSVtk mRNA (data not shown), which is consistent with earlier reporter transgene studies using transcriptional control elements from the murine CCSP gene (27, 33). The cell type specificity of HSVtk expression in the CCTk-171 and -30 lines was determined by immunofluorescent colocalization of HSVtk protein with the Clara cell marker CCSP (Fig. 1). Transgene-negative littermates express CCSP but not HSVtk mRNA in nonciliated epithelial cells distributed throughout the airway (Fig. 1B, TG−). In both the CCTk-171 and -30 lines, HSVtk protein was detected exclusively in CCSP-immunopositive cells, with no detectable expression in other cell populations including type 2 cells (Fig. 1B, CCTk171 and CCTk30). HSVtk protein was detected throughout the cytoplasm but was highly enriched in the perinuclear region of the cell (Fig. 1B, xz and yz). The cellular abundance of HSVtk protein is similar between the CCTk-171 and -30 lines. These data indicate that the CCTk transgene is expressed specifically in Clara cells throughout the airway epithelium and that the mouse CCSP promoter elements used more faithfully recapitulate the pattern of endogenous CCSP expression than is achieved with rat CCSP promoter elements (32).

GCV-dependent Clara cell ablation in CCTk transgenic mice. To define the cellular selectivity and kinetics of GCV-mediated cellular ablation, wild-type and
CCtk transgenic mice were implanted with miniosmatic pumps containing either saline or 375 mg/ml of GCV. GCV-exposed CCtk mice experienced progressive tachypnea and cyanosis beginning at 6–8 days of continuous GCV exposure. No such symptoms were observed among either saline-exposed CCtk transgenic controls or GCV-exposed wild-type mice (data not shown). Changes in the mRNA abundance of the Clara cell-specific markers CCSP and CYP2F2 were quantified in total lung RNA by S1 nuclease protection assay. Steady-state mRNA abundance of lineage-specific markers did not vary significantly between wild-type animals exposed to either saline or GCV (data not shown), indicating that neither the surgical protocol nor GCV modulates expression of these Clara cell-specific markers. In contrast, transgene-positive animals treated with GCV for either 6 or 12 days exhibited a dramatic decline in the abundance of both CCSP and CYP2F2 mRNAs compared with either saline-treated transgenic mice (Fig. 2) or GCV-treated transgene-negative mice (data not shown). At the 6-day time point, CCSP mRNA was 14.22 ± 0.3% of the control value. CCSP message levels were further reduced to 1.65 ± 0.7% of the control value at the 12-day time point (Fig. 2), a value equivalent to the threshold of detection. Changes in CYP2F2 mRNA abundance paralleled the decline observed in CCSP mRNA abundance (Fig. 2). Because the presence of the CCSP promoter-regulated HSVtk transgene does not affect steady-state levels of the endogenous CCSP message (Fig. 2), the reduction in CCSP message levels in GCV-treated CCtk mice is due to the loss of CCSP-expressing cells similar to that observed after Clara cell injury by agents such as naphthalene (34). We conclude that exposure of CCtk mice to GCV results in a rapid decline in Clara cell-specific message levels resulting from depletion of the Clara cell population.

To define the kinetics of GCV-mediated Clara cell injury, expression of the stress response gene metallothionein (MT) (Ref. 2 and references therein) was determined. No changes in MT mRNA abundance were observed in total lung RNA from either GCV-exposed wild-type mice or saline-exposed CCtk mice (data not shown), supporting the conclusion that the exposure protocol itself does not adversely affect the lung. In contrast, the abundance of MT mRNA showed a dramatic increase in total lung RNA of GCV-exposed CCtk transgenic mice, exhibiting 501 ± 55 and 1,342 ± 220% increases at the 6- and 12-day time points, respectively (Fig. 2). These data indicate that depletion of the Clara cell population results in the initiation of a stress response by remaining lung cells.

The cellular specificity of HSVtk-dependent, GCV-mediated cell ablation was assessed by immunohistological detection of regional changes in the abundance of CCSP and the ciliated cell marker ACT (16). As noted above, CCSP is expressed in nonciliated cells of both the proximal (Fig. 3A, Control) and distal airways (Fig. 3B, Control) in saline-treated CCtk mice. In accordance with the known distribution of ciliated cells (4), ACT-immunopositive cells were more abundant in the proximal than in the distal airway (compare Fig. 3A, Control and B, Control). The number of CCSP-immunopositive cells in both the proximal (Fig. 3A, d6) and distal airways (Fig. 3B, d6) was reduced after a 6-day exposure to GCV. No CCSP mRNA-expressing cells (data not shown) and only rare necrotic CCSP-immunopositive cells were detected by day 12 of treatment (Fig. 3, A, d12 and B, d12). The few CCSP-immunopositive cells were randomly distributed throughout the airway and did not show a preferential association with epithelial structures such as branch points or NEBs. Depletion of Clara cells was verified with a second Clara cell-specific immunohistochemical marker, CYP2F2 (data not shown). This analysis indicates that GCV treatment of CCtk mice results in virtually complete ablation of Clara cells from all airway locations.

Ablation of HSVtk-expressing cell populations through administration of GCV may adversely affect adjacent cells through a phenomenon known as the bystander effect (39). Evidence of this phenomenon in CCtk mice was sought through morphometric analysis of the ciliated cell population as a function of changes to the total population of airway epithelial cells. The effect of Clara cell depletion on epithelial cell density was determined and is expressed as the number of nuclei per millimeter of BM. The overall cell density in treated tissue was 61.72% of the control value (Fig. 3D). Although changes in cell density were observed in all regions of the airway, those in the terminal bronchioles were more dramatic than those in the most proximal regions of the airway (Fig. 3, A, d12 and B, d12). The effect of Clara cell depletion on the density of ciliated...
cells was determined by morphometric analysis of ACT staining intensity and is reported as intensity per micrometer of BM. The density of ACT-immunopositive cells in the proximal airway does not vary with GCV treatment at either the 6- or 12-day time point (Fig. 3C; and data not shown). However, significant depletion of ciliated cells was observed in terminal bronchioles after a 12-day GCV exposure (Fig. 3B, d12). Loss of both Clara and ciliated cells resulted in apparent denudation of the BM in the terminal bronchioles (Fig. 3B, d12, inset). Selective ablation of Clara cells in the proximal airway suggests that the depletion of ciliated cells in
the terminal bronchioles is secondary to loss of Clara cells from this site rather than the bystander effect.

PNE cell hyperplasia in the absence of Clara cells. Earlier studies (29, 31, 34) using Clara cell-selective lipophilic pollutants have shown that Clara cell ablation is followed by the rapid appearance of focal populations of proliferating CCSP-positive cells that colocalize to hyperplastic NEBs. Although the differentiation potential of NEB-associated Clara cells is not known, Reynolds et al. (29) and others (17, 36) have speculated that this progenitor population contributes to the development of PNE cell hyperplasia. To determine whether CCSP-expressing progenitors are critical for the development of PNE cell hyperplasia, we quantified the abundance of PNE cells after permanent GCV-mediated Clara cell ablation in CCtk mice. Hyperplasia of PNE cells was observed after 12 days of continuous GCV exposure as evidenced by a threefold increase in the total number of PNE cells per millimeter of BM (Fig. 4A). Similarly, the percent of BM occupied by CGRP-immunoreactive cells (data not shown) and the number of NEBs per millimeter of basement membrane was increased twofold in treated animals (Fig. 4A).

These data indicate that NEB hyperplasia and hyper trophy observed in GCV-exposed CCtk transgenic mice occurs independently of Clara cell involvement.

The role of cellular proliferation in PNE cell and NEB hyperplasia after Clara cell ablation was assessed with the proliferation marker PCNA (40). Adjacent section analysis of tissues from animals exposed to GCV for 12 days demonstrated one or more PCNA-positive nuclei within 77.4% of CGRP-immunoreactive regions (Fig. 4B). No other PCNA-positive cells were identified within the conducting airway epithelium of CCtk mice continuously exposed to GCV for 12 days. These data support the notion that PNE proliferation is sufficient for the development of NEB hypertrophy and hyperplasia. Moreover, PNE cell hyperplasia in the absence of CCSP-expressing cells argues that Clara cell proliferation and differentiation are not required for establishment of this pathology.

DISCUSSION

Chronic ablation of Clara cells in CCtk mice. We have established CCtk mice, allowing temporally controlled ablation of airway Clara cells in conducting airways of the lung. CCtk transgenic mice represent a tool to define the contribution of CCSP-expressing epithelial cells toward airway maintenance and repair. We demonstrated that accumulation of HSVtk protein is confined to Clara cells as defined by coexpression with the endogenous Clara cell product CCSP. This pattern of transgene expression was distinct from that previously generated with rat CCSP promoter elements (32). Promoter elements from the rat CCSP gene confer appropriate expression of a chloramphenicol acetyltransferase (CAT) reporter gene within Clara cells of proximal airways, yet fail to confer CAT expression within all Clara cells of terminal bronchioles. Furthermore, the rat promoter supported measurable expression of CAT within alveolar type 2 epithelial cells (32).

The specificity of the mouse CCSP promoter is further illustrated by treatment of CCtk mice with GCV, which results in rapid and specific depletion of all CCSP-expressing cells. Clara cell ablation was associated with PNE cell hyperplasia, desquamation of epithelial cells, and exposure of the terminal bronchiolar basement membrane. Ciliated cells of the proximal airway were not affected by GCV treatment, although a marked effect on the normally sparsely distributed ciliated cells of the terminal bronchioles was noted. In contrast with other models of Clara cell injury and repair, ablation of CCSP-expressing cells in GCV-exposed CCtk mice was not associated with epithelial regeneration.

CCSP-expressing cells in lung homeostasis. The role of the CCSP-expressing cell in lung homeostasis is particularly evident in the terminal bronchioles where cells defined morphologically as Clara cells function as
a progenitor for the ciliated cell population (7). Differential depletion of ciliated cells from this location in GCV-treated CCtk mice suggests that CCSP-expressing cells may not only be necessary for replacement of ciliated cells after injury but could serve to protect existing ciliated cells in injured airways. In contrast with the terminal bronchioles, ciliated cell density in the proximal airways is not altered in GCV-treated CCtk mice. Although the mechanisms underlying the differential susceptibility of the proximal and terminal bronchiolar ciliated cell populations have not been addressed in the current study, this phenomenon may reflect fundamental differences in the interaction of Clara cells and ciliated cells within these different airway regions. As such, an alternative explanation for the loss of ciliated cells in terminal bronchioles of GCV-exposed CCtk mice is their unique susceptibility to cytotoxic GCV metabolites produced in neighboring Clara cells. Structural and functional heterogeneity among Clara cells is well documented (26) and may contribute to this effect.

PNE cell proliferation is sufficient for development of NEB hypertrophy and hyperplasia and is not dependent on Clara cell involvement. Despite a general lack of airway repair, we were able to demonstrate proliferation and hyperplasia of PNE cells after GCV-mediated ablation of CCSP-expressing cells. Reynolds et al. (29) previously demonstrated that Clara cell injury is a stimulus for proliferation and hyperplasia of PNE cells. However, when considering mechanisms that may account for PNE cell hyperplasia, the finding that multiple cell types proliferate in the NEB microenvironment raises the possibility that a non-PNE cell progenitor may yield progeny cells with the capacity to undergo PNE cell differentiation (29). This notion is supported by studies in which labeling methodologies were used to deduce a lineage relationship between PNE and adjacent non-PNE cells in the prenatal (12) and normal adult lungs (17) and by the demonstration of PNE cell hyperplasia in the absence of associated cell proliferation (36). Findings from the present study establish PNE cells as a progenitor population sufficient for development of NEB hypertrophy and hyperplasia. We conclude that CCSP-expressing Clara cells functioning either as progenitors or through elaboration of PNE cell mitogens are not necessary for the process of NEB hyperplasia and hypertrophy in the GCV-treated CCtk mouse.

Progenitor populations of the airway epithelium. Although the existence and identity of stem cells in conducting airways of the mammalian lung remains an issue of debate, characterization of such cells is critical for understanding the basis of lung neoplasia and development of gene therapy approaches (20). A number of proliferative cell types, including the nonciliated bronchiolar (Clara) cells, PNE cells, and basal cells have been described in conducting airways and have been implicated in the development of non-small cell, small cell, and squamous cell lung carcinoma, respectively (reviewed in Refs. 18, 20). Future studies with CCtk mice acutely exposed to GCV represent a unique model in which the contribution of various progenitor populations to regeneration of Clara cells can be determined and the possibility of a pluripotent airway stem cell can be tested.

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