Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium

Yaron Daniely,1,5 Grace Liao,1 Darlene Dixon,2 R. Ilona Linnoila,4 Adriana Lori,3 Scott H. Randell,3 Moshe Oren,5 and Anton M. Jetten1

1Cell Biology Section, Division of Intramural Research, and 2Laboratory of Pathology, National Institute of Environmental Health Sciences, Research Triangle Park 27709; 3Cystic Fibrosis/Pulmonary Monatory Research and Treatment Center, The University of North Carolina, Chapel Hill, North Carolina 27599; 4Experimental Pathology Section, Cell and Cancer Biology Branch, National Cancer Institute, Rockville, Maryland 20850; and 5Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel 76100

Submitted 20 June 2003; accepted in final form 22 February 2004

Daniely, Yaron, Grace Liao, Darlene Dixon, R. Ilona Linnoila, Adriana Lori, Scott H. Randell, Moshe Oren, and Anton M. Jetten. Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. Am J Physiol Cell Physiol 287: C171–C181, 2004; 10.1152/ajpcell.00226.2003.—The trachea and esophagus originate from the foregut endoderm during early embryonic development. Their epithelia undergo a series of changes involving the differentiation of stem cells into unique cell types and ultimately forming the mature epithelia. In this study, we monitored the expression of p63 in the esophagus and the trachea during development and examined in detail morphogenesis in p63−/− mice. At embryonic day 15.5 (E15.5), the esophageal and tracheobronchial epithelium contain two to three layers of cells; however, only the progenitor cells express p63. These progenitor cells differentiate first into ciliated cells (p63+/β-tubulin IV+) and after birth into mature basal cells (p63+/K14+/K5+/BS-I-B4+). In the adult pseudostratified, columnar tracheal epithelium, K14+/K5+/BS-I-B4+ basal cells stain most intensely for p63, whereas ciliated and mucosecretory cells are negative. In stratified squamous esophageal epithelium and during squamous metaplasia in the trachea, cells in the basal layer stain strongest for p63, whereas p63 staining declines progressively in transient amplifying and squamous differentiated cells. Generally, p63 expression is restricted to human squamous cell carcinomas, and adenocarcinomas and Barrett’s metaplasia do not stain for p63. Examination of morphogenesis in newborn p63−/− mice showed an abnormal persistence of ciliated cells in the esophagus. Significantly, in both tissues, lack of p63 expression results in the development of a highly ordered, columnar ciliated epithelium deficient in basal cells. These observations indicate that p63 plays a critical role in the development of normal esophageal and tracheobronchial epithelia and appears to control the commitment of early stem cells into basal cell progeny and the maintenance of basal cells.

A homolog of the tumor suppressor and transcription factor p53, p63, exhibits remarkable structural similarity to p53 (68). The p63 gene encodes at least six different isoforms that are generated by transcription from two different promoters in combination with alternative splicing (32, 63, 68). As p53, the transactivating (TA) isoforms contain an NH2-terminal acidic activation domain (TAD), a DNA binding domain, and an oligomerization domain, whereas the NH2-terminal truncated isoforms (∆Np63) lack the TAD. The isoforms are further distinguished by unique COOH termini. The COOH terminus of the p63α isoforms contains a sterile α motif that is present in several signaling molecules and may function as a protein interaction module (59). The TA isoforms are efficient activators of transcription (32, 63, 68). The ∆N isoforms function as repressors of transcription, either by competing for DNA binding sites or by directly binding to the TA isoforms and rendering them inactive transcription complexes (15, 68). ∆N isoforms also can affect gene expression in a positive manner (11, 12, 15, 57). The latter may involve alternative activation domains and posttranscriptional controls or effects through other transcriptional regulators.

Recent studies of p63-null mice revealed that during embryonic development, p63 plays a critical role in the morphogenesis of several tissues (38, 70). Mice deficient in p63 die soon after birth and display a number of striking developmental defects, including the absence of epidermis, hair follicles, teeth, prostate, and mammary glands, as well as several abnormalities in limb development (38, 58, 70). p63 is particularly highly expressed in progenitor or stem cell populations of a variety of epithelial tissues (38, 43, 58, 65, 70). It is highly expressed in epidermal stem cells, and the lack of a stratified epidermis in p63−/− mice suggests a role in the regulation of differentiation and/or maintenance of epithelial progenitor cells (2, 33, 38, 43, 47, 70). Recent studies (29) have indicated distinct functions for TAp63 and ∆Np63 in the initiation of epithelial stratification and the maintenance of the proliferative potential of basal keratinocytes. Evidence indicating a regulatory role for p63 in development was derived from patients with various autosomal dominant syndromes (7, 9, 37, 62). These disorders have phenotypes reminiscent of p63 knockout mice and have been linked to the presence of heterozygous mutations in the p63 gene (7, 63). Although the role of p53 in cancer is well established, the involvement of p63 in cancer remains relatively unclear (67). The p63 gene maps to human chromosome 3q27-ter, a region known to be amplified frequently in human cancer, particularly in patients with squamous cell carcinomas (18, 25, 35, 40, 61, 64).

In this study, we investigated the expression of p63 during development of the esophagus and trachea and examined how the lack of p63 expression affects the morphogenesis of these tissues.
epithelia (67). The trachea and the esophagus have a common origin and develop from the foregut endoderm at embryonic day 9.5 (E9.5) in the mouse (66). At the time of tracheoesophageal separation (E10.5–E11), the epithelia consist of two to four layers of cells (45) and are likely derived from a common stem cell. Stem cells, defined by the International Society of Stem Cell Research as cells that have the capacity to self-renew as well as to differentiate in more mature cells (http://www.isscr.org/glossary/index.htm), are also responsible for regenerating injured tissue and maintaining tissue homeostasis. Complicating this issue are recent changes in stem cell concepts: stem cells in adult tissues have been reported to have much greater plasticity and differentiation potential than previously thought, and cells well along a differentiation pathway can revert to stem cells (6). In both the tracheobronchial and esophageal epithelia, stem cells differentiate first into ciliated cells, followed by basal cells (50, 66). After birth, the esophageal epithelium becomes a stratified squamous epithelium that in Barrett’s metaplasia transforms into a simple columnar, mucussecretory epithelium as a result of gastroesophageal reflux disease (20). The tracheobronchial epithelium differentiates after birth into a pseudostratified mucociliary epithelium that, during injury and vitamin A deficiency, transforms into a stratified, squamous epithelium (22). However, the stem cell-progeny relationships in these epithelia have not yet been determined with certainty.

In this study, we demonstrate that p63 is abundant in early progenitor cells of both the esophageal and tracheobronchial epithelia in E15.5 embryos and subsequently becomes confined to the K14+/K5+/BS-I-B4+ basal cells and in increasingly reduced levels in transient amplifying cells. Significantly, in both tissues, lack of p63 expression results in the development of both tissues, lack of p63 expression results in the development of normal esophageal and tracheobronchial epithelia and appears to control the commitment of early stem cells into basal cell progeny.

MATERIALS AND METHODS

p63-deficient mice. C57/BL6/p63+/− mice were described previously and kindly provided by Dr. Frank D. McKeon (Department of Cell Biology, Harvard Medical School, Boston, MA) (70).

Immunohistochemistry. Formalin-fixed, paraffin-embedded specimens of human tumors and normal tissues were obtained from the Cooperative Human Tissue Network and the archives of the Laboratory of Pathology of the National Cancer Institute (Bethesda, MD). Sections of human tracheal explants cultured in the presence or absence of 0.1 μM retinoic acid (60) were obtained from Dr. Jonathan Kurie (Program in Cancer Biology, M.D. Anderson Cancer Center, Houston, TX). All human tissues used were collected after approval was obtained from the appropriate institutional review boards at the individual institutions. Mouse tissues were fixed in 4% paraformaldehyde in PBS for 4 h at 4°C, dehydrated, and embedded in paraffin. Sections were examined by immunohistochemistry for lectin binding with the use of peroxidase-labeled lectin Bandeiraea simplicifolia (BS-I-B4; Sigma, St. Louis, MO) or with mouse monoclonal antibodies specific for p63 (4A4; 1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), the basal cell marker keratin 14 (K14; 1:300; Novocastra Laboratories, Newcastle-upon-Tyne, UK), keratin 5 (K5; 1:200; Research Diagnostics, Flanders, NJ), and a marker for ciliated cells, β-tubulin IV (AM-2510-01, 1:1,500; InnoGenex, San Ramon, CA). Staining was performed using a Vectastain Elite ABC system (Vector Laboratories, Burlingame, CA) and diaminobenzidine (DAKO, Carpenteria, CA). Sections were either counterstained with a 1:5 dilution of eosin phloxine stain (Poly Scientific, Bay Shore, NY) or methyl green or stained with hematoxylin and eosin.

For dual staining of p63 and K5, sections were incubated simultaneously with both primary antibodies, and, after washing, Cy3- or Cy2-conjugated donkey anti-mouse and anti-guinea pig secondary antibodies, respectively, were added (no species cross-reaction grade; Jackson ImmunoResearch, West Grove, PA). Slides were viewed on a Zeiss 510 Meta laser scanning confocal microscope (Carl Zeiss, Thornwood, NY).

Western blot analysis. Cells were washed in PBS and then collected in sample buffer (60 mM Tris•HCl, pH 6.8, 2% SDS, 10% glycerol, 10 mM DTT, 1 mM phenylmethylsulfonyl fluoride, aprotinin, and leupeptin) and phosphatase inhibitor mixture I and II (Sigma). Proteins were examined by Western blot analysis with the use of the 4A4 mouse monoclonal anti-p63 or rabbit anti-cornifin (34) antibody. Peroxidase-conjugated anti-mouse or anti-rabbit IgG purchased from Chemicon (Temecula, CA) was used as secondary antibody. Antibodies were diluted in PBS containing 1% milk powder and 0.05% Tween 20. Detection was performed with SuperSignal chemiluminescent substrate (Pierce, Rockford, IL), and luminal and peroxide were purchased from Pierce.

Tissue culture. Human tracheobronchial epithelial cells were obtained from Clonetics (Walkersville, MD) and cultured in complete human tracheobronchial epithelial (TBE) medium as described previously (39). Cells were induced to undergo squamous cell differentiation by the addition of TPA (30 ng/ml).

RESULTS

At E15.5 of mouse development, the epithelial lining of the esophagus consisted of a layer of two to three cells (Fig. 1A), in agreement with previous observations (50). Immunohistochemical analysis showed that all cells that made contact with the basement membrane stained intensely for p63, whereas cells in the superficial layer were p63− (Fig. 1B). These p63− cells likely constitute early progenitor (i.e., stem) cells. They distinguish themselves from basal cells in the adult esophagus in that they do not express K14 (Fig. 1C) and do not bind BS-I-B4 (not shown), which are markers for basal cells (14, 16, 21, 48, 49). The p63+ cells in the upper layers may represent preciliated cells, because these cells stain weakly for the ciliated cell marker β-tubulin IV (Fig. 1D), and ciliated cells were occasionally seen at E15.5 and more frequently observed at later stages of development (not shown). Most if not all ciliated cells resided in the most superficial layer and did not make contact with the basement membrane. Ciliated cells were most numerous at approximately E17, and their number declined rapidly thereafter (50) (not shown). At birth, the epothelial lining is largely a stratified, nonsquamous epithelium in which ciliated cells can still be observed sporadically (Fig. 2D). The few ciliated cells present appear to be shed from the epithelium. The basal cell population may play an important role by expediting this process. The nuclei of cells in the basal layer of the esophageal epithelium of newly born mice remained p63+ (Fig. 2B). Cells that appeared to migrate into the suprabasal layer either did not stain or stained less intensely for p63. At birth, many of the p63+ cells begin to differentiate into basal cells and stain K14 (Fig. 2C) and BS-I-B4 (not shown). Although the degree of maturation and K14 expression can differ considerably between different pups (2 different images in Fig. 2C), basal cells at birth usually do not stain as homogeneously and as strongly for K14 and...
BS-I-B4 as do basal cells in the adult esophagus (Fig. 3, C and D), supporting the hypothesis that these cells are in the process of maturing into basal cells.

After birth, the esophageal epithelium develops into a stratified squamous epithelium. Depending on species, the epithelial lining of the esophagus in the adult remains nonkeratinized, as it does in humans (Fig. 3E), or differentiates into a keratinized, stratified squamous epithelium, as it does in mice (Fig. 3A). The stratified squamous epithelium contains several cell populations: stem cells, transient amplifying cells, and terminally differentiated cells. Stem cells reside in the basal layer and make contact with the basement membrane; they divide infrequently and have a long lifespan, whereas transient amplifying cells have a limited life span and divide more frequently (55, 56). Cells in the basal layer of the squamous epithelium in adult mice stained strongest for p63 (Fig. 3B and F), keratin 14 (K14) (C and G), β-tubulin IV (Tub. IV) (D and H). Epithelia consist of a layer of 2–3 cells. Only cells making contact with basement membrane are p63⁺. Bar in E indicates 20 μm.

In humans, the basal layer of the esophageal epithelium consists of two zones: one overlying the papillae [i.e., the papillary basal layer (PBL)] and the other between the papillae [i.e., the interapillary basal layer (IBL)] (Fig. 3, E and F). Previous studies have provided evidence that stem cells are located mainly in the IBL (55, 56). In both the IBL and the PBL, the basal cells closest to the basement membrane stained most intensely for p63 (Fig. 3F) and K14 (not shown). Some heterogeneity was observed within this layer, however, with some cells staining more intensely than others. Staining for p63 generally declined in the epibasal layers, which consist mainly of transient amplifying cells that transit further into the more suprabasal layers, where they undergo squamous differentiation. Therefore, the highest level of p63 expression appears to be associated with cells that are the least committed to terminal differentiation. This is in agreement with findings in the epidermis, where epidermal stem cells express the highest level of p63, and expression is diminished in transient amplifying cells (38, 43, 70).

In the setting of Barrett’s metaplasia, a disorder in which the stratified epithelium is replaced by a simple columnar epithelium consisting of mucosecretory cells, none of the cells were p63⁺ (Fig. 3G). Basal cells in submucosal glands and cells in the basal layer of certain multilayered glandular epithelia were

---

**Fig. 1.** Expression of p63 in the esophageal and tracheal epithelia of embryonic day 15.5 (E15.5) mouse embryos. Sections of E15.5 mouse embryos were examined by hematoxylin and eosin (H&E) staining (A and F) and immunohistochemical analysis using antibodies specific for p63 (B and F), keratin 14 (K14) (C and G), β-tubulin IV (Tub. IV) (D and H). Epithelia consist of a layer of 2–3 cells. Only cells making contact with basement membrane are p63⁺. Bar in E indicates 20 μm.
p63 (Fig. 3H). These p63+ cells differed from the p63+ basal cells in stratified squamous esophageal epithelium in that they were negative for K14 and BS-I-B4 (not shown). Barrett’s metaplasia is mainly a result of duodenogastroesophageal reflux disease (20). The origin of the columnar cells in Barrett’s metaplasia is still controversial. It has been suggested that Barrett’s metaplasia arises from esophageal stem or basal cells through reprogramming of their differentiation process, from the glandular ducts lining the esophagus, or from cells at the gastroesophageal junction (20, 55). Barrett’s metaplasia has a
high probability of developing into esophageal adenocarcinoma. Adenocarcinomas (n = 3) of esophageal origin did not stain positively or contained few weakly positive cells (not shown); in contrast, all squamous cell carcinomas (n = 9) stained strongly for p63 (Fig. 3H), although with different intensities. Some disagreement exists about the expression of p63 in adenocarcinomas. The absence of p63 staining in Barrett’s metaplasia and adenocarcinoma reported in this study is consistent with recent observations by Glickman et al. (16) but appears to contrast with those of Hall et al. (17), who demonstrated weak staining in Barrett’s metaplasia and strong staining in adenocarcinomas.

As in the esophagus, the tracheobronchial epithelium in E15.5 mice consists of a layer of two to three cells (Fig. 1E). Only the putative stem cells adherent to the basement membrane were p63+ and did not stain for K14, β-tubulin IV (Fig. 1, F–H), K5, or BS-I-B4 (not shown). During development, stem cells first differentiate into ciliated (p63+/ β-tubulin IV+ ) and secretory cells and later, at birth, begin to differentiate into basal cells (p63+/K14+/K5+/BS-I-B4+), thereby generating a pseudostratified epithelium (Fig. 4, A and C) (49). Similarly to basal cells in the esophagus, the basal cells in the tracheal epithelium of newborn mice stained strongly for p63 (Fig. 4B) and sporadically and weakly for K14, K5, and BS-I-B4 (Fig. 4D and data not shown) but began to stain more intensely for K14, K5, and BS-I-B4 after birth, in agreement with findings described in previous reports (49). In adult mice as well as in humans, the tracheobronchial lining consists of a pseudostratified epithelium containing ciliated, basal, and mucosecretory cells. K14+ cells stained positively for p63, whereas ciliated and mucous cells were negative (Fig. 4 and Fig. 5, A and B), in agreement with recent studies (8). Figure 6, B–D, shows that in the normal adult mouse trachea, the expression of p63 also correlates closely with the expression of the basal cell marker K5, in agreement with observations in squamous cell carcinomas (24). Basal cells in mucous cell hyperplasia stained positively for p63 (Fig. 5D), as did basal cells in the submucosal glands of human and mouse (Figs. 5C and 6A). In human basal cell hyperplasia, many cells in basal and suprabasal layers stain positively for p63 (Fig. 5E). Examination of sections from human lung tumors with different histologies showed that expression of p63 is highly correlated with squamous cell carcinomas (6 of 6 squamous cell, 0 of 3 large cell, 0 of 5 adenocarcinoma, and 0 of 4 small cell lung carcinoma samples were p63 positive; not shown), in agreement with previous observations (35, 44, 64). In human lung carcinomas containing mixed tumor types, p63 immunoreactivity was confined predominantly to the region with squamous histology. In squamous cell carcinomas, p63 was localized to the nuclei of less differentiated cells, whereas more differentiated regions did not stain for p63.

In a number of circumstances, such as vitamin A deficiency and injury (22, 31), the tracheobronchial epithelium undergoes basal cell hyperplasia and metaplasia. Organ culture of human trachea in the presence of retinoic acid maintains the normal pseudostratified epithelium, in which only basal cells stain positive for p63 (Fig. 7), whereas in the absence of retinoids, hyperplastic and squamous metaplastic lesions develop. In
hyperplastic lesions, many cells in the basal and suprabasal layers stain positively for p63 (Fig. 7B), whereas in squamous metaplastic regions, only basal cells are p63⁺ (Fig. 7C), as reported for several tissues (3, 9, 16, 30, 46).

TBE cells in culture exhibit many characteristics of basal cells, including the abundant presence of tonofilaments and the expression of K5 and K14 (Figs. 5 and 6) (14, 21, 24, 49). In the presence of retinooids, these cells can be induced to undergo normal differentiation into mucous-secreting and ciliated cells (27, 51). However, TBE cells undergo hyperplasia and squamous metaplasia in the absence of retinoic acid when cultured cells are grown to confluence or are treated with the phorbol ester TPA (13, 34, 36, 52). To examine the expression of p63 during squamous differentiation, logarithmic cultures of human TBE cells were treated with TPA or vehicle. As shown in Fig. 7D, the level of p63 protein was significantly decreased in TPA-treated compared with untreated TBE cells, and the level of the squamous cell marker cornin was dramatically enhanced. These results indicate that in the tracheobronchial epithelium, as in the esophagus and the epidermis, the expression of p63 correlates inversely with squamous differentiation (16, 28, 33, 43, 64).

To obtain more insight into the role of p63 in the morphogenesis of these epithelia, we examined the effect of the lack of p63 expression on the development of these epithelia in p63⁻/⁻ mice. Because p63⁻/⁻ mice die shortly after birth (38, 70), we compared the structure of these epithelia in newborn wild type and p63⁻/⁻ mice. Mice deficient in p63 developed a trachea and an esophagus, suggesting that the lack of p63 does not appear to affect the early stages of development of these tissues, including budding from the foregut endoderm or the tracheoesophageal separation. However, in contrast to the esophagus of newborn wild-type mice, which consists of several layers of cells with few ciliated cells and many K14⁺ basal cells (Fig. 2, A–D), the esophageal epithelium of p63⁻/⁻ mice consisted of a columnar epithelium of largely ciliated cells that lacked K14⁺ basal cells (Fig. 2, E–H), in agreement with previous observations (70). In contrast to the cells in wild-type mice, these ciliated cells made contact with the basement membrane. Similar changes were observed in the tracheobronchial epithelium of newborn p63⁻/⁻ mice: the number of ciliated cells was greatly enhanced, and the epithelial lining consisted of a well-organized, columnar, ciliated epithelium lacking basal cells, as indicated by the abundant staining for β-tubulin IV and the total absence of K14 staining (Fig. 4, D–H).

DISCUSSION

In this study, we show that at E15.5 of mouse development, most if not all cells in the basal layer of both the esophageal and tracheobronchial epithelium stain intensely for p63. We think that these p63⁺/K14⁺/BS-I-B4⁺ cells or a subpopulation of these cells may constitute stem cells (Fig. 8, models A and B). In both epithelia, these stem cells have the capacity to differentiate into ciliated cells (p63⁻/TubIV⁺), which are the first differentiated cells to appear during development, and secretory cells (49). Later, at or shortly after birth, the basally located cells begin to express increasing levels of K14 and K5 and to stain for BS-I-B4, as Randell et al. (49) also observed, suggesting that they are gradually differentiating into mature (p63⁺/K14⁺/K5⁺/BS-I-B4⁺) basal cells (Fig. 8). With the lack of additional stem cell markers, it is not possible to determine whether all p63⁺/K14⁺/BS-I-B4⁻ stem cells differentiate into basal cells that then may constitute the new progenitor cell population in the adult epithelium or whether some sporadic p63⁺/K14⁺/BS-I-B4⁻ or possibly p63⁻/K14⁺/BS-I-B4⁻ stem cells remain. Stem cells are thought to divide infrequently and to have a great renewal capacity (5, 23, 56). A recent study in mouse trachea demonstrated that high K5 promoter activity is
regions thought to contain epithelial stem cells. Confocal microscopy (B) of adult epithelium and in the tracheal gland duct (C), in basal cells in the superficial epithelium (A) and in prebasal cells (D). p63 also is present in p63
expressions demonstrate that p63 is required for the development of basal cells and, in consequence, a normal stratified squamous esophageal epithelium and a pseudostratified tracheobronchial epithelium (Fig. 8). The impact that the lack of p63 expression has on the development of these epithelia is in agreement with the hypothesis that p63 plays a critical role in the regulation of differentiation of stem cells into basal cell progeny or in the maintenance of basal cells. This function of p63 appears to be consistent with the observed role of p63 in basal cells in other epithelia (1, 28–30, 38, 46, 58, 70). In addition, our observations demonstrate that p63+/K14−/BS-I-B4− stem cells present in p63−/− fetal mice retain the ability to undergo differentiation into ciliated and mucosecretory cells in both the tracheal and esophageal epithelium (70) and that the lack of p63 expression may even promote differentiation of these cells into ciliated cells. Therefore, induction of p63 may be required only for the generation of p63+/K14−/BS-I-B4− prebasal cells and subsequently the p63+/K14+/BS-I-B4+ mature basal cells, as recent evidence suggests (29) (Fig. 8, models C and D).

During injury and under vitamin A deficiency, the normal pseudostratified tracheobronchial epithelium is transformed into a stratified squamous epithelium (22). This involves enhanced proliferation of p63+/K14+/K5+/BS-I-B4+ basal cells and their differentiation into transient amplifying cells and subsequently into terminally differentiated squamous cells (Fig. 8). The latter program of differentiation is similar to that associated with epithelial cells residing in the basal layer that exhibit high renewal potential (54). In the current study, we have demonstrated that expression of p63 in adult mouse trachea closely correlates with the expression of K5 (Fig. 6). These observations are in agreement with the hypothesis that p63+/K5+ basal cells or a subpopulation of these cells function as stem cells in the adult tracheobronchial epithelium. This conclusion is in line with the findings of studies showing that the highest level of p63 expression is associated with epidermal keratinocytes exhibiting the highest proliferative capacity (i.e., stem cells) (38, 42, 43, 70).

Our observations in p63−/− mice show that the trachea and the esophagus still develop, indicating that p63 is not required for the budding of the esophagus and the trachea from the foregut endoderm or for the separation of the esophagus and the trachea (66). p63 also is not required for the differentiation of stem cells into ciliated and secretory cells (70). However, the esophageal and tracheobronchial epithelial linings of newborn p63−/− mice are greatly different from those of wild-type mice and consist of a well-organized, largely columnar ciliated epithelium that appears to lack basal cells. These observations indicate that p63 is required for the development of basal cells and, in consequence, a normal stratified squamous esophageal epithelium and a pseudostratified tracheobronchial epithelium (Fig. 8). The latter program of differentiation is similar to that observed during normal squamous differentiation in normal human tracheobronchial epithelial (TBE) cells. Human TBE cells growing in explant cultures exhibit high renewal potential (54). In the current study, we have demonstrated that expression of p63 in adult mouse trachea closely correlates with the expression of K5 (Fig. 6). These observations are in agreement with the hypothesis that p63+/K5+ basal cells or a subpopulation of these cells function as stem cells in the adult tracheobronchial epithelium. This conclusion is in line with the findings of studies showing that the highest level of p63 expression is associated with epidermal keratinocytes exhibiting the highest proliferative capacity (i.e., stem cells) (38, 42, 43, 70).

Our observations in p63−/− mice show that the trachea and the esophagus still develop, indicating that p63 is not required for the budding of the esophagus and the trachea from the foregut endoderm or for the separation of the esophagus and the trachea (66). p63 also is not required for the differentiation of stem cells into ciliated and secretory cells (70). However, the esophageal and tracheobronchial epithelial linings of newborn p63−/− mice are greatly different from those of wild-type mice and consist of a well-organized, largely columnar ciliated epithelium that appears to lack basal cells. These observations indicate that p63 is required for the development of basal cells and, in consequence, a normal stratified squamous esophageal epithelium and a pseudostratified tracheobronchial epithelium (Fig. 8). The latter program of differentiation is similar to that observed during normal squamous differentiation in normal human tracheobronchial epithelial (TBE) cells. Human TBE cells growing in explant cultures exhibit high renewal potential (54). In the current study, we have demonstrated that expression of p63 in adult mouse trachea closely correlates with the expression of K5 (Fig. 6). These observations are in agreement with the hypothesis that p63+/K5+ basal cells or a subpopulation of these cells function as stem cells in the adult tracheobronchial epithelium. This conclusion is in line with the findings of studies showing that the highest level of p63 expression is associated with epidermal keratinocytes exhibiting the highest proliferative capacity (i.e., stem cells) (38, 42, 43, 70).

Our observations in p63−/− mice show that the trachea and the esophagus still develop, indicating that p63 is not required for the budding of the esophagus and the trachea from the foregut endoderm or for the separation of the esophagus and the trachea (66). p63 also is not required for the differentiation of stem cells into ciliated and secretory cells (70). However, the esophageal and tracheobronchial epithelial linings of newborn p63−/− mice are greatly different from those of wild-type mice and consist of a well-organized, largely columnar ciliated epithelium that appears to lack basal cells. These observations indicate that p63 is required for the development of basal cells and, in consequence, a normal stratified squamous esophageal epithelium and a pseudostratified tracheobronchial epithelium (Fig. 8). The latter program of differentiation is similar to that observed during normal squamous differentiation in normal human tracheobronchial epithelial (TBE) cells. Human TBE cells growing in explant cultures exhibit high renewal potential (54). In the current study, we have demonstrated that expression of p63 in adult mouse trachea closely correlates with the expression of K5 (Fig. 6). These observations are in agreement with the hypothesis that p63+/K5+ basal cells or a subpopulation of these cells function as stem cells in the adult tracheobronchial epithelium. This conclusion is in line with the findings of studies showing that the highest level of p63 expression is associated with epidermal keratinocytes exhibiting the highest proliferative capacity (i.e., stem cells) (38, 42, 43, 70).

Our observations in p63−/− mice show that the trachea and the esophagus still develop, indicating that p63 is not required for the budding of the esophagus and the trachea from the foregut endoderm or for the separation of the esophagus and the trachea (66). p63 also is not required for the differentiation of stem cells into ciliated and secretory cells (70). However, the esophageal and tracheobronchial epithelial linings of newborn p63−/− mice are greatly different from those of wild-type mice and consist of a well-organized, largely columnar ciliated epithelium that appears to lack basal cells. These observations indicate that p63 is required for the development of basal cells and, in consequence, a normal stratified squamous esophageal epithelium and a pseudostratified tracheobronchial epithelium (Fig. 8). The latter program of differentiation is similar to that observed during normal squamous differentiation in normal human tracheobronchial epithelial (TBE) cells. Human TBE cells growing in explant cultures exhibit high renewal potential (54). In the current study, we have demonstrated that expression of p63 in adult mouse trachea closely correlates with the expression of K5 (Fig. 6). These observations are in agreement with the hypothesis that p63+/K5+ basal cells or a subpopulation of these cells function as stem cells in the adult tracheobronchial epithelium. This conclusion is in line with the findings of studies showing that the highest level of p63 expression is associated with epidermal keratinocytes exhibiting the highest proliferative capacity (i.e., stem cells) (38, 42, 43, 70).

Our observations in p63−/− mice show that the trachea and the esophagus still develop, indicating that p63 is not required for the budding of the esophagus and the trachea from the foregut endoderm or for the separation of the esophagus and the trachea (66). p63 also is not required for the differentiation of stem cells into ciliated and secretory cells (70). However, the esophageal and tracheobronchial epithelial linings of newborn p63−/− mice are greatly different from those of wild-type mice and consist of a well-organized, largely columnar ciliated epithelium that appears to lack basal cells. These observations indicate that p63 is required for the development of basal cells and, in consequence, a normal stratified squamous esophageal epithelium and a pseudostratified tracheobronchial epithelium (Fig. 8). The latter program of differentiation is similar to that observed during normal squamous differentiation in normal human tracheobronchial epithelial (TBE) cells. Human TBE cells growing in explant cultures exhibit high renewal potential (54). In the current study, we have demonstrated that expression of p63 in adult mouse trachea closely correlates with the expression of K5 (Fig. 6). These observations are in agreement with the hypothesis that p63+/K5+ basal cells or a subpopulation of these cells function as stem cells in the adult tracheobronchial epithelium. This conclusion is in line with the findings of studies showing that the highest level of p63 expression is associated with epidermal keratinocytes exhibiting the highest proliferative capacity (i.e., stem cells) (38, 42, 43, 70).
normal embryonic development, esophageal and tracheobronchial progenitors (p63/H11001) which then differentiate into terminally differentiated squamous cells (p63/H11002). The lack of basal cells in the esophageal and tracheobronchial epithelia of p63−/− mice suggests that p63 regulates the differentiation of progenitors into basal cells or the survival of these cells. The downregulation of p63 during squamous differentiation suggests an additional role for p63 in the maintenance or differentiation of stem cells. Barrett’s metaplasia may arise from reprogramming of p63+/+BS-I-B4+ glandular cells or p63+/+BS-I-B4− basal cells. C and D: in an alternative model, p63+/+BS-I-B4− stem cells can differentiate into ciliated and mucosecretory cells and give rise to p63+/+BS-I-B4− cells. The latter constitute an intermediate stage, prebasal cells, which then mature into p63+/+BS-I-B4− basal cells. B, BS-I-B4 binding (marker for murine tracheal basal cells but for human); TA cells, transient amplifying cells; RA, retinoic acid; Tub, β-tubulin IV.

Fig. 8. Models of stem cell-progeny relationships in the tracheobronchial and esophageal epithelia: role of p63. A and B: during normal embryonic development, esophageal and tracheobronchial progenitors (p63+/K14+/BS-I-B4−) differentiate into ciliated cells (p63+/Tub+/K14+) and later into basal cells (p63+/K14+/K5+/BS-I-B4−). In the mouse tracheobronchial epithelium, basal cells mature after birth. (K14 and K5 expression and BS-I-B4 binding are induced.) Basal cells have self-renewal capacity and in the presence of retinoids give rise to mucosecretory (p63+/K14+/K5+/BS-I-B4−) and ciliated (p63+/Tub+/K14+) cells, thereby generating a pseudostratified epithelium. During injury or vitamin A deficiency, basal cells proliferate and differentiate into transient amplifying cells and subsequently into squamous cells, thereby generating a stratified squamous epithelium. In the esophageal epithelium (B), ciliated cells in the superficial layer shed into the lumen, whereas after birth, basal cells mature (K14 and K5 expression and BS-I-B4 binding increase dramatically), divide, and give rise to transient amplifying cells (p63, K14, K5, and BS-I-B4 decline), which then differentiate into terminally differentiated squamous cells (p63+/K14+/K5+/BS-I-B4−), thereby forming a stratified squamous epithelium. In Barrett’s esophagus, the nonkeratinizing epithelium is replaced by a columnar epithelium consisting of mucosecretory cells (p63+/K14+/BS-I-B4−). The lack of basal cells in the esophageal and tracheobronchial epithelia of p63−/− mice suggests that p63 regulates the differentiation of progenitors into basal cells or the survival of these cells. The downregulation of p63 during squamous differentiation suggests an additional role for p63 in the maintenance or differentiation of stem cells. Barrett’s metaplasia may arise from reprogramming of p63+/K14+/BS-I-B4− glandular cells or p63+/K14+/BS-I-B4− basal cells. C and D: in an alternative model, p63+/K14+/BS-I-B4− stem cells can differentiate into ciliated and mucosecretory cells and give rise to p63+/K14+/BS-I-B4− cells. The latter constitute an intermediate stage, prebasal cells, which then mature into p63+/K14+/BS-I-B4− basal cells. B, BS-I-B4 binding (marker for murine tracheal basal cells but for human); TA cells, transient amplifying cells; RA, retinoic acid; Tub, β-tubulin IV.

of squamous differentiation of basal cells in the esophagus. Squamous differentiation in vivo and in cultured cells is accompanied by downregulation of p63 expression. The precise functions of p63 in basal keratinocytes and the downregulation of p63 during differentiation are not precisely understood (2, 4, 12, 33, 47). It has been suggested that p63 might be required for maintaining the undifferentiated phenotype of basal keratinocytes and that its downregulation might be necessary for basal cells to differentiate into squamous cells and/or in the execution of the differentiation program after cells become committed. The reduced expression of p63 in transient amplifying cells would be in agreement with such a hypothesis but would not prove a role for p63 in the control of this differentiation process. Activation of phosphoinositide 3-kinase, which inhibits epidermal differentiation, recently was reported to positively regulate the expression of ΔNp63α (4), in agreement with a role for ΔNp63α in the survival and proliferative capacity of keratinocytes. Recent studies (12, 26, 29) revealed distinct roles for TAp63 or ΔNp63α in epidermal keratinocytes and demonstrated a role for p63 in the maintenance of the proliferative potential of basal cells and in the initiation of the epithelial stratification program. The association between strong expression of p63 and hyperplasia and/or squamous metaplasia and squamous cell carcinomas may suggest a role for p63 in these pathological processes and is consistent with these proposed functions (10, 16, 19, 24, 25, 35, 40, 42, 44, 53, 64). Defects in the control mechanisms regulating the expression of p63 may promote the undifferentiated phenotype, proliferation, and/or inhibition of apoptosis and therefore may play a role in tumorigenesis (4, 10, 41, 69).
As a result of gastroesophageal reflux disease, the esophagus transforms into Barrett’s metaplasia, consisting of a simple, columnar mucosecretory epithelium (20) and lacking p63 expression. The stem cell-progeny relationships in the esophagus are still poorly understood. Barrett’s metaplasia may arise via reprogramming of the differentiation program of basal cells, from the glandular ducts, or from cells at the gastroesophageal junction (20, 55). It is interesting to note that the basal layer of the glands lining the human esophagus stain positively for p63 but do not stain for the basal cell markers K14 or BS-I-B4+ (not shown) (16). This phenotype is similar to that of the early stem cells in E15.5 mouse esophagus. One might speculate that these p63+/K14+/BS-I-B4− cells in the glands might be closely related to the early stem cells and might have the ability to differentiate into mucosecretory cells. Through reprogramming of their differentiation program and loss of p63 expression, these p63+/K14+/BS-I-B4− glandular cells could function as the progenitors in Barrett’s metaplasia. However, such a mechanism would not explain the development of Barrett’s-like metaplasia in rodents that do not have glands. Alternatively, reprogramming (i.e., dedifferentiation) of p63+/K14−/BS-I-B4+ basal cells into p63+/K14+/BS-I-B4− stem cells might function as the progenitors in Barrett’s metaplasia (20, 55).

In summary, our study demonstrates that p63 plays a critical role in the normal morphogenesis of the tracheobronchial and esophageal epithelia. The lack of basal cells in p63−/− mice suggests that p63 is required for the differentiation of early stem cells into basal cell progeny and/or the maintenance and/or survival of the basal cell population. Although expression of p63 has been reported to be critical to the survival of stem cells in a number of epithelia (38, 58, 70), the esophagus and trachea of p63−/− mice still contain p63+/K14−/BS-I-B4− stem cells that are able to differentiate into ciliated and mucosecretory cells (70). This raises the question of whether p63 is needed only to generate the basal cell progeny. Based on this idea, an alternative stem cell progeny model (Fig. 8C) may be considered in which the adult trachea retains few p63+/K14−/BS-I-B4− stem cells that are able to generate basal, mucous, and ciliated cells. In this model, the p63+/K14−/BS-I-B4− cells constitute prebasal cells, an intermediate step in the differentiation program to mature p63+/K14+/BS-I-B4− basal cells. A recent study provides support for this hypothesis (29).

To date, however, no evidence exists indicating that p63+/K14−/BS-I-B4− stem cells remain in the adult tracheal epithelium. Future studies using additional stem cell markers are needed to distinguish these two possibilities.

ACKNOWLEDGMENTS

We thank Dr. Stephen M. Hewitt of the National Cancer Institute for the esophageal carcinoma tissue microarray slides and Dr. Frank D. McKeon (Harvard University) for providing the p63 heterozygous mice and comments on the manuscript.

GRANTS

This work was supported by American Cancer Society Postdoctoral Grant 01-182-01-TBE (to Y. Daniely) and National Cancer Institute Grant R01-CA-40099 (to M. Oren).

REFERENCES


24. Kaufmann O, Fietze E, Mens J, and Dietel M. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential di
P63 IN ESOPHAGEAL AND TRACHEOBRONCHIAL EPITHELIA


