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Clustering of mutant mitochondrial DNA copies suggests stem cells are common in human bronchial epithelium

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Abstract

Tissue maintenance stem cells, as opposed to transition and/or terminal cells in the epithelium, are possible progenitor cells for human tumors, but little is known about their frequency in human tissues. It occurred to us that the colonies of mutants that should be created when a stem cell mutates and transmits the rare mutation to its descendent transition and terminal cells should, given a quantitative mutation assay, define the average number of cells in a maintenance turnover unit and permit calculation of stem cell number. To test this concept we used a combination of high fidelity PCR and constant denaturant capillary electrophoresis to enumerate mitochondrial point mutations and define their number and distribution among multiple small samples of approximately one million cells containing about 400 million copies of mitochondrial DNA. The bulk of the data were best explained by a model in which most stem cells, defined here as long-lived cells, give rise to colonies of approximately 8–128 cells. In addition, we found that about 1.5% of colonies contained hundreds or even thousands of homoplasmic mutant cells. These expanded turnover units suggest the bronchial epithelium may contain large clusters of cells with mutations, and possibly phenotypic alterations as well.
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1. Introduction

The constant cell renewal characteristic of epithelial tissues is performed by maintenance stem cells. Most of the time, maintenance stem cells divide asymmetrically to create another stem cell, thus replacing itself, and an initial transition cell (reviewed in [1]). The transition

Abbreviations: mtDNA, mitochondrial DNA; CDGE, constant denaturant gel electrophoresis; CDCE, constant denaturant capillary electrophoresis; TMR, tetramethylrhodamine

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cells then undergo a limited number of cell divisions and ultimately give rise to fully differentiated terminal cells that undergo programmed cell death. Stem cells are of particular interest because they are immortal (have a very low death rate) and can thus accumulate and perpetuate the several rare genetic events postulated as requirements for tumor initiation [2,3].

The epithelial cells of the upper airways of the lung give rise to approximately 50% of all primary lung tumors even though they cover less than 1% of the lung surface [4]. Despite the possibility that they are the progenitor cells for lung cancer, bronchial epithelial maintenance stem cells remain poorly understood.

To gain insight into the organization of human tissue as maintenance turnover units of a stem cell and descendent transition and terminal cells, an ideal approach would be to identify stem cells by staining with specific markers. Although such markers have been identified for the skin epidermis and hemato-leukopoietic tissue [5–7], there are no validated stem cell markers for most human tissues, including bronchial epithelium.

It occurred to us that the clusters of mutant cells that we found distributed throughout rodent and human tissues [8,9] might represent the maintenance turnover units derived from mutations occurring in the maintenance stem cell of the turnover unit. Nuclear mutations would amplify through the transition and stem cells to terminal cells [10–13]. Mitochondrial mutations would also be expected to be transmitted to all descendents of a stem cell [14], but would amplify by a more complex route including stochastic extinction [15,16]. Thus, an alternative approach to stem cell enumeration is to determine the number of mutant cells in clusters distributed within a tissue. The size distribution of such clusters of rare mutants would allow estimation of the sizes of such turnover units.

This approach was originally validated in mice; a nuclear mutation that occurred in a single stem cell in colonic epithelium was ultimately transmitted to all cells within a crypt [10]. In human skin samples, immunohistochemistry for *p*53 mutations has been used to identify small patches of clones containing *p*53 mutations. The number of epidermal cells per clone has been estimated as 60–3000 [11] and 10 to several hundred in humans [12], and approximately 16 in mouse [13]. These clusters of mutated cells were more frequent and their sizes were larger in skin that had been exposed to sunlight and in older individuals. However, interpretation is complicated in the case of skin because solar irradiation both induces mutations and selects for the growth of certain types of mutants [13]. The accumulation of mitochondrial DNA mutations in human colonic crypt stem cells and their progeny have been visualized and reconstructed in three dimensions based on biochemical defects in cytochrome *c* oxidase activity [14].

Progenitor cells in the bronchial epithelium have been identified in steady state lung and in lung after mechanical injury [17–22]. Both basal cells and nonciliated secretory cells have been shown to have proliferative capacity using a variety of labeling methods in normal lung and in tracheal grafts. Because both of these cell types have been shown to have proliferative potential, a definitive model establishing the progenitor–progeny relationship in normal bronchial epithelium has not emerged [23].

We have measured the numerical distributions of several different mitochondrial point mutations throughout a series of epithelial sheets derived from smokers and nonsmokers. Our technical approach involved measuring the number of mutant copies for mutations that had occurred in a 100 bp mitochondrial DNA target sequence in a human tissue or cell sample [24,25]. This method allowed us to scan the target sequence (10,030–10,130 bp) for the most prominent mutations. A series of 12 single base pair substitutions present as fractions of $\sim 10^{-6}$ and greater of the total mtDNA pool were detected and measured [9]. Two of these mutations have been observed polymorphisms [26], two others are silent, and we assume that the others are neutral as well. The extent of variability in the number of mutant copies for these mutations across samples provided information about the organization of the bronchial epithelium and the frequency of stem cells, defined here based on the ability of these cells to give rise to a colony of progenitor cells.

2. Materials and methods

2.1. Sample collection

Bronchial epithelial cells were obtained via brush biopsy from healthy adult volunteers and by dissection from deceased organ donors in protocols approved by the MIT and University of Rochester Medical Center institutional review boards [9]. Informed consent

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