Isolating bronchial epithelial cell preparations from gross lung specimens

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Abstract Tissue microdissection is appropriate for separating pure cells from heterogeneous tissues. Recently, we have focused on whole-genome DNA methylation patterns of lung squamous cell carcinoma (SCC) and needed to obtain the appropriate counterpart cells of lung SCC. However, in some regions of human tissues, such as in bronchial epithelium, it is difficult to apply tissue microdissection as a means to isolate pure cells from a heterogeneous mixture of cells. Accordingly, we developed the pop brush method to retrieve sufficient amounts of pure bronchial epithelium from gross lung specimens and this method enables us to study epigenetic variations of lung SCC.

Keywords Tissue microdissection · Whole-genome methylation patterns analysis · Lung squamous cell carcinoma (SCC) · Pop brush method · Bronchial epithelial cell as a counterpart of lung SCC

Tissue microdissection is a useful tool for separating pure cells from heterogeneous tissues. Since the development of this technique, the isolation of a homogeneous population of pure cells from a heterogeneous tissue section has allowed for more accurate results following whole-genome-scale analyses such as gene expression profiling and whole-genome methylation analyses (Bleul et al. 2001; Kitahara et al. 2001; Grove et al. 2002; Miura et al. 2002; Nie et al. 2002; Sato et al. 2003). Recently, we have focused on the whole-genome methylation pattern analysis of lung squamous cell carcinoma (SCC). The most established developmental stages of lung SCC are as follows: (1) in response to outside toxins, normal bronchial epithelium may undergo hyperplasia and squamous metaplasia; (2) the formation of dysplasia, leading to squamous cell carcinoma in situ; and finally (3) the development of invasive squamous cell carcinoma. In order to isolate the appropriate counterpart cells from cancer cells, we tried to separate squamous cancer cells of lungs and normal epithelial from the surgical specimens of lung SCC patients from whom informed consent was obtained. In this manner, we successfully obtained pure cancer cells by tissue microdissection. However, since normal epithelial cells reside in just the most outer membrane within a very narrow region of bronchial walls shown as Fig. 1a, it was difficult to obtain sufficient amounts of these cells for the whole-genome methylation assay. Therefore, it has been considered labor-

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Figure 1. The limitations in obtaining homogenous bronchial epithelial cells from gross lung specimens by tissue microdissection. Since epithelial cells line the most outer membrane of lung tissues and reside within a very narrow region, it was hard to isolate them using a UV laser and IR spotting. In (b), the *red line* is the UV cutting line and the *red circles* represent IR spotting. As shown in (c), none of the epithelial cells were captured using this technique. (a) Gross lung specimen before tissue microdissection. (b) A view of tissue microdissection showing the UV cutting line and IR spots. (c) Gross lung specimen after tissue microdissection.

intensive and time-wasteful to collect bronchial epithelial cells by tissue microdissection. Additionally, as shown in Fig. 1b, c, even though microdissection was completed, bronchial epithelium remained because infrared spotting was not strong enough to detach the cells from the slide in these narrow regions. Here, we developed the pop brush method to separate only the normal bronchial epithelial cells from lung tissues that were not contaminated with other type of cells.

1. Bronchial washing to clean the inner membrane of lungs

Immediately following dissection, lung tissues were washed first with distilled water and then with phosphate-buffered saline (PBS) containing antibiotics. The inner parts

of all bronchi were washed using a syringe and all traces and sputum inside the bronchi were removed.

2. Scrape the inner membrane of the bronchi to obtain epithelial cells

We developed the use of a pop brush to scrape the inner membrane of bronchi and to obtain epithelial cells. At first, lung tissues were immobilized with a syringe on the plate. Next, we scraped the inner wall of the bronchus with a pop brush keeping tension of lung tissues. We usually repeated bronchial scraping to isolate enough epithelial cells for analysis.

3. Preparing genomic DNA from pop brush

After scraping was completed, the end of the pop brush was cut and put into ATL buffer for preparing genomic DNA as shown in Fig. 2a. Next, we followed the “Isolation of genomic DNA from swabs” protocol in the QIAamp DNA Micro Handbook (Qiagen, Hilden, Germany). Ultimately, more than 10- μ g genomic DNA was obtained from normal epithelial cells and this amount was sufficient for analyzing genomic DNA by polymerase chain reaction (PCR).

In order to validate the complete and delicate removal of epithelial cells from bronchi, we made frozen slides of the bronchial tissue used for scraping. Next, staining with hematoxylin and eosin dye was performed and the slides were observed using a light microscope. In this manner, the removal of epithelial cells from the inner membrane of the bronchi was confirmed as shown in Fig. 2b.

The QIAamp DNA micro kit was used to isolate the genomic DNA from normal epithelial cells and PCR was performed on these specimens using a primer pair specific to human C-Jun.

For these reactions, 34 cycles of PCR were performed on 50 ng of genomic DNA. As shown in Fig. 2c, a single band was detected following amplification by PCR and separation by gel electrophoresis. These results show that the genomic DNA of normal epithelial cells from bronchi obtained using the technique described is sufficient for further analysis by PCR.

The genomic and epigenomic study of a pure population of cells has been increasingly used to accurately examine various molecular events happening within these cells (Kuzmin et al. 2002; Kwong et al. 2002; Kim et al. 2003; Chan et al. 2004). Often, tissue microdissection is sufficient to obtain a pure population of cells from a heterogeneous mixture of cells. Some regions, however, such as those containing bronchial epithelial cells are elongated

Figure 2. Validating the preparation of normal bronchial epithelial cells from gross lung specimens. We confirmed the genomic DNA preparation by comparing microscopic observations of the lung bronchus epithelium before and after scraping and by using PCR. (a) *Left panel*, pop brush; *right panel*, scraped brush in the

tube containing buffer. (b) *Left panel*: before scraping; *right panel*: after scraping. (c) PCR results with c-*JUN* primer set using epithelial cell genomic DNA as a template. c-*JUN* primer sequences: forward, 5-TTGTTTGGTGGGTATCCTG-3, and reverse, 5-ATGCAGAAAAGAGGTTAGGG-3.

and too narrow to allow for tissue microdissection, particularly since cellular component such as genomic DNA and RNA may be damaged by the UV light used during this technique. Also, this technique is time consuming and requires many tissue slides to obtain sufficient amounts of bronchial epithelium required for whole-genome analyses. Here, we developed the pop brush method to obtain sufficient amounts of bronchial epithelial

cells from gross lung tissues. Finally, we suggest that this method provides us with enough homogeneous normal counterparts of lung SCC for studying genetic and epigenetic variations.

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