〈Brief Note〉

Observation of surface structures using cell transfer from Papanicolaou-stained preparation

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Summary Glutaraldehyde and osmium tetroxide are used in scanning electron microscopy for fixation, and cells are observed following the completion of various steps. This technique is routinely employed to observe cell surfaces, and is particularly useful for the diagnosis of hairy cell leukemia. Cell transfer is performed by scraping cells smeared on a preparation and transferring them to a different preparation. In the present study, cells were transferred from a Papanicolaou-stained specimen, and were observed using scanning electron microscopy.

Key words: Scanning electron microscopy, Cell transfer, Cytology

1. Introduction

Glutaraldehyde and osmium tetroxide solutions are the most commonly used means of fixation in scanning electron microscopy (SEM).

In the present study, cells were photographed in a Papanicolaou (Pap) - stained specimen and were then transferred in order to determine whether their surface structures were observable by SEM. Cell transfer was initially performed by scraping cells smeared on a preparation and transferring them to a different preparation. This method is used to repair broken specimens and return them to their original state. Surface structures were observed with this method. The present study introduced our method and experience, which have not yet been published in Japan.

2. Materials and Methods

Written informed consent was obtained from all volunteers, and our study was conducted in compliance with the rules for human experimentation at our institution.

Definitely diagnosed carcinoma of the breast, papillary adenocarcinoma of the thyroid, and adenocarcinoma (pleural effusion) were used.

1) After photographs has been taken, Pap-stained preparations were immersed in xylene, the cover glasses were removed, and Entellan new mounting agent was applied to the surfaces on which cells were

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smeared. 2) The slide glasses were maintained in a horizontal position at 37℃ overnight in order to solidify the mounting agent. 3) The slide glasses were placed in a 50℃ hot bath for approximately 2 hours, and the softened mounting agent was peeled off using a knife and forceps. 4) Self-made film slides (blue slides prepared by cutting an X-ray film into the size of a slide glass and removing the X-ray-sensitive film), and the cells that adhered to the mounting agent were transferred to a blue slide for scanning electron microscopy. 5) The regions containing the target cells were cut out using a utility knife into small pieces (2-3 mm) and placed on a blue slide. 6) The blue film was extended in a hot bath and dried on an extender for approximately 1 hour to complete cell transfer. The remaining region was similarly dried using an extender, Entellan new mounting agent was removed using xylene, and the sample was mounted following the standard procedure to preserve preparations. 7) The blue slides were placed in xylene/100% alcohol, 100% alcohol, 100% alcohol, and t-butyl alcohol in this order, with 2 exchanges of the solution every 30 minutes. 8) t-Butyl alcohol was added to a level that completely immersed the sample, followed by freezing in a refrigerator. 9) The samples were dried at 0-5℃ in a t-butyl alcohol freeze dryer. 10) The sample was placed on the stage, metal-coated, and observed by SEM.

3. Results

Clusters of breast adenocarcinoma cells were clearly observed under light microscopy (LM). Tumor cell cytoplasm was strongly stained light green (Fig. 1). SEM revealed that the surface (Fig. 1) was covered with globular processes (Fig. 2).

Papillary adenocarcinoma of the thyroid had a high N/C ratio under LM and intranuclear cytoplasmic inclusion (Fig. 3) and grooves were also present.

Regarding the surface structure of papillary adenocarcinoma of the thyroid, intranuclear cytoplasmic inclusions were slightly concave under SEM, as shown in Figure 4.

Under LM, adenocarcinoma cells in coelomic fluid formed aggregates in many cases. As shown in Figure 5, the N/C ratio of these malignant cells was high, the chromatin pattern was coarsely granular, and nucleoli were clearly observed.

Figure 6 shows that, under SEM, the surface of adenocarcinoma cells was covered with long, narrow microvilli.

4. Discussion

Samples for scanning electron microscopy are generally prepared by double fixation with glutaraldehyde and osmium tetroxide. In the present study, we transferred cells from Pap-stained preparations to other slides (custom-made blue slides), subsequently performed various processes, and then observed the samples using SEM. Brown et al.[1] reported methods to restore preparations on broken slide glasses while Sherman et al.[2] described various immunostaining methods that are applicable for cell transfer, in which cells are transferred to another slide glass. In our method, the cover glass was removed from a Pap-stained preparation, and cells were transferred to self-made film slides. Various procedures were employed, and the preparations were observed using scanning electron microscopy. Kaneko et al.[3] previously reported a light microscopic-scanning-transmission electron microscopic method, i.e., a continuous observation method[4].

In these methods, cells were smeared on a blue slide, followed by fixation with glutaraldehyde and Pap staining. After light microscopic photographs had been taken, the cover glass was removed, the blue slide was cut into 4-mm square pieces using a utility knife, treated with various processes, and observed using scanning and transmission electron microscopy. They stated that the surface structure and cytoplasmic organelles of adenocarcinoma cells were clearly observed. Kobayashi et al.[5] also obtained favorable results with bar-shaped chromatin and adenocarcinoma cells.

An application of this technique has been the observation of columnar epithelial cells in natural urine, namely, hyaline casts, granular casts, red blood cell casts, white blood cell casts, and fatty casts.

The observation of columnar epithelial cells,

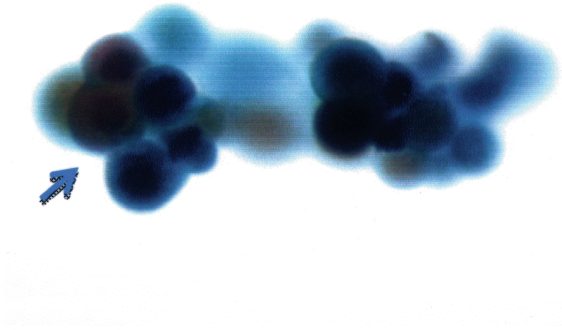

Fig. 1 Breast cancer.
A cluster of tumor cells was observed (arrow).
(Pap stain x200)

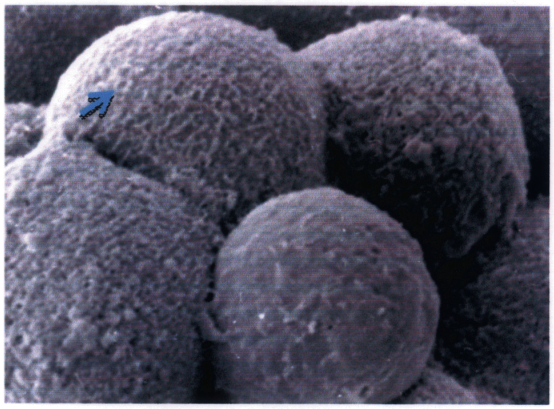

Fig. 2 Scanning electron micrograph of Figure 1.
The surfaces of tumor cells were covered with globular processes (arrow). (SEM x1,500)

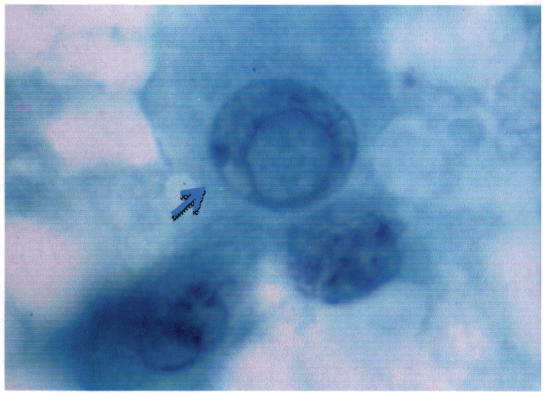

Fig. 3 Papillary adenocarcinoma of the thyroid.
An intracytoplasmic nuclear inclusion was observed (arrow). (Pap stain x500)

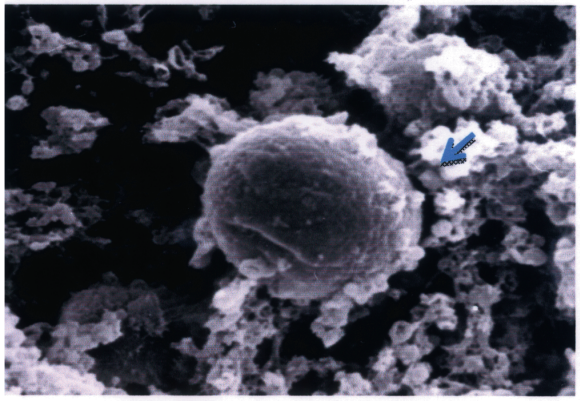

Fig. 4 Scanning electron micrograph of Figure 3.
A slight nuclear elevation was observed (arrow).
(SEM x1,000)

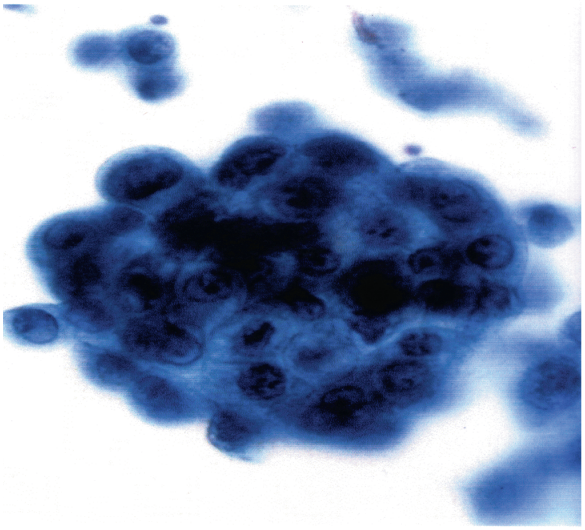

Fig. 5 Adenocarcinoma cell of effusion.
A cluster of tumor cells was observed. Tumor cells had oval nuclei and distinct nucleoli.
(Pap stain x300)

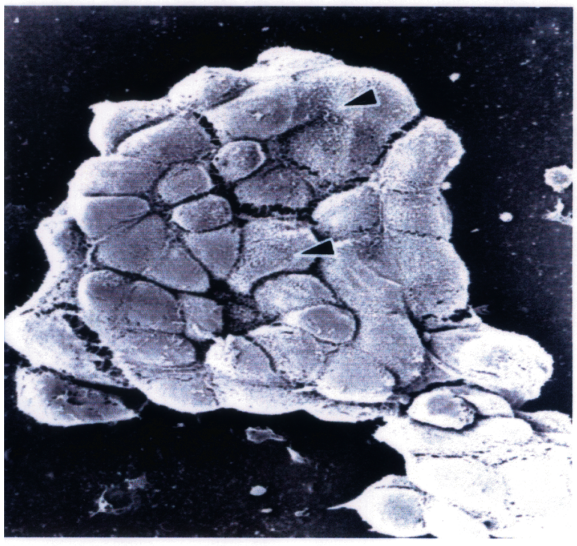

Fig. 6 Scanning electron micrograph of Fig. 5.
The surfaces of tumor cells were covered with many microvilli (arrowheads). (SEM x500)

except for hyaline casts, is important as it indicates renal parenchymal lesions. Furthermore, the identification of columnar epithelial cells is possible through observations of the surface structure, and has been used to diagnose glomerulonephritis.

Difficulties have been associated with identifying the cells in the body cavities of cancer patients. The results of the present study suggest that LM-SEM is of diagnostic value because it is capable of distinguishing between mesothelial cells, malignant mesothelioma, and adenocarcinoma cells. It is desirable for LM and SEM observations to be introduced into various clinical aspects in order to improve diagnoses by cytologic examinations, assess therapeutic effects, decide appropriate drug selection, and perform prognostic presumptions.

We observed the surface structures of transferred cells and obtained favorable results, which confirmed that cells were transferable from Pap-stained preparations. We intend to investigate the application of this method in other fields in future studies.

References

1 Brown GG, Tao LC: Restoration of broken cytology slides and creation of multiple slides from a single smear preparation. Acta Cytol, 36: 259-263, 1992.

2 Sherman ME, Jimenez-Joseph D, Gangi MD, Rojas-Corona RR: Immunostaining of small cytologic specimens facilitation with cell transfer. Acta Cytol, 38: 18-22, 1994.

3 Kaneko C, Niimi H, Shinzato M, Shamoto M: Comparative studies of the same adenocarcinoma cells, macrophages and mesothelial cells by light microscopy, scanning electron microscopy and transmission electron microscopy. Diagn. Cytopathol, 11: 333-342, 1994.

4 Kaneko C, Shamoto M, Niimi H, Osada A, Shimizu M, Shinzato M: Studies on intranuclear inclusions and nuclear grooves in papillary thyroid cancer by light, scanning electron and transmission electron microscopy. Acta Cytol, 40: 417-442, 1996.

5 Kobayashi TK, Ishida Y, Kaneko C, Tsubota K: Bar-shaped nuclear chromatin in conjunctival samples: with cytologic features and ultrastructural correlation, Acta Cytol, 42: 129-1132, 1998.