学 位 論 文 の 要 旨

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学位論文名　Comparison of Ultra-Magnifying Endocytoscopic and Hematoxylin-Eosin-Stained Images of Lung Specimens
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論 文 内 容 の 要 旨

INTRODUCTION

Endocytoscopy (ECS) enables real-time observation of lesions at ultra-magnification. ECS has been used in the gastrointestinal field in clinical practice. Endocytoscopic images of colorectal lesions are similar to their hematoxylin-eosin (H&E)-stained images. Nevertheless, some respiratory field studies have reported endocytoscopic observations, and no quantitative studies have compared the endocytoscopic findings with the pathologic findings. This study aimed to obtain the endocytoscopic findings of pulmonary lesions for quantitative comparison of the nuclear features with those of H&E-stained images.

MATERIALS AND METHODS

This prospective, single-center, observational study was performed at the Shimane University Hospital. We conducted this study from September 2019 to September 2021 in 40 patients who underwent surgical resection of pulmonary lesions at the Department of Cardiothoracic Surgery, Division of Thoracic Surgery, Shimane University. Thoracic surgeons performed surgical resection of the lesion. A pathologist cut into the center of the lesion in the resected specimens. We stained the cut surface with a drop of 0.25–0.5% methylene blue. The tip of the endocytoscope was placed directly on the cut surface, and we immediately observed the stained lesion on the monitor screen. We attempted to observe the normal lung area in a similar manner. These observations were recorded using a video recorder. The specimens were fixed with formalin and stained with H&E.

We obtained H&E-stained images of the pathologic tissues, and every image was processed using the ImageJ. We extracted the stained nuclear component and obtained the following five nuclear features; nuclear number per area, mean nucleus area, median circularity, coefficient of variation (CV) of roundness, and median Voronoi area. We conducted dimensionality reduction analyses for these features, followed by assessments of the

inter-observer agreement among two pathologists and two pulmonologists to evaluate endocytoscopic videos. The study protocol was approved by the Research Ethics Committee of Shimane University.

RESULTS AND DISCUSSION

In total, 40 lesions from 40 cases were diagnosed histopathologically. Of the 40 lesions, 36 were malignant and 4 were benign cases. Thirty-eight lesions and 38 normal lung tissue specimens were observed by ECS.

To identify the features obtained from H&E-stained and endocytoscopic images, we determined the nuclear features of the normal lung, benign lesions, and malignant lesions via image analyses. All 40 lesions were included in the analysis of the H&E-stained Images. Nuclear feature analysis revealed that the nuclear number per area and mean nucleus area were significantly higher in the malignant lesions than in the normal lung tissues. Moreover, the median circularity, CV of roundness, and median Voronoi area were significantly higher in normal tissues than in malignant lesions. The nuclear number per area was also higher, and the median Voronoi area was lower in benign lesions than in normal lung tissues. Similarly, we performed nuclear extraction and the quantification of the nuclear features from the endocytoscopic images. Thirty-three of 40 lesions were included in the analysis of the endocytoscopic images, and 19 of 40 normal lung tissues were included in the analysis of the endocytoscopic images. The results were consistent with the H&E-stained image analysis. All five features were concordant between H&E-stained and endocytoscopic images.

We compared the values of H&E-stained and endocytoscopic nuclear features extracted from similar specimen areas of the same cases where an H&E-stained and endocytoscopic image were both obtained. However, no feature values were correlated between the H&E-stained and endocytoscopic images. To obtain the data trend for all five nuclear features, we presented heat maps of the normalized nuclear feature values in the H&E-stained and endocytoscopic images. Subsequently, we performed the PCA and UMAP, respectively, using normalized values. PCA and UMAP clustering and embedding revealed analogous cluster locations between the H&E-stained images and endocytoscopic images. We distinguished the normal lung-prone and malignant lesion-prone areas in the UMAP clusters. Thus, the five nuclear features displayed similar trends in both H&E staining and ECS.

To investigate agreements between observers of ECS, the pathologists and pulmonologists evaluated 36 cases from the recorded endocytoscopic videos. We assessed the diagnostic accuracy and inter-observer agreement between the three categories (ECS-adeno, ECS-non-adeno, and ECS-benign). The total diagnostic accuracy of pathologists 1 and 2 was 58.3% and 52.8%, respectively, and their inter-observer agreement was 0.38, i.e., fair. The total diagnostic accuracy of pulmonologists 1 and 2 was 50% and 47.2%, respectively, and their

inter-observer agreement was 0.33, i.e., fair. Inter-observer agreement between each two of the four observers was between 0.31 and 0.43, i.e., fair or moderate.

This study revealed that the five nuclear features significantly differed between the malignant lesions and normal lungs in both the H&E-stained and endocytoscopic images. To our knowledge, this is the first report of ECS in various types of pulmonary lesions and a quantitative investigation of endocytoscopic images.

We found that five nuclear features are beneficial for distinguishing between normal and malignant tissue in the resected specimens in this study. All five features were concordant between the H&E-stained and endocytoscopic images. Despite significant differences in the five nuclear features between the malignant lesions and normal lungs in the H&E-stained and endocytoscopic images, all five nuclear features were not correlated with the H&E-stained and endocytoscopic images in each feature of the same lesion. The combination of all five nuclear features provided information on the malignant and normal lung cells since the UMAP-mapped data locations of the malignant lesions and normal lungs of the endocytoscopic images were considerably similar to those of the H&E-stained images. In clinical practice, pathologists determine the malignancy of a lesion based on the combination of multiple features of the cells and extracellular matrix. Our findings might also provide evidence for the importance of the five nuclear features in distinguishing lung malignancy. Considering ex vivo application, ECS will likely be useful in determining whether the specimen of the pulmonary lesions is qualified for pathologic diagnosis during bronchoscopy. Evaluating the nuclear features of biopsy specimens using ex vivo ECS may reduce the number of biopsies by assessing the specimen's amount and quality. In addition, ex vivo ECS may assist in the rapid pathologic diagnosis of tissues obtained by surgery, bronchoscopic biopsy, or CT-guided biopsy. We examined the inter-observer agreement to investigate the diagnostic ability of ECS for surgically resected specimens. Although the pathologists are experts in pathological images, agreements were only fair between the pulmonologists and between the pathologists that carried out an ECS evaluation. As they have little experience observing endocytoscopic videos with only two training videos, training could improve the diagnostic ability of ECS. In addition, as a new approach in the gastroenterology field, crystal violet and methylene blue double staining and artificial intelligence (AI) are used to improve the diagnostic ability of ECS. Moreover, AI may facilitate the on-site identification of histological types of biopsy specimens based on the nuclear features of endocytoscopic images.

CONCLUSION

The nuclear features of pulmonary lesions and normal lung tissue were similar in both the endocytoscopic and H&E-stained images. Endocytoscopic imaging could provide nuclear information similar to that of H&E-stained images.