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Original contribution

Tumor microenvironment in functional adrenocortical adenomas: immune cell infiltration in cortisol-producing adrenocortical adenoma☆,☆☆

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Summary The tumor microenvironment plays pivotal roles in various human neoplasms. However, that of benign tumor, particularly hormone-secreting endocrine tumors, has remained virtually unknown. Therefore, we firstly attempted to analyze the tumor microenvironment of autonomous hormone-secreting adrenocortical adenomas. We first histologically evaluated 21 cortisol-producing adrenocortical adenoma (CPA) and 13 aldosterone-producing adrenocortical adenoma (APA) cases. Quantitative histologic analysis revealed that intratumoral immune cell infiltration (ICI) was more pronounced in CPAs than in APAs. We then evaluated the cytokine and chemokine profiles using polymerase chain reaction arrays in APAs and CPAs. Angiogenic chemokines, C-X-C motif chemokine ligand (CXCL) 1 and CXCL2, were significantly more abundant in CPAs than in APAs using subsequent quantitative polymerase chain reaction and immunohistochemical analyses. We then examined the vascular density between these 2 adenomas, and the density was significantly higher in overt CPAs than in APAs. Of particular interest, CXCL12-positive vessels were detected predominantly in CPAs, and their infiltrating immune cells were C-X-C motif chemokine

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receptor 4 (CXCR4) positive. These results above indicated that CXCL12-CXCR4 signaling could partly account for ICI detected predominantly in CPAs. We then further explored the etiology of ICI in CPAs by evaluating the senescence of tumor cells possibly caused by excessive cortisol in CPAs. The status of senescence markers, p16 and p21, was significantly more abundant in CPAs than in APAs. In addition, all CPA cases examined were positive for senescence-associated β-galactosidase. These results all indicated that exposure to local excessive cortisol could result in senescence of tumors cells and play essential roles in constituting the characteristic tissue microenvironment of CPAs.

1. Introduction

Tumor microenvironment has been recently reported to play pivotal roles in tumor development, progression, and others in various human malignancies, which could also possibly serve as a therapeutic target [1]. The tumor microenvironment consists of parenchymal or tumor and stromal cell components with extracellular matrix. Of these components above, the immune system, including cytokines and chemokines, is generally considered one of the most important factors involved in both tumorigenesis and antitumor response [1]. However, few have studied its status in benign neoplasms, and in particular, the microenvironment of hormone-secreting endocrine tumors has remained virtually unknown.

Adrenocortical adenoma is the most common primary benign adrenal tumor [2] and could hormonally be further subclassified into nonfunctioning and functioning ones. Among functioning adenomas, 2 major subtypes are aldosterone-producing adenoma (APA) and cortisol-producing adenoma (CPA) [2]. Steroids have been known to exert enormous effects on both local and systemic immunities [3,4]. Glucocorticoid is also known to exert profound anti-inflammatory effects and has been widely used to control various inflammatory conditions [3]. Aldosterone is also known as a modulator of immunity, and excessive aldosterone could cause direct organ damages in the heart, vasculature, and kidneys [4]. Therefore, functioning adrenocortical adenomas are most likely to be constantly exposed to high concentrations of immune-modulating steroids, but their tissue microenvironments, especially their effects on the tumor immunity, have not been explored at all. In adrenocortical adenomas, the presence of intratumoral macrophages and lymphocytes infiltration has been reported [5,6]. In addition, APA cases harboring massive infiltration of mast cells have been also reported [7,8]. However, a detailed examination of these immune cells, their prevalence, and the relevance to the secreting steroids has not been performed at all to the best of our knowledge. Therefore, in this study, we firstly studied the tumor microenvironment of autonomous hormone-secreting adrenocortical adenomas including APAs and CPAs, particularly focusing on intratumoral immune cell infiltration (ICI).

2. Materials and methods

2.1. Patients and adrenal tissues

All the specimens examined in this study were retrieved from surgical pathology files of the Department of Pathology, Tohoku University Hospital. They were all Japanese who underwent surgical treatment from 2011 to 2015 and had been fixed in 10% formalin and embedded in paraffin, with portion of the specimens frozen in liquid nitrogen and kept in deep freezer at −80°C until use. Both immunohistochemical and quantitative polymerase chain reaction (PCR) analyses were performed in 21 CPA cases, clinically associated with overt Cushing syndromes (13 cases) and with subclinical Cushing syndrome (8 cases), 13 APA cases, and 2 nonfunctioning adenoma (NFA) cases. Hematoxylin and eosin (H&E) stain and immunohistochemical analysis were performed on the tissues fixed in 10% formalin and embedded in paraffin. The histopathological diagnosis of CPA and APA had been made as previously reported [9,10]. Clinicopathological features of these 36 cases studies are summarized in Table 1. Among the frozen specimens, 3 CPA and 3 APA cases were randomly selected and submitted for further PCR array analysis.

In addition, histopathological features of all the 83 CPA cases surgically removed from 1999 to 2015 were carefully reviewed and 21 cases harboring relatively abundant ICI were evaluated to further characterize the features of these immune cells. Five CPA cases were freshly prepared for histochemical staining of senescence-associated β-galactosidase (SA-β-gal). All research protocols of this study were approved by the Ethics Committee of Tohoku University Graduate School of Medicine (approval number 2015-1-651).

2.2. Histologic and immunohistochemical analyses

In this study, the percentage of the areas occupied by immune cells over total tumor area was analyzed using "HALO TM Area Quantification ver. 1.0" program (Indica laboratories, Corrales, NM) to quantitatively evaluate the status of tumor-infiltrating immune cells [11].

Immunostaining was performed using a biotin-streptavidin method with Histofine SAB-PO kit (Nichirei, Tokyo, Japan). The characteristics of the primary antibodies used in this study