Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

An immunohistochemical panel consisting of EZH2, C-KIT, and CD205 is useful for distinguishing thymic squamous cell carcinoma from type B3 thymoma

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ARTICLE INFO

Keywords:
Thymoma
Thymic carcinoma
EZH2
C-KIT
CD205
Immunohistochemistry

ABSTRACT

Type B3 thymoma and thymic squamous cell carcinoma (SqCC) often cause a diagnostic problem due to their histological similarities. The aim of this study is to identify EZH2 as a novel and powerful biomarker that can effectively distinguish thymic SqCC from type B3 thymoma, and find optimal combinations among 11 markers. A total of 53 patients, comprising 26 with type B3 thymoma and 27 with thymic SqCC, were allocated to the discovery or validation cohorts, and immunohistochemical staining was performed and analyzed. The expression level of each marker was scored, and receiver-operator characteristic curve analysis was performed to evaluate their diagnostic accuracies. This analysis identified EZH2, C-KIT, and CD205 as useful markers for distinguishing thymic SqCC, and a combined panel approach using them further improved diagnostic accuracy in both the discovery and validation cohorts. In the combined cohorts analysis, EZH2 was the single best marker with 88.9% sensitivity and 100% specificity [area under the curve (AUC) = 0.967]. The sensitivity and specificity were 85.2% and 100% (AUC = 0.962) for C-KIT, and 100% and 73.1% (AUC = 0.844) for CD205. The combined panel had the highest sensitivity and specificity at 96.3% and 100%, which was significantly or marginally higher than those of EZH2, C-KIT, and CD205 alone (P = 0.071, 0.034, and 0.005, respectively). The present findings indicate that EZH2 is useful as a novel diagnostic marker for distinguishing thymic SqCC and that the panel approach can be used as an effective differential diagnostic tool in daily practice.

1. Introduction

Primary thymic epithelial neoplasms are rare anterior mediastinal tumors that originate from thymic epithelial cells. The tumors have been classified into thymoma and thymic carcinoma, and the World Health Organization (WHO) classification of thymic epithelial tumors, which is currently the most widely used histological classification, divides the tumors into types A, AB, B1, B2, B3, and thymic carcinoma, on the basis of the morphology of the tumor cells and the lymphocyte-to-epithelial cell ratio of the tumor [1]. Although the role of this classification with regard to prognosis has not yet been completely validated [2], the prognosis of type B3 thymoma appears to fall between that of thymic squamous cell carcinoma (SqCC) and other types of thymoma [3], making the differentiation between type B3 thymoma and thymic SqCC an important task for the pathologist.

Currently, thymic SqCC is differentiated from type B3 thymoma based on both the histological features described above and several immunohistochemical markers, such as C-KIT and CD5 [1,4–9]. However, differential diagnosis of type B3 thymoma and thymic SqCC histologically is sometimes difficult due to their morphological similarities, particularly in small biopsy specimens [2,3,9,10]. Although C-KIT and CD5 have been reported to be useful for distinguishing thymic SqCC, they also had limited sensitivities for distinguishing thymic carcinoma [2–7]. Thus, although more immunohistochemical markers to distinguish thymic SqCC from type B3 thymoma have been reported [2,3,10], reliable diagnostic markers still remain to be identified. That is, except for C-KIT and/or CD5, no markers have yet been reliably validated across multiple studies assessing the diagnostic significance of the candidates.

This study aims to determine whether the immunohistochemical

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https://doi.org/10.1016/j.prp.2018.01.009
Received 27 October 2017; Received in revised form 14 January 2018; Accepted 24 January 2018

expression of 11 markers, CD205, RANK, RANKL, CD40, FGF7, FGFR2, EZH2, BMI1, H3K27me3, C-KIT and CD5 (Supplementary Table S1), are significantly different between type B3 thymoma and thymic SqCC and, if they are, whether they can effectively distinguish these conditions using the discovery cohort. The first 6 markers are related to the development and reconstitution of the normal thymus and were selected because thymomas tend to recapitulate normal thymic epithelium to various degrees [11]. The next 3 markers, including EZH2, BMI1 and H3K27me3, were selected because they are related to HDAC, which was reported to correlate with the aggressiveness of thymic carcinoma and be a target for Belinostat, a pan-HDAC inhibitor for the group of heavily pretreated thymic malignancies [12]. C-KIT and CD5, 2 routinely used markers in daily practice, were selected for comparison of differential diagnostic performance.

Since single markers usually do not reach sufficient sensitivity and specificity for differential diagnosis [13,14], multiple markers were selected for combination into a panel to establish whether the panel was superior to the individual markers for distinguishing thymic SqCC from type B3 thymoma. The results from the discovery cohort were evaluated further by comparing them with data from the validation cohort and finally, their diagnostic accuracies were more precisely estimated and compared using the combined discovery and validation cohorts.

2. Materials and methods

2.1. Patient selection and study design

Seventy-two patients who were diagnosed with type B3 thymoma or thymic carcinoma between 1997 and 2013 at the Seoul National University Hospital were retrospectively identified from the hospital's electronic database. Hematoxylin and eosin (HE)-stained slides of the tumors were reviewed and reclassified by two pathologists (BSK and JKW) according to the WHO 2015 classification scheme [1], and 53 patients were finally selected, including 26 with type B3 thymoma and 27 with thymic SqCC. C-KIT and CD5 slides were not used in the initial pathological slide review and the diagnosis was done by morphological consensus. Most of the patients underwent surgery as initial treatment for a primary tumor, and formalin-fixed, paraffin-embedded primary tumor tissues were available for all 53 patients. The clinicopathological data, including age, sex, and their Masaoka-Koga stage [15,16], were obtained from the medical records and pathology reports. The cases were allocated to two cohorts in chronological order: those who were treated between 1999 and 2007 to the discovery cohort, and those who were treated between 2008 and 2013 to the validation cohort (Fig. 1). This study was approved by the institutional review board for human subject research at Seoul National University Hospital.

2.2. Tissue microarray

Tissue microarray (TMA) blocks (Superbiochips Laboratories, Seoul, Korea) were prepared. Briefly, after a representative tumor area was carefully selected and marked on an HE-stained slide, two core tissue biopsies (2-mm diameter) were taken from the corresponding donor paraffin block and arranged in a recipient paraffin block using a trephine. Cases were considered to represent a tumor if the tumor occupied more than 10% of the core area. Representative figures of type B3 thymoma and thymic SqCC are presented in Fig. 2A and B.

2.3. Immunohistochemistry

TMA blocks were sectioned into 4-mm slices and affixed onto glass slides. Sections from the discovery cohort were subjected to immunostaining with 11 antibodies using BenchMark XT (Ventana, Tucson, AZ, USA), BOND-MAX (Leica Microsystems, Bannockburn, IL, USA) or autostainer 360 (Lab Vision, Fremont, CA, USA) systems, according to the manufacturer's instructions. Details of the 11 antibodies used in this study are described in Supplementary Table S1. After analysis on the discovery cohort, 3 out of 11 antibodies were selected for validation (see Sections 3.2 and 3.3), and sections from the validation cohort were immunostained with these 3 antibodies in the same way as above.

For CD205, RANK, RANKL, CD40, FGF7, FGFR2, C-KIT, and CD5, the staining intensity of the cytoplasm and/or membrane was scored as 0 (not stained), 1+ (weakly stained), 2+ (moderately stained), and 3+ (strongly stained). Cases were considered stained only if the extent of staining was more than 10%, and less than 10% overall staining was considered negative. For EZH2, BMI1, and H3K27me3, the percentage of stained tumor nuclei was calculated and scored as 0–100%.

2.4. Statistical analysis

The clinicopathological variables were analyzed using Student's *t*-test or Fisher's exact test. The immunohistochemical expression in the discovery cohort was analyzed using the Mann–Whitney test or Fisher's exact test. A Benjamini-Hochberg correction was performed for multiple testing of 11 markers, and markers with a false discovery rate (*Q* value) < 0.01 were selected for subsequent analysis.

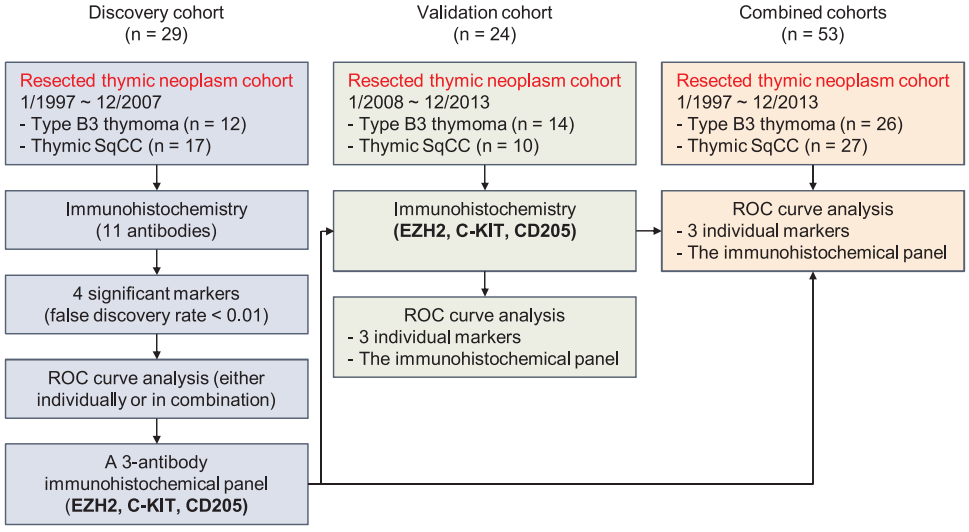

Fig. 1. Flow chart of study design. ROC, receiver-operator characteristic; SqCC, squamous cell carcinoma.