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Desmoglein 3 and p40 immunoreactivity in neoplastic and nonneoplastic thymus: a potential adjunct to help resolve selected diagnostic and staging problems

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ABSTRACT

The potential usefulness of the squamous markers p40 and desmoglein 3 (DSG-3) for the diagnosis and staging of selected thymic lesions is uncertain. We investigated their expression and distribution pattern in 66 thymomas, 12 thymic squamous carcinomas, 6 undifferentiated thymic carcinomas, 5 hyperplastic thymi, and 5 normal thymi. p40 nuclear and DSG-3 cytoplasmic/membranous immunoreactivity in greater than or equal to 10% of thymic epithelial cells was interpreted as positive, and DSG-3 distribution pattern was classified as organotypic and nonorganotypic. All nonneoplastic thymic tissues, 100% of thymic squamous carcinomas, 97% of thymomas, and 50% of undifferentiated thymic carcinomas were positive for p40. Expression of p40 in almost all thymomas and in 50% of undifferentiated carcinomas that lacked squamous features suggests that p40 is not a good marker for the diagnosis of thymic squamous carcinoma. All normal and hyperplastic thymi, 51.5% of thymomas, and 0% of thymic squamous carcinomas expressed DSG-3 in an organotypic pattern, and 13.6% of thymomas and 83% of thymic squamous carcinomas were DSG-3 positive in a nonorganotypic pattern. Findings suggest that nonorganotypic DSG-3 expression favors the diagnosis of squamous cell carcinoma over thymoma. In 26 (60.5%) of the 43 cases where neoplastic and nonneoplastic thymus were present on the same slide, the presence/absence or distribution pattern of DSG-3 immunoreactivity was different in the 2 components, suggesting that this marker can be helpful in staging thymomas with incomplete encapsulation. The presence of DSG-3–positive and DSG-3–negative thymomas raises the possibility that these tumors may originate from 2 different types of thymic epithelial cells.

1. Introduction

Thymomas and thymic carcinomas are unusual malignant neoplasms that are generally easy to distinguish from other mediastinal tumors based on histopathologic criteria and immunohistochemistry [1]. The World Health Organization (WHO) classifies thymomas into A, B1 to B3, AB, and other less frequent subtypes [2]. Lymphoid-poor thymomas A and B3 can generally be distinguished from normal or hyperplastic thymic tissues, whereas other thymomas have histopathologic features that can closely resemble the normal thymus making it difficult to distinguish them from nonneoplastic thymic tissue in small biopsies or resected but incompletely encapsulated tumors. Furthermore, it can be difficult to accurately diagnose the presence of transcapsular invasion in incompletely encapsulated thymomas or lesions where the capsule has been cut tangentially, as the neoplastic component can blend almost imperceptively into the adjacent nonneoplastic thymic tissue. According to the widely used Masaoka-Koga staging system, assessment of transcapsular invasion is important to distinguish stage I from stage I thymomas [3]. Squamous carcinoma, the most frequent histologic type of primary thymic carcinoma, can be difficult to distinguish from thymomas B3, which Suster and Moran [4] categorized as "atypical thymomas" because they are usually lymphoid-poor and composed of solid sheets of epithelioid cells with oval or elongated nuclei, amphophilic or slightly eosinophilic cytoplasm, and equivocal single-cell keratinization and/or intercellular bridges—morphologic features that resemble squamous carcinoma [2]. This diagnostic problem becomes more difficult in tumors with combined B3 and squamous carcinoma elements [1,2].

There is limited information regarding the expression of "squamous markers" in thymic epithelial tumors and nonneoplastic thymus and whether they could be useful in cases that are difficult to diagnose and/or stage. Dotto et al [5] described the expression of p63, an epithelial marker that is a member of the p53 family, in a variety of thymomas and thymic carcinomas but did not describe whether the pattern of immunoreactivity could help distinguish among these lesions. p40 (ΔN-63a), another member of the p53 family, is an isoform of p63 that is highly expressed in squamous carcinoma, basal cell carcinoma, and transitional cell carcinoma [6-11]. Chilosi et al [12] reported the

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expression of p40 in cortical and medullary thymic epithelial cells, with lesser intensity in Hassall corpuscles and in 54 thymomas encompassing various WHO subtypes. Their study did not evaluate expression of p40 in thymic carcinomas or whether the pattern of p40 expression in nonneoplastic thymus and B1 thymoma was helpful in diagnosis or staging. Desmoglein 3 (DSG-3), a calcium-binding glycoprotein, is a component of desmosomes in epidermal cells, and it is often expressed as membranous immunoreactivity in squamous carcinomas of the lung and other origins [8,13-18]. To our knowledge, DSG-3 expression has not been studied in the thymus or thymic neoplasms. In this study, we investigated DSG-3 and p40 immunoreactivity in normal thymus, hyperplastic thymus, a variety of thymomas, and thymic squamous carcinomas and assess the potential value of p40 and DSG-3 immunoreactivity to help resolve diagnostic and staging problems in selected cases.

2. Materials and methods

2.1. Cases studied

After institutional review board approval, 57 thymomas, 7 thymic squamous carcinomas, 5 hyperplastic thymus glands, and 5 normal thymus glands were identified in the surgical pathology database of Cedars–Sinai Medical Center (CSMC). Patients ranged from aged 2 to 82 years at diagnosis. The 5 normal thymus glands were excised from children aged 2 days to 3 years who underwent cardiac surgery. The thymomas were classified according to WHO criteria [2] into types A (n = 2), AB (n = 16), B1 (n = 15), B2 (n = 16), and B3 (n = 8). Diagnoses were confirmed by 2 of the authors (AW and AM). As the CSMC database included only a small number of B3 thymomas and thymic squamous carcinomas, additional B3 thymomas (n = 9), squamous carcinomas (n = 5), and undifferentiated thymic carcinomas (n = 6) were obtained from Japan by one of the coauthors (KH). Some of these cases had preceded the 1999 WHO classification of thymic neoplasms and had been diagnosed by several Japanese pathologists using variable terminology; they were reclassified according to WHO criteria [2] by KH.

Sections from 43 of the 84 neoplasms included a portion of adjacent nonneoplastic thymic tissue allowing us to compare the presence and patterns of immunoreactivity in tumor and adjacent nonneoplastic thymic tissue.

2.2. Immunohistochemical staining

All selected slides were immunostained for DSG-3 and p40 at CSMC. Briefly, immunohistochemical detection of DSG-3 (BC11) and of p40 was performed on 4-μm sections of formalin-fixed, paraffin-embedded tissue using mouse monoclonal predilute antibody and rabbit polyclonal predilute antibody, respectively, both from Biocare (Concord, CA). Staining was done on the Ventana Benchmark Ultra (Tucson, AZ) automated slide stainer. For DSG-3 staining, Ventana protease 1 enzyme (16 minutes at room temperature) was used for pretreatment. For p40 staining, the onboard heat-induced epitope retrieval method in high pH CC1 buffer (Ventana) was used. Staining was visualized using the Ventana Optiview DAB Detection System. The slides were subsequently counterstained with Mayer hematoxylin. Squamous carcinoma and tonsil served as positive and negative controls, respectively.

2.3. Immuohistochemical analysis

For each stain, immunoreactivity in 10% or more of the thymic epithelial cells present was recorded as positive. Immunoreactivity for each marker was recorded as positive or negative and nuclear or membranous + cytoplasmic and grouped by diagnosis. The distribution pattern of DSG-3-positivity was further classified into organotypic (when the positive cells were arranged in a growth pattern that resembled normal thymic tissue) (Fig. 1) or nonorganotypic (when the positive cells were arranged in other growth patterns) (Fig. 2). In slides that showed both tumor and adjacent nonneoplastic thymus, findings were recorded separately.

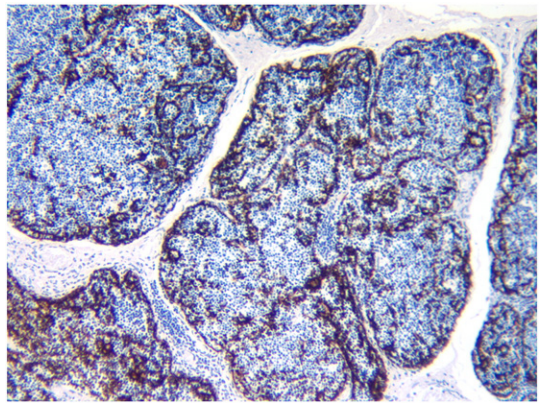

Fig. 1. Organotypic distribution pattern of immunoreactivity for DSG-3 in a normal thymus. Original magnification, ×100.

3. Results

3.1. Immunoreactivity for p40

All nonneoplastic thymic samples and 79 (94%) of the 84 neoplasms were p40 positive including 97% of the thymomas, 100% of the thymic squamous carcinomas, and 50% of the undifferentiated thymic carcinomas as shown in Table 1. In each case, the immunoreactivity was exclusively nuclear, confined to the thymic epithelial cells and observed in virtually all of the thymic epithelial cells (Fig. 3). p40-immunoreactivity was observed in the subcapsular, cortical, and medullary regions with focal staining observed in Hassall corpuscles. Of the 66 thymomas (comprising 12% of the B3 thymomas), only 2 (3%) were p40 negative (where present, nonneoplastic thymic tissue showed p40-positive thymic epithelial cell nuclei and served as a control). Interestingly, 50% of the 6 "undifferentiated carcinomas" from Japan were p40 positive (Fig. 4), although these lesions did not exhibit morphologic features of squamous carcinoma in hematoxylin and eosin–stained sections.

3.2. Immunoreactivity for DSG-3

Desmoglein 3 immunoreactivity was more variable as shown in Tables 1 and 2. All normal and hyperplastic thymic tissues were DSG-3 positive in an organotypic distribution pattern that highlighted the glandular architecture. Interestingly, the DSG-3 immunoreactive cells

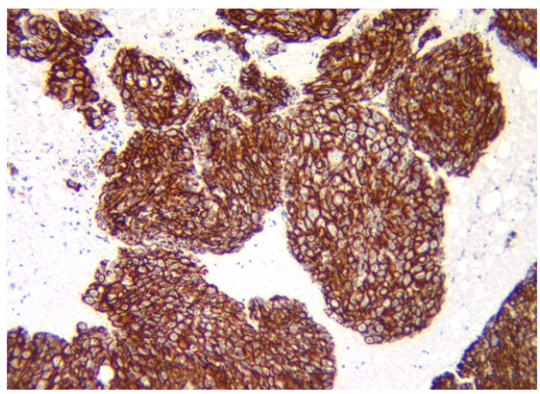

Fig. 2. Nonorganotypic distribution pattern of immunoreactivity for DSG-3 showing thick membranous staining in a thymic squamous carcinoma. Original magnification, ×100.