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Original contribution

Primary mediastinal seminomas: a comprehensive immunohistochemical study with a focus on novel markers☆

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Summary Primary mediastinal seminomas are unusual tumors that can present in a pure form or as part of a mixed germ cell tumor. Contrary to testicular seminomas, little is known about the expression of novel immunohistochemical markers in mediastinal seminomas. This study investigates the immunohistochemical features of these tumors with a focus on novel markers. Thirty-two cases of primary mediastinal seminomas were reviewed; and representative whole-tissue sections were selected for immunohistochemical studies using antibodies directed against high molecular weight cytokeratin 5/6 (CK5/6), low molecular weight cytokeratin (CAM5.2), octamer-binding transcription factor 3/4 (OCT3/4), spalt-like transcription factor 4 (SALL4), GATA binding protein 3 (GATA-3), sry-related HMG box 2 (SOX2), SOX17, human T cell leukemia/lymphoma 1 (TCL1), glypican 3, melanoma associated antigen C2 (MAGEC2), and paired box gene 8 (Pax8). The percentage of positive tumor cells as well as the intensity of staining was evaluated and scored. Thirty-one cases (97%) expressed SOX17, whereas 29 cases (91%) were positive for OCT3/4 and SALL4, respectively. Twenty-eight cases (88%) expressed MAGEC2 and CAM5.2, respectively. Two cases (6%) were positive for Pax8, and a single case (3%) was positive for TCL1. None of the cases stained with CK5/6, GATA-3, SOX2, or glypican 3. Similar to testicular seminomas, mediastinal seminomas show consistent expression of OCT3/4, SALL4, SOX17, and MAGEC2 and are negative for SOX2, glypican 3, GATA-3, and CK5/6. Pax8 positivity is only inconsistently identified in mediastinal seminomas. Contrary to their testicular counterparts, mediastinal tumors show diffuse expression of low-molecular-weight cytokeratin in up to 90% of cases and are commonly negative for TCL1. Although there is some immunohistochemical overlap between testicular and mediastinal seminomas, considerable differences also exist and should be acknowledged when dealing with these tumors.

1. Introduction

Extragonadal germ cell tumors account for less than 10% of all germ cell tumors and have been described in the central nervous system, mediastinum, and retroperitoneum [1]. They

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are believed to originate from ectopic primordial germ cells that are displaced during embryologic migration [2]. Approximately 95% of malignant tumors arising in the testis are germ cell tumors [1]. In the mediastinum, germ cell tumors account for approximately 10% to 20% of all mediastinal tumors, whereas seminomas constitute merely 3% to 4% of tumors at this site [3,4]. The treatment for germ cell tumors differs quite significantly from that of the more common mediastinal tumors, making correct diagnosis critical for patient management and prognosis.

In this context, the use of immunohistochemical techniques may be invaluable to facilitate the diagnostic process. Not surprisingly, most of what is known about the immunohistochemical phenotype of seminomas derives from studies on testicular tumors. Although the morphologic features of seminoma are similar irrespective of primary tumor site, differences have been described between the expression of immunohistochemical markers and genetic profiles of testicular and mediastinal seminomas [5-8]. Several more recent publications have investigated the expression pattern of various novel immunohistochemical markers—including stem cell markers—in testicular seminomas [9-16], whereas such investigations in mediastinal seminomas are still incomplete. The purpose of this study was to evaluate the diagnostic utility of a comprehensive panel of immunohistochemical antibodies—with a particular focus on novel markers—in mediastinal seminomas and compare them with the reported expression pattern in their testicular counterparts.

2. Materials and methods

Thirty-two cases of primary mediastinal seminomas were identified from the surgical pathology files at MD Anderson Cancer Center, Houston. Clinical and radiologic correlation had verified the absence of any tumor in the gonads or elsewhere confirming the mediastinum to be the primary site. Tissue was obtained from surgical resection specimens in all cases. Representative unstained sections obtained from paraffin blocks were available in all cases to perform immunohistochemical studies. The sections were incubated with 3% hydrogen peroxide in methanol and fetal bovine serum to block endogenous peroxidase activity and nonspecific protein-protein interactions, respectively. Immunostaining was performed using a horseradish peroxidase–labeled polymer system. Tissue sections were incubated with antibodies against high molecular weight cytokeratin 5/6 (CK5/6), low molecular weight cytokeratin (CAM5.2), octamer-binding transcription factor 3/4 (OCT3/4), spalt-like transcription factor 4 (SALL4), GATA binding protein 3 (GATA-3), sry-related HMG box 2 (SOX2), SOX17, human T cell leukemia/lymphoma 1 (TCL1), glypican 3, melanoma associated antigen C2 (MAGEC2), and paired box gene 8 (Pax8) (Table 1). 3,3′-Diaminobenzidine was used as a chromogen for antigen localization. Adequate positive and negative controls were run for all antibodies tested. The immunostaining was scored on a sliding scale of 0 to 4+ according to the percentage of reactive cells (0, negative; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, 76%-100%); and the staining intensity was graded as weak, intermediate, or strong. The study was approved by the institutional review board.

3. Results

The patients were all male with an age range from 19 to 56 years (mean, 32.7 years). All patients were diagnosed with primary mediastinal seminoma with no clinical or radiologic evidence of germ cell tumor elsewhere. All tumors were pure seminomas with no evidence of any additional germ cell tumor elements.

3.1. Morphologic characteristics

On low-power examination, the tumors were composed of sheets or discrete nests of tumor cells separated by fibrovascular septa and infiltrated by small mature lymphocytes (Fig. 1A). Individual cells had a round-to-polygonal shape, indistinct cell borders, and clear to eosinophilic

Table 1 Antibody information

Antibody	Company	Clone	Dilution	Antibody incubation time	Staining pattern
CK5/6	Dako, Carpinteria, CA	D5/16B4	1:50	30 min	Cytoplasmic
CAM5.2	BD Biosciences, San Jose, CA	CAM5.2	Ready to use	40 min	Cytoplasmic
OCT3/4	Leica Microsystems, Buffalo Grove, IL	N1NK	1:100	30 min	Nuclear
SALL4	Santa Cruz Biotechnology, Dallas, TX	EE-30	1:100	30 min	Nuclear
GATA-3	Cell Signaling Technology, Danvers, MA	D13C9	1:1000	60 min	Nuclear
SOX2	Cell Signaling Technology, Danvers, MA	D6D9	1:100	60 min	Nuclear
SOX17	Origene Technologies, Rockville, MA	2G8	1:1000	30 min	Nuclear
TCL1	MyBioSource, San Diego, CA	Rabbit polyclonal	1:50	60 min	Nuclear
Glypican 3	BioMosaics, Burlington, VT	1G12	1:200	60 min	Cytoplasmic
MAGEC2	Proteintech, Chicago, IL	Rabbit polyclonal	1:800	30 min	Nuclear
Pax8	Proteintech, Chicago, IL	Rabbit polyclonal	1:300	30 min	Nuclear