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CD56 expression in ovarian granulosa cell tumors, and its diagnostic utility and pitfalls

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Abstract

Objective. The purpose of this study is to investigate CD56 staining in ovarian granulosa cell tumor and its morphological mimics in order to determine the value of CD56 staining in a diagnostic setting.

Materials and methods. Tissue samples taken from 82 ovarian tumors, 26 extra-ovarian tumors and 11 normal ovaries were immunohistochemically stained using monoclonal anti-CD56 antibody. Ovarian tumors comprised 32 granulosa cell tumors, 3 Sertoli-stromal cell tumors, 14 fibrothecomas, 6 carcinoid tumors, 1 large cell neuroendocrine carcinoma, 17 endometrioid adenocarcinomas and 9 poorly differentiated serous adenocarcinomas. Extra-ovarian tumors comprised 22 uterine endometrial stromal sarcomas and 4 pulmonary small cell carcinomas. Normal ovaries contained 47 ovarian follicles.

Results. All of the 32 granulosa cell tumors, all of the 3 Sertoli-stromal cell tumors, all of the 4 small cell carcinomas, 1 of 1 large cell neuroendocrine carcinoma, 11 of 14 fibrothecomas, 5 of 6 carcinoid tumors, 17 of 22 endometrial stromal sarcomas and 7 of 9 poorly differentiated serous adenocarcinomas were positive for CD56. No immunoreactive cells were observed in 17 endometrioid adenocarcinomas or 47 ovarian follicles. All the immunoreactive cells showed membranous staining except for fibrothecomas where vague cytoplasmic staining was seen.

Conclusion. CD56, known as a neuroendocrine marker, is a sensitive marker of granulosa cell tumors, but since granulosa cell tumors and neuroendocrine tumors may be morphologically similar, CD56 positivity represents a significant diagnostic pitfall. CD56 is useful in distinguishing between granulosa cell tumor and normal ovarian follicles or endometrioid adenocarcinoma. Lack of membranous CD56 expression in fibrothecoma may help differentiate it from granulosa cell tumor. However, CD56 is of limited use for distinguishing between granulosa cell tumor and poorly differentiated carcinoma or endometrial stromal sarcoma. Appropriate and cautious interpretation of CD56 expression should lead to a more accurate diagnosis of granulosa cell tumor.

Keywords: CD56; Granulosa cell tumor; Neuroendocrine tumor; Ovarian carcinoma; Endometrial stromal sarcoma; Ovarian follicle; Immunohistochemistry; Diagnostic utility and pitfalls

Introduction

Granulosa cell tumor (GCT) is composed of two different clinicopathologic subtypes: adult and juvenile. Adult GCT accounts for approximately 1% to 2% of all primary ovarian tumors and 95% of all GCTs [1–3]. The tumor occurs most often in postmenopausal women, with a peak incidence at between 50 and 55 years of age. Adult GCT is considered to be a low-grade malignant tumor that may recur up to two to three decades after the diagnosis. Ninety percent of adult GCTs are stage I with a 10-year survival of 86% to 96% [1,2]. Microscopically, adult GCTs grow in a wide variety of patterns. The better-differentiated tumors typically show a microfollicular, macrofollicular, insular or trabecular pattern. The less-differentiated forms include

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watered-silk, gyriform or diffuse pattern. The nuclei are haphazardly oriented; oval, round, or angular; pale; often have characteristic nuclear grooves; and lack significant pleomorphism. Juvenile GCT usually occurs in women less than 30 years of age, and in half of them, it occurs during the first decade of life [4]. Juvenile GCT usually appears to be more malignant than the adult type. Microscopically, juvenile GCTs grow in diffuse sheets or nodules which are often punctured by various sized follicular spaces. The nuclei are characterized by hyperchromatism, considerable mitoses and lack of nuclear grooves. The tumor cells usually show luteinization.

Because of the diversity of the morphological patterns described above, GCT may be confused with various types of primary or metastatic tumors. Carcinoid tumor and large cell neuroendocrine carcinoma mimic the insular or trabecular pattern of GCT. Poorly differentiated carcinoma, small cell carcinoma of pulmonary or hypercalcemic type, fibrothecoma and endometrial stromal sarcoma (ESS) mimic the diffuse pattern of GCT. Non-neoplastic ovarian follicular cyst and small cell carcinoma of hypercalcemic type mimic the macrofollicular pattern of adult GCT or juvenile GCT. Endometrioid adenocarcinoma occasionally mimics the microfollicular or trabecular pattern of GCT or Sertoli-stromal cell tumor (ST). Although an accurate diagnosis is crucial in patient management, the morphological diversity of GCT sometimes makes it difficult to make an accurate diagnosis simply by routine HE staining.

Immunohistochemistry using anti-inhibin-alpha and anti-epithelial membrane antigen (EMA) antibodies is very useful for making an accurate diagnosis of GCT [5,6]. Inhibin-alpha positivity and EMA negativity of GCT usually help differentiate GCT from undifferentiated, poorly differentiated, small cell carcinoma and endometrioid adenocarcinoma. However, Young called pathologists' attention to the occasional inhibin-negativity of GCT [7]. McCluggage reported that EMA expression is occasionally observed in juvenile GCT, and concluded that the notion that ovarian sex cord-stromal tumors are always EMA-negative is not true [8]. Undifferentiated, poorly differentiated or small cell carcinoma occasionally lose or show a decrease in EMA immunoreactivity. Thus, an excessive trust in inhibin-alpha and EMA immunostaining may represent a significant diagnostic pitfall. Calretinin is also a sensitive marker for GCT, but it is less specific [9]. CD99 is also reported to be positive for GCT, but it is a less sensitive and specific marker [10]. Furthermore, these markers cannot distinguish between neoplastic and non-neoplastic granulosa cells. A new useful diagnostic marker may help improve the precision of a diagnosis of GCT.

CD56 glycoprotein family consists of three major members with molecular masses of 120, 140 and 180 kDa (CD56-120, -140, and -180), which are generated from a single gene by alternative splicing [11]. They belong to the immunoglobulin family of cell surface adhesion proteins involved in direct cell–cell adhesion [12,13]. They play an important role in organogenesis, and are expressed in neural, peripheral neuroectodermal, and neuroendocrine tissues and tumors [14–18]. CD56 is also well known as a sensitive diagnostic marker of neuroendocrine tumors such as carcinoid tumor and small cell carcinoma in the lung [14].

In the ovary, CD56–140 expression was detected in human cultured granulosa cells, and modification of the CD56 isoform pattern in the process of luteinization has also been noted [11]. CD56 is speculated to be involved in folliculogenesis and the formation of the corpus luteum in humans [11]. However, CD56 expression of GCT has not been immunohistochemically investigated previously.

We have experienced some consultation cases of ovarian GCT initially misdiagnosed as carcinoid tumor or small cell carcinoma based on the finding of CD56 positivity and inhibin-alpha negativity. Since CD56 expression in GCT has never been demonstrated in the literature, the finding of CD56 positivity is likely to create significant confusion.

Accordingly, we decided to investigate the immunohistochemical expression of CD56 in both ovarian GCT and its morphological mimics using paraffin-embedded tissue samples, and we clarified its diagnostic utility and pitfalls. Furthermore, more specific neuroendocrine markers, chromogranin A and synaptophysin were also investigated.

Materials and methods

Patient samples

One hundred and nineteen paraffin-embedded tissue samples taken from 82 ovarian tumors and 26 extra-ovarian tumors and 11 normal ovaries were studied. The ovarian tumors included 30 adult GCTs (2 sets of primary and recurrent tumors were included), 2 juvenile GCTs, 3 STs (1 well-differentiated and 2 intermediate differentiation), 14 fibrothecomas, 6 primary ovarian carcinoid tumors (3 insular and 3 strumal carcinoid), 1 large cell neuroendocrine carcinoma, 17 endometrioid adenocarcinomas (14 grade 1 and 3 grade 2) (11 typical and 6 sex cord-like variant) and 9 poorly differentiated high-grade serous adenocarcinomas. Twenty adult GCTs showed predominant typical morphology such as insular, trabecular, microfollicular or macrofollicular pattern (typical type), whereas 10 showed predominant diffuse pattern (diffuse type). Cases of luteinized adult GCT and cellular fibrothecoma were not included in this study. The extra-ovarian tumors included 22 uterine ESSs (20 typical and 2 sex cord-like variant) and 4 pulmonary small cell carcinomas. It should be noted that the ESSs in all the cases studied corresponded to "low-grade ESSs" in the old terminology. Tissue samples from 11 normal ovaries contained 47 normal ovarian follicles (5 preantral follicles, 13 mature follicles, 23 atretic follicles and 6 corpora lutea). Degenerated follicles were excluded from this study.

Immunohistochemical procedure

Monoclonal antibodies to CD56 (clone 1B6, Novocastra Laboratories, UK), chromogranin A (clone DAK-A3, Dako, CA) and polyclonal antibody to synaptophysin (Dako, CA) were utilized, and the working dilutions of each of the antibodies were 1:200, 1:100 and 1:100, respectively. Surgically resected specimens were fixed with 10% formalin and embedded in paraffin. Four-micrometer-thick sections on silane-coated slides were stained using the universal immuno-peroxidase polymer method with a ChemMate ENVISION kit (Dako, CA) and the automated immunohistochemical staining machine "Autostainer" (Dako, CA) according to the manufacturer's instructions. After deparaffinization, rehydration and inhibition of endogenous peroxidase, sections were exposed to the primary antibodies for 30 min at room temperature. After incubation of the ChemMate kit reagent for 30 min at room temperature, the sections were then finally incubated in 3,3′-diaminobenzidine, counterstained with hematoxylin, and mounted. High-temperature antigen retrieval was required for CD56 (95 °C, 40 min; pH 9 buffer solution, Dako, CA) and synaptophysin (95 °C, 20 min; pH 6, citrate buffer), but was unnecessary for