New Developments in Immunohistochemistry for Gynecologic Pathology

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THE UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER
Immunohistochemistry in Gynecologic Pathology

• Majority of diagnoses made through gross and microscopic morphology
• However, the morphologic features of different diseases are not always discreet
• IHC can provide additional data from which to make the diagnosis
Immunohistochemistry in Gynecologic Pathology
Problems and New Developments

- Cervical dysplasia vs. Metaplasia, Reactive Changes, Atrophy
- Germ Cell Tumors (YST) vs. Somatic Carcinoma (CCC, Endometrioid)
- Gynecologic Carcinoma vs. Breast Carcinoma
Immunohistochemistry in Gynecologic Pathology
Problems and New Developments

• Cervical Dysplasia vs. Metaplasia, Reactive Changes, Atrophy
Immunohistochemistry in Gynecologic Pathology
Problems and New Developments

Cervical Dysplasia

Ki-67
p16
ProExC
Cervical Dysplasia

Ki-67

- Ki-67 (MIB 1) is a marker of proliferation
- Non-dysplastic mucosa - scattered nuclear staining of basal and parabasal cells
- Dysplastic mucosa – increase in the number of cells and the level of the epithelium involved
Cervical Dysplasia

p16

- p16 (cyclin dependent kinase inhibitor 4) is a tumor suppressor protein.
- It binds cyclin – CDK 4/6 complexes and inhibits progression to the S-phase.
- In the lower Gyn tract, overexpression of p16 has been found to be a surrogate marker for high risk HPV.
Cervical Dysplasia

p16

• In the cervix, diffuse strong staining for p16 (overexpression) correlates well with high risk HPV associated neoplasia.

  - High grade squamous dysplasia (90%; low grade may also stain)
  - Invasive squamous carcinoma (95%)
  - Adenocarcinoma in-situ (>90%)
  - Invasive endocervical adenocarcinoma (>90%)
Cervical Dysplasia
ProExC

• Topoisomerase IIα
  – Topoisomerases are enzymes involved in DNA replication, transcription, recombination and chromatin remodeling
  – Increased expression found in cancers of the cervix, lung, and colon

• Minichromosome maintenance protein 2
  – MCM proteins have roles in regulation of DNA replication (marker of proliferating cells)
  – Upregulated in dysplasia and carcinoma

• Found useful in LBC, limited experience in tissue
Biomarker (ProEx™ C, p16\textsuperscript{INK4A}, and MiB-1) distinction of high-grade squamous intraepithelial lesion from its mimics

Alvaro P Pinto\textsuperscript{1}, Nicolas F Schlecht\textsuperscript{1}, Terri YC Woo\textsuperscript{1}, Christopher P Crum\textsuperscript{1} and Edmund S Gibas\textsuperscript{8}

\textsuperscript{1}Department of Pathology, Federal University of Parana, Curitiba, Parana, Brazil; \textsuperscript{2}Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, USA; \textsuperscript{3}Division of Women’s and Perinatal Pathology, Department of Pathology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA and \textsuperscript{4}Division of Cytology, Department of Pathology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA

Topoisomerase IIs and minichromosome maintenance protein 2 are proteins associated with aberrant S-phase induction. The current study evaluated the performance of these biomarkers (ProEx™ C; TriPath Oncology, Burlington, NC) compared with p16\textsuperscript{INK4A} and MiB-1 in distinguishing high-grade squamous intraepithelial lesions (HSILs) from HSIL mimics. We collected archival cervical biopsy, cone, and curettage specimens from 96 cases in which the differential diagnosis of HSIL vs reactive epithelial changes was considered. Hematoxylin-
and eosin-stained slides were reviewed independently by three pathologists and scored for the presence or absence of SIL. Immunostains for ProEx C, p16, and MiB-1 were available for 95, 96, and 98 samples, respectively, and classified blinded to histological interpretation. Strong nuclear and cytoplasmic staining for p16 and staining for MiB-1 and ProEx C that extended beyond the lower one-third of the epithelium were scored as positive. \(z\)-test and receiver operating characteristic analysis were conducted to statistically compare biomarker immunostaining performance against majority histological interpretation of SIL. Agreement between pathologists was also assessed by the \(k\)-statistic. Inter-observer agreement ranged from fair to moderate (\(k = 0.37-0.57\)). All three biomarkers correlated strongly with the majority diagnosis of SIL (\(P < 0.001\)). Positive staining for ProEx C, p16, and MiB-1 was observed in 87\% (\(N = 82/95\)), 84\% (\(N = 81/96\)), and 94\% (\(N = 92/96\)) respectively, of all SIL, and negative in 71\% (\(N = 28/95\)), 63\% (\(N = 61/96\)), and 52\% (\(N = 50/96\)) respectively, of all non-SIL. The combination of p16/ProEx C predicted more SIL (92\%, \(N = 33/36\)) and non-SIL (91\%, \(N = 34/36\)) than p16 plus MiB-1 (91\%, \(N = 35/38\) and 43\%, \(N = 10/23\)), although this difference was not statistically significant. ProEx C appears to provide an equivalent level of sensitivity and a higher level of specificity for HSIL alone or in conjunction with p16. Its principal value may be in providing a lower false positive rate for non-SIL relative to MiB-1.

Keywords: cervical biopsy; cervical intraepithelial neoplasia; high-grade squamous intraepithelial lesion; MiB-1; p16\textsuperscript{INK4A}; ProEx C

It is well established that most cervical cancers develop from precancerous lesions.\textsuperscript{1} High-grade squamous intraepithelial lesions (HSILs) are at greatest risk for a malignant outcome due to their stronger association with cancer-associated human papillomaviruses (HPVs), underscoring the importance of accurate histological classification.\textsuperscript{2}\textsuperscript{3}

Histopathology is the method of choice for confirming the diagnosis of a squamous intraepithelial lesion (SIL) that is usually first detected by cytological screening. Diagnosis variability has been documented among observers and depends, in part, on the grade of the abnormality.\textsuperscript{4} Reactive/suppressible epithelial changes, immature squamous metaplasia, and atrophy are well-recognized mimics of HSIL and frequently cause problems in histological interpretation.\textsuperscript{5}
Cervical Dysplasia
ProExC

Pinto AP, Schlecht NF, Woo TYC, Crum CP, Cibas ES

- 96 cases HSIL vs. Reactive epithelial changes
- H&Es reviewed by 3 pathologists
- ProExC, p16, MiB1
- Staining above the lower 1/3
## Cervical Dysplasia

**ProExC**

Pinto AP, Schlecht NF, Woo TYC, Crum CP, Cibas ES

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<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>p16</td>
<td>84%</td>
<td>63%</td>
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<tr>
<td>MiB1</td>
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<tr>
<td>ProExC</td>
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<td>p16/MiB1</td>
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<td>92%</td>
<td>61%</td>
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Original contribution

Evaluation of p16INK4a, minichromosome maintenance protein 2, DNA topoisomerase IIα, ProE8 C, and p16INK4a/ProE8 C in cervical squamous intraepithelial lesions

Jiahui Shi MD, PhD, Haiyan Liu MD, Myra Wilkerson MD, Yajue Huang MD, PhD, Steven Meschter MD, William Dupree MD, Conrad Schuerch MD, Fan Lin MD, PhD

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†Department of Pathology, Lehigh Valley Hospital, Allentown, PA 18103, USA
‡Department of Pathology, Temple University Hospital, Philadelphia, PA 19140, USA

Received 27 November 2006; revised 23 January 2007; accepted 24 January 2007

Keywords:
- Cervical biopsy
- p16INK4a
- ProE8 C
- p16/ProE8 C
- Squamous intraepithelial lesions

Summary: p16INK4a has been shown to be overexpressed in nearly all high-grade squamous intraepithelial lesions (HSILs). Other cell-cycle regulators, such as minichromosome maintenance protein 2 (MCM2), DNA topoisomerase IIα (TOP IIα), and ProE8 C (a cocktail of MCM2 and TOP IIα), have also demonstrated some value in identifying squamous intraepithelial lesions. Data on direct comparison of these cell cycle regulatory proteins in the detection of squamous intraepithelial lesions, with a focus on low-grade squamous intraepithelial lesions (LSILs), are limited. We immunohistochemically evaluated the diagnostic value of p16, MCM2, TOP IIα, ProE8 C, and a cocktail of p16 and ProE8 C in 65 cervical biopsy specimens, including 14 cases of benign squamous mucosa (group 1), 24 cases of LSILs (group 2), and 14 cases of HSILs (group 3). The staining intensity and distribution were recorded. The results demonstrated that positive staining for p16 and the p16/ProE8 C was observed in 100% of cases in group 1, whereas 79%, 86%, and 78% of cases were positive for CM2, TOP IIα, and ProE8 C, respectively. ProE8 C and the p16/ProE8 C showed positive staining in 94% and 100% of cases in group 2, respectively. In contrast, immunoreactivity for p16, MCM2, and TOP IIα was detected in only 76% of cases in group 2. Importantly, all 8 p16-negative cases in group 2 were positive for p16/ProE8 C (P = 0.003). Our data indicate that (1) p16 is a more sensitive and specific marker for identifying HSILs; (2) ProE8 C is a better marker for the detection of HSILs; and (3) p16/ProE8 C provides the highest diagnostic value for the detection of both HSILs and LSILs.

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1. Introduction

After the remarkable introduction of Papenicolau cervical smear screening, the incidence of cervical cancer dramatically decreased in the United States and worldwide. Because of this, deaths caused by carcinoma of the cervix, once the leading cause of cancer deaths in women in the United States, have significantly declined to the present rank as the eighth leading cause of cancer mortality [1,2]. It is generally believed that most cervical cancers develop from precursor lesions, that is, from high-grade squamous
Cervical Dysplasia
ProExC


• 62 cervical biopsies
  – 14 benign
  – 34 LSIL
  – 14 HSIL
• ProExC, p16, Ki-67
• Staining above basal and parabasal cells
Cervical Dysplasia
ProExC


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<tr>
<td>p16/ProExC</td>
<td>-</td>
<td>100%</td>
<td>100%</td>
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BD ProEx C: A Sensitive and Specific Marker of HPV-associated Squamous Lesions of the Cervix

Riem E. Badr, MD, Ann E. Wolts, MD, Pui Chung, BS, HTL, and Shikha Bose, MD

Abstract: BD ProEx C (ProEx C) is a recently developed immunocytochemical assay that targets the expression of topoisoenzymes II-alpha and minichromosome maintenance protein-2, 2 genes shown to be overexpressed in cervical cancers. Recent studies validated this reagent in liquid-based cytology specimens and suggested its usefulness as an adjunct in the diagnosis of challenging cases. Limited information is available on its expression in tissue sections. This study aims to assess ProEx C expression patterns in benign, atypical, and dysplastic lesions of the cervix and to compare these patterns with p16 and Ki67 expression and with the presence of human papilloma virus DNA as determined by in situ hybridization. ProEx C positivity was limited to the basal layers of the epithelium in 75% of benign cervixes. In the remaining 25%, staining extended into the lower half of the epithelium particularly in areas of squamous metaplasia and immature squamous metaplasia. In 92% of high-grade dysplasia [cervical intraepithelial neoplasia (CIN) II and III] strong positive staining for ProEx C involved the lower and upper halves of the epithelium. Condylomas and CIN I showed greater variability in staining pattern with ProEx C positivity extending into the upper half of the epithelium in 48% of cases. Statistically significant correlation were noted with presence of human papilloma virus DNA and with p16/Ki67 expression. Atypical squamous metaplasia showed varied staining with 34% being positive. To summarize and reduce ProEx C is a reliable marker for high-grade CIN that can be applied to tissue sections to confirm the diagnosis of high-grade CIN and to triage cases of atypical squamous metaplasia. ProEx C may also have a potential role in triaging patients with low-grade CIN. ProEx C expression patterns are similar to those for p16 and Ki67 with most discrepancies occurring in the CIN I category.

Key Words: cervical intraepithelial neoplasia, human papilloma virus, ProEx C, p16, Ki67

From the Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

Support: BD ProEx C methodology was provided free of cost for this study by BD Diagnostics—TriPath, Burlington, NC.

None of the authors received financial assistance from or has any financial interest in any of the manufacturers cited.


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Am J Surg Pathol • Volume 32, Number 6, June 2008

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Cervical Dysplasia
ProExC

Badr RE, Walts AE, Chung F, Bose S

• 119 cases, reviewed by 2 pathologists
  – 38 negative (including immature squamous metaplasia)
  – 24 LSIL
  – 37 HSIL
  – 21 atypical squamous metaplasia
• ProExC, p16, Ki-67
• Staining in the upper ½ positive
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<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>p16/ProExC</td>
<td>89%</td>
<td>93%</td>
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Cervical Dysplasia
ProExC

Guo M, Baruch AC, Silva EG, Jan YJ, Sniege N, Deavers MT

• 136 cervical biopsies
  – 20 benign
  – 27 LSIL
  – 60 HSIL
  – 29 Carcinoma

• ProExC, p16

• Staining in upper ½ positive
# Cervical Dysplasia

**ProExC**

Guo M, Baruch AC, Silva EG, Jan YJ, Sniege N, Deavers MT

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<td>p16/ProExC CIN2</td>
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<td>p16/ProExC CIN3</td>
<td>75%</td>
<td>93%</td>
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Problem and New Development
Cervical Dysplasia
ProExC

• Useful as a marker for dysplasia in biopsies
• Interpretation
  – Establishing threshold for positive
• $p16 + \text{ProExC}$ may have increased specificity
Problem
Germ Cell Tumors (YST) vs. Somatic Carcinomas (CCC, Endometrioid)

- Glandular variant of YST can mimic Endometrioid Carcinoma
- Papillary, solid, hepatoid pattern can mimic Clear Cell Carcinoma
- Mixed somatic carcinoma + Yolk Sac Tumor
Problem
YST vs. Somatic Carcinoma

• AFP 80% (1+) YST, 0 - 35% Ca (CCC)

• EMA 0% (rare cells) YST, ~100% Ca (CCC, Endometrioid)

• CK7 0% (rare cells) YST, ~100% Ca (CCC, Endometrioid)
SALL4

- Zinc finger transcription factor
- Important in embryonic development, regulates OCT4 transcription and embryonic stem cells
SALL4

- Seminoma / Dysgerminoma 100%
- Embryonal Carcinoma 100%
- Yolk Sac Tumor 100%
- Choriocarcinoma 67 – 100%
SALL4

- Esophageal Adenocarcinoma 2% (focal)
- Gastric Adenocarcinoma 15 – 33% (focal)
- Colonic Adenocarcinoma 8% (focal)
- Ovarian Carcinoma 0 – 3% (focal)
- Uterine Adenocarcinoma 0%
SALL4 Is a Novel Sensitive and Specific Marker of Ovarian Primitive Germ Cell Tumors and Is Particularly Useful in Distinguishing Yolk Sac Tumor From Clear Cell Carcinoma

Dengfeng Cao, MD, PhD* Shuangping Guo, MD, † Robert W. Allen, MD, ‡ Kyle H. Molberg, MD, § and Yan Peng, MD, PhD$ 

Abstract: Ovarian primitive germ cell tumors (GCTs) are uncommon tumors and sometimes pose diagnostic challenges. Among them, yolk sac tumor (YST) poses the greatest diagnostic difficulty and can be mistaken for clear cell carcinoma (CCC). Current immunohistochemical markers such as alpha-fetoprotein (AFP), glypican-3, cytokeratin (CK) 7, and epithelial membrane antigen (EMA) used to distinguish YST from CCC lack adequate sensitivity and specificity. Here by immunohistochemistry, we investigated a novel marker SALL4 in 98 GCTs (29 YSTs, 18 dysgerminomas, 6 gonadoblastomas, 6 embryonal carcinomas, 15 immature and 12 mature teratomas, 7 carcinoid tumors, 3 serous carcinoids, and 2 serous ovarian) with particular interest of exploring SALL4 to distinguish YST from CCC. Our hundred sixty-three non-GCTs including 45 CCCs were also stained. We found that SALL4 is strongly positive in more than 90% tumor cells in all YSTs, dysgerminomas, gonadoblastomas, and embryonal carcinomas. Variable SALL4 staining is seen in 11 of 15 immature teratomas. All other GCTs included in this study are negative for SALL4. Except 3 CCCs with focal SALL4 staining (<15% tumor cells), SALL4 is negative in the remaining 160 non-GCTs. We also compared SALL4 with AFP, glypican-3, CK7, and EMA in all YSTs and CCCs. AFP and glypican-3 are positive in 24 (83%) and 20 (69%) YSTs, respectively, whereas 16 (32%) and 10 (20%) CCCs show positive AFP and glypican-3 staining, respectively. Three (10%) and 4 (14%) YSTs show focal (<2% tumor cells) CK7 and EMA staining, respectively. CK7 and EMA are positive in all 45 CCCs but 3 (7%) and 1 (2%) cases show staining in less than 30% tumor cells, respectively. Our findings indicate that SALL4 is a novel sensitive and specific marker for ovarian primitive GCTs. SALL4 is particularly useful in distinguishing YST from CCC and better than AFP, glypican-3, CK7, and EMA.

Key Words: SALL4, ovary, germ cell tumor, yolk sac tumor, clear cell carcinoma

Am J Surg Pathol 2009;33:894-904

In the most recent World Health Organization classification, ovarian germ cell tumors (GCTs) are divided into three major categories: primitive GCTs, immature and mature teratomas; and monodermal teratomas and somatic-type tumors associated with dermoid cysts. The primitive ovarian GCTs are uncommon but malignant tumors, accounting for approximately 3% of ovarian cancers in the Western countries. The primitive ovarian GCTs include dysgerminoma, dysgammaglobulina, yolk sac tumor (YST), embryonal carcinoma (EC), polyembryoma, nongerminomatous choriocarcinoma, and mixed GCTs. Given the chemosensitivity demonstrated by malignant primitive ovarian GCTs and the increasing adoption of fertility-sparing surgery for malignant GCTs, correct pathologic diagnosis of ovarian primitive GCTs is critical.

Ovarian primitive GCTs can sometimes pose diagnostic challenges. EC can mimic high-grade serous carcinoma and dysgerminoma can mimic non-GCTs with clear cytoplasm such as serous cell carcinoma (CCC) or lipid-rich steroid cell tumor. However, YST is still the one primitive GCT that poses the greatest diagnostic difficulty for pathologists. One common error in ovarian tumor pathology is misinterpretation of YST as CCC or vice versa. Although most YSTs occur in children and young adults, rarely they can occur in postmenopausal women and thus age alone is not a reliable discriminating factor. Most patients with YST have elevated serum alpha-fetoprotein (AFP), however, AFP information sometimes is not available to the pathologist at the time of pathologic diagnosis or even not measured. Morphologically, YST is notorious for displaying multiple histologic patterns. Solid, tubular, cystic, or papillary pattern in YST can closely mimic CCC especially in limited biopsy material. The endometrioid-like glandular pattern of YST can mimic endometrioid...
SALL4

Cao D, Guo S, Allan RW, Molberg KH, Peng Y

• 29 Yolk Sac Tumor

• 45 Clear Cell Carcinoma
SALL4

Cao D, Guo S, Allan RW, Molberg KH, Peng Y

- YST 100% (4+)
- CCC 7% (1+)
Glypican 3

- Membrane protein involved in cell growth and differentiation
- Oncofetal protein
Glypican 3

- Hepatoblastoma: 100%
- Hepatocellular carcinoma: ~80%
- Hepatic dysplastic nodules: ~50%
- Hepatic adenoma: 0%
- Macrolegetenerative nodules: 0%
- Wilms’ tumor: 100%
- Melanoma: 0% - >80%
- Squamous carcinoma, lung: ~50%
- Liposarcoma: ~50%
Glypican 3

- YST: 69 – 100%
- Choriocarcinoma: 100%
- Serous carcinoma: <1 – 11%
- Endometrioid adenocarcinoma: 5 – 6%
- Mucinous carcinoma: 0 – 4%
- Clear cell carcinoma: 17 – 64%
Oncofetal Protein Glypican-3 Distinguishes Yolk Sac Tumor From Clear Cell Carcinoma of the Ovary

Ghada E. Elsheba, MSc, Lisa L. Pate, MD, and Teri A. Longacre, MD

Abstract: Clear cell carcinoma (CCC) of the ovary is the surface epithelial neoplasm most often confused with primitive germ cell tumors, particularly yolk sac tumor (YST) and dysgerminoma. OCT3/4 has proven to be a sensitive and relatively specific marker for the latter entity, but existing markers for YST are limited. Recent studies suggest that glypican-3 (GPC3), an oncopetal protein expressed in fetal liver and malignant tumors of hepatocytic lineage, is also expressed in germ cell tumors, particularly YST. To investigate whether GPC3 is useful in distinguishing YST from ovarian CCC, we studied the expression of GPC3 in a large series of ovarian neoplasms and compared it to the expression profiles of CK7 and alpha-fetoprotein. Tissue microarrays containing over 400 benign and malignant ovarian neoplasms, including 34 CCCs were stained with monoclonal GPC3 (clone 1G11, Biomark, Burlington, VT). These arrays contained a wide assortment of ovarian surface epithelial neoplasms and sex cord stromal neoplasms, as well as germ cell tumors. Full paraffin tissue sections from 32 YSTs and 10 CCCs were also assessed. All but one YST (97%), including those associated with mixed germ cell tumor were positive for GPC3, whereas all teratomas and embryonal carcinomas were negative. Both cytologic and membrane staining were present in the positive cases, with no background staining. The syncytiotrophoblastic cells in the germ cell tumors and placental villi included in the arrays were also positive for GPC3. Most CCCs (85%) were completely negative for GPC3, as were 95% serous, 94% endometrioid, and 100% mucinous tumors. Five CCCs exhibited focal, moderate to strong GPC3 expression and in 2 the expression was focal and weak. All other tumors, including dermoid ovary were negative for GPC3. GPC3 seems to be a promising diagnostic marker for differentiating YST from ovarian CCC (P = 0.0001). Because GPC3 is associated with alpha-fetoprotein expression, further studies are required to determine the utility of GPC3 in differentiating YST from CCC with hepatoid differentiation.

Key Words: yolk sac tumor, endometrial stroma tumor, ovary, clear cell carcinoma, glypican-3, CK7, AFP, immunohistochemistry

From the Department of Pathology, Stanford University, Stanford, CA. Presented in part at the 96th meeting of the United States and Canadian Academy of Pathology, San Diego, CA, March 2007. Reprints Teri A. Longacre, MD, Department of Pathology, School of Medicine, Stanford University, Room 2255, 300 Pasteur Drive, Stanford, CA 94305 (e-mail: longacre@stanford.edu).

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Materials and Methods
All the specimens used in this study were retrieved from archives of the Division of Surgical Pathology, Department of Pathology, Stanford University, Stanford, CA.

Am J Surg Pathol • Volume 32, Number 4, April 2008
Glypican 3

Esheba GE, Pate LL, Longacre TA

- 33 Yolk Sac Tumor
- 42 Clear Cell Carcinoma
- 32 Endometrioid Adenocarcinoma
- 195 Serous Carcinoma
- 10 Mucinous Carcinoma
- Glypican, AFP, CK7
Glypican 3

Esheba GE, Pate LL, Longacre TA

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<td>97%</td>
<td>91%</td>
<td>3% (&lt;20% cells)</td>
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<tr>
<td>CCC</td>
<td>17%</td>
<td>29%</td>
<td>100%</td>
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SALL4 Is a Novel Sensitive and Specific Marker of Ovarian Primitive Germ Cell Tumors and Is Particularly Useful in Distinguishing Yolk Sac Tumor From Clear Cell Carcinoma

Dengfeng Cao, MD, PhD, Shuangping Guo, MD, Robert W. Allen, MD, Kyle H. Molberg, MD, and Yan Peng, MD, PhD

Abstract: Ovarian primitive germ cell tumors (GCTs) are uncommon tumors and sometimes pose diagnostic challenges. Among them, yolk sac tumor (YST) poses the greatest diagnostic difficulty and can be mistaken for clear cell carcinoma (CCC). Current immunohistochemical markers such as alpha-fetoprotein (AFP), glypican-3, cytokeratin (CK) 7, and epithelial membrane antigen (EMA) used to distinguish YST from CCC lack adequate sensitivity and specificity. Hereby, we investigated a novel marker SALL4 in 98 GCTs (29 YSTs, 28 dysgerminoma, 16 gonadoblastoma, 6 embryonal carcinomas, 15 immature and 12 mature teratomas, 7 carcinoid tumors, 3 serous carcinoids, and 2 serous ovaries) with particular interest of exploring SALL4 to distinguish YST from CCC. One hundred sixty-three non-GCTs including 45 CCCs were also stained. We found that SALL4 is strongly positive in more than 90% tumor cells in all YSTs, dysgerminomas, gonadoblastomas, and embryonal carcinomas. Variable SALL4 staining is seen in 11 of 15 immature teratomas. All other GCTs included in this study are negative for SALL4. Except 3 CCCs with focal SALL4 staining (< 15% tumor cells), SALL4 is negative in the remaining 160 non-GCTs. We also compared SALL4 with AFP, glypican-3, CK7, and EMA in all YSTs and CCCs. AFP and glypican-3 are positive in 24 (83%) and 20 (69%) YSTs, respectively, whereas 16 (32%) and 13 (25%) CCCs show positive AFP and glypican-3 staining, respectively. Three (10%) and 4 (14%) YSTs show focal (< 2% tumor cells) CK7 and EMA staining, respectively. CK7 and EMA are positive in all 45 CCCs but 3 (7%) and 1 (2%) cases show staining in less than 30% tumor cells, respectively. Our findings indicate that SALL4 is a novel sensitive and specific marker for ovarian primitive GCTs. SALL4 is particularly useful in distinguishing YST from CCC and better than AFP, glypican-3, CK7, and EMA.

Key Words: SALL4, ovary, germ cell tumor, yolk sac tumor, clear cell carcinoma

In the most recent World Health Organization classification, ovarian germ cell tumors (GCTs) are divided into 3 major categories: primitive GCTs; immature and mature teratomas; and monodermal teratomas and somatic-type tumors associated with dermoid cysts. The primitive GCTs include dysgerminoma, embryonal carcinoma (EC), yolk sac tumor (YST), embryonal carcinoma (EC), polycystic, and gestational choriocarcinoma, and mixed GCTs. Given the chemosensitivity demonstrated by malignant primitive ovarian GCTs and the increasing adoption of fertility-sparing surgery for malignant GCTs, correct pathologic diagnosis of ovarian primitive GCTs and distinguishing them from non-GCTs is critical.

Ovarian primitive GCTs can sometimes pose diagnostic challenges. EC can mimic high-grade serous carcinoma and dysgerminoma can mimic non-GCTs with clear cytoplasm such as clear cell carcinoma (CCC) or lipid-rich steroid cell tumor. However, YST is still the one primitive GCT that poses the greatest diagnostic difficulty for pathologists. One common error in ovarian tumor pathology is misinterpretation of YST as CCC or vice versa. Although most YSTs occur in children and young adults, rarely they can occur in postmenopausal women and thus age alone is not a reliable discriminating factor. Most patients with YST have elevated serum alpha-fetoprotein (AFP), however, AFP information sometimes is not available to the pathologist at the time of pathologic diagnosis or even not measured. Morphologically, YST is notorious for displaying multiple histologic patterns. The endodermal-like glandular pattern of YST can mimic endometrioid...
## Glypican 3

Cao D, Guo S, Allan RW, Molberg KH, Peng Y

<table>
<thead>
<tr>
<th></th>
<th>Glypican</th>
<th>AFP</th>
<th>CK7</th>
<th>EMA</th>
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<tbody>
<tr>
<td>YST</td>
<td>69%</td>
<td>83%</td>
<td>10% (&lt;2% cells)</td>
<td>14% (&lt;2% cells)</td>
</tr>
<tr>
<td>CCC</td>
<td>28%</td>
<td>35%</td>
<td>100%</td>
<td>100%</td>
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</table>
CDX2

- Homeobox gene involved in hindgut development
- Marker of intestinal differentiation
CDX2

• Ovary
  – Mucinous tumor
  – Endometrioid Adenocarcinoma
  – Teratoma
• 13 Yolk Sac Tumors
  – 6 pure
  – 3 mixed
  – 3 Yolk Sac Tumor + Somatic Carcinoma
  – 1 lymph node metastasis
CDX2

Ngae MY, Deavers MT

- 100% positive
- 11 - 1+, 1 - 2+, 1 - 3+
- Reticular, glandular, papillary, solid and polyvesicular-vitelline patterns stained
- Teratoma (intestinal glands) positive
Ovarian Germ Cell Tumors

- SALL4: Dysgerminoma, Yolk Sac Tumor, Choriocarcinoma, Embryonal Carcinoma
- OCT 4: Dysgerminoma, Embryonal Carcinoma
- Glypican 3: Yolk Sac Tumor, Choriocarcinoma
- CDX2: Yolk Sac Tumor
Problem and New Developments: YST vs. Clear Cell Carcinoma, Endometrioid Adenocarcinoma

- SALL4, Glypican 3, CDX2, AFP
- CK7, EMA
Problem
Gynecologic Carcinoma vs. Breast Carcinoma

- The gyn tract is a common site of metastases for breast carcinoma
- Patients with breast carcinoma are at increased risk for developing a second primary (BRCA)
- Gyn carcinomas can metastasize to the breast, axilla, and supraclavicular lymph nodes
- Overlapping histologic and immunohistochemical features
- Carcinoma of unknown primary
Problem

Gynecologic Carcinoma vs Breast Carcinoma

- Cytokeratin 7: + in both
- ER/PR: + in both
- GCDFP: +40-65% Breast
  + ~2% Ovarian
- Mammaglobin: +60-80% Breast
  +15-60% Endometrioid
  +0-3% Serous
Problem

Gynecologic Carcinoma vs Breast Carcinoma

- CA125: +15% Breast
  +75% Ovarian

- WT1: +3% Breast
  +80-90% Serous
  +~20% Endometrioid
PAX8
(Paired box gene 8)

• Transcription factor for organogenesis of the thyroid, kidney, and Mullerian system

• Regulates WT1 expression
PAX8

**Thyroid**
- Papillary Carcinoma 100%
- Follicular Carcinoma 100%
- Medullary Carcinoma 75%
- Anaplastic Carcinoma 79%
  (TTF-1 20%)

**Lung**
Adenocarcinoma, Squamous, Large Cell 0-1%
PAX8

Kidney

- Clear Cell 98%
- Papillary 90%
- Chromophobe 82%
- Medullary 100%
- Oncocytoma 95%
- Sarcomatoid 71%
- Nephrogenic adenoma 100%
PAX8

Gyn Tract

- Ovarian Ca ~80%
  - Serous 96-100%
  - Endometrioid 67-89%
  - Clear Cell 100%
  - Mucinous 8%

- Endometrial Ca 80% (?)
  - Clear Cell Ca 100%
Expression of Pax8 as a Useful Marker in Distinguishing Ovarian Carcinomas From Mammary Carcinomas

Daisuke Nonaka, MD, Luis Chiriboga, PhD, and Robert A. Soslow, MD

Abstract: The ovary is a common site of involvement for metastasis and the breast is one of the most common sources. Metastatic breast carcinomas can mimic primary ovarian carcinomas. Pax8 is a crucial transcription factor for organogenesis of the thyroid gland, kidney, and Müllerian system, and it also regulates Wt1 tumor suppressor gene (WT1) expression. A total of 124 cases of ovarian carcinomas (84 serous papillary, 18 endometrioid, 12 mucinous, 10 clear cell) and 245 cases of invasive breast carcinomas (178 ductal, 65 lobular) were immunostained with Pax8 and Wt1 by tissue microarrays to see the differential expression. Pax8 reaction was found in 104 of 124 ovarian carcinomas (83.9%) generally in diffuse staining, including 81 of 14 serous papillary carcinomas (96.4%), 16 of 18 endometrioid carcinomas (88.9%), 19 of 10 clear cell carcinomas (100%), and 1 of 12 mucinous carcinomas (0.8%), whereas WT1 expression was seen in 78 of 124 ovarian carcinomas (62.9%), including 77 of 14 serous papillary carcinomas (86.9%), and 6 of 18 endometrioid carcinomas (27.3%), with no expression in all 10 clear cell carcinomas and 12 mucinous carcinomas. All the mammary carcinomas were completely negative for Pax8, but WT1 expression was seen in 25 of 281 cases (22.7%). Pax8 is a useful marker in the differential diagnosis of ovarian and breast carcinomas, and it seems to be superior to WT1 for the diagnosis of all benign and malignant ovarian neoplasms. Notably, Pax8 and WT1 expression is generally negative or very focal.

Key Words: Pax8, WT1, ovarian carcinoma, breast carcinoma, ovary, mammary gland

Materials and Methods
The following cases of ovarian surface epithelial carcinomas and invasive breast carcinomas were studied: 124 cases of ovarian surface epithelial carcinomas including 84 serous papillary carcinomas, 18 endometrioid carcinomas, 10 clear cell carcinomas, and 12 mucinous carcinomas; 245 cases of invasive breast carcinomas including 173 ductal carcinomas and 65 lobular carcinomas. Among the 173 cases of invasive ductal carcinomas, there were 16 cases of grade 1, 109 cases of grade 2, and 50 cases of grade 3. Histologic diagnosis was based on recent classifications.

Formalin-fixed, paraffin-embedded tissues of all the above-mentioned cases were used for tissue microarray construction. Tissue microarrays were assembled using a Manual Tissue Arrayer 1 (Bucher Instruments, Sunnyvale, CA) A representative area of each tumor was identified on the conventional sections, and 1 to 3 cylinders per tissue were arrayed using a punch biopsy (needles with a diameter of 1.0 mm). Immunohistochemical studies were performed on all the above-mentioned tissues using anti-Pax8 rabbit polyclonal, Protein Tech Group Inc, Chicago, IL and WT1 (mouse monoclonal 6F-H2, Daico, Carpinteria, CA).

The sections were deparaffinized in xylene (3 changes), rehydrated through graded alcohols (3 changes 100% ethanol, 3 changes 95% ethanol), and rinsed in distilled water. Heat-induced epitope retrieval was performed in 1,000 Watt microwave oven at 90% power in
Nonaka D, Chiriboga L, Soslow RA

- 124 Ovarian Carcinomas
  - 84 serous, 18 endometrioid, 12 mucinous, 10 clear cell

- 243 Invasive Breast Carcinomas
  - 178 ductal, 65 lobular

- PAX8 and WT1
PAX8

Nonaka D, Chiriboga L, Soslow RA

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<thead>
<tr>
<th></th>
<th>PAX 8</th>
<th>WT1</th>
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<tr>
<td>Serous</td>
<td>96%</td>
<td>87%</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>89%</td>
<td>28%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>8%</td>
<td>0%</td>
</tr>
<tr>
<td>Clear Cell</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Ductal</td>
<td>0%</td>
<td>3%</td>
</tr>
<tr>
<td>Lobular</td>
<td>0%</td>
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Immunohistochemical Panel to Identify the Primary Site of Invasive Micropapillary Carcinoma

Tamara L. Lotan, MD, Huihui Ye, MD, Jonathan Mekada, MD, Xue-Ru Wu, MD, Ic-Ming Shih, MD, PhD, and Jonathan I. Epstein, MD

Abstract: Invasive micropapillary carcinoma (IMC) is generally an aggressive morphologic variant that has been described in the bladder, lung, breast, salivary gland, gastrointestinal tract, and ovary. Given the morphologic similarities between IMCs arising from different organ systems and the high propensity of this histologic subtype for lymphatic metastasis, it may be necessary to use immunohistochemical (IHC) markers to determine the primary site of an IMC. Few studies have compared the IHC profiles of IMCs originating from different sites. We studied a panel of 11 IHC markers for their ability to distinguish urothelial, lung, breast, and ovarian IMC using a tissue microarray constructed with primary tumor tissue from 47 patients with IMC (13 bladder, 6 lung, 16 breast, and 12 ovarian). For each tumor, receptor classification as IMC was verified by reverse polarity MUC1 expression. We found that immunostaining for urethelial, CK5/6, TTF-1, estrogen receptor (ER), WT-1, and/or PAX8, and maimaglobin was the best panel for determining the most likely primary site of IMC. The test results to identify urothelial IMC were similar and CK5+ and ER-, whereas ER+, high molecular weight cytokeratin, and chromogranin a were specific and positive. Lung IMC was uniformly TTF-1 positive. Breast IMC was ER and PAX8 positive, maimaglobin positive, and PAX5/WT-1 negative, while ovarian IMC was ER positive, maimaglobin negative, and PAX8/WT-1 negative. In the metastatic setting, or when IMC occurs without an associated site or conventional carcinoma component, staining for urethelial, CK5/6, TTF-1, ER, and WT-1, and/or PAX8, and maimaglobin in the best panel for accurately classifying the likely primary site of IMC.

Key Words: micropapillary carcinoma, urothelial, bladder, breast, lung, ovary, immunohistochemistry

MATERIALS AND METHODS

Tissue Selection

Formalin-fixed paraffin-embedded primary tumor tissue from 47 IMC cases was collected from the files of the Johns Hopkins Hospital and arrayed in duplicate on 2 tissue microarrays with protocol approval from the...
PAX8

Lotan TL, Ye H, Melamed J, Wu X-R, Shih I-M, Epstein JI

- 16 Micropapillary Ca Breast
- 11 Micropapillary/Low Grade Serous Ca Ovary
- PAX8 and WT1
Lotan TL, Ye H, Melamed J, Wu X-R, Shih I-M, Epstein JI

<table>
<thead>
<tr>
<th></th>
<th>MP Ovary</th>
<th>MP Breast</th>
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<tbody>
<tr>
<td><strong>PAX8</strong></td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>WT1</strong></td>
<td>91%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Euscher ED, Deavers MT

- 8 patients with both Breast and Mullerian carcinomas.
  - 6 ductal (1 in-situ), 2 lobular
  - 5 ovarian, 2 peritoneal, 1 fallopian tube (serous)
- 3 of the Mullerian carcinomas initially interpreted as metastatic Breast
- 1 consultation – metastatic Breast vs. Mullerian
PAX8

Euscher ED, Deavers MT

Ovarian/Peritoneal/Tubal Serous 100%

Breast 0%
Problems and New Development

PAX8

Potential utility in Gyn Pathology

- Marker of Clear Cell Ca
- Serous Carcinoma vs Mesothelioma
- Ovarian or Endometrial Origin vs. Other (exceptions- kidney, thyroid)
  vs. Breast