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Tubo-Ovarian Transitional Cell Carcinoma and High Grade Serous Carcinoma Show Subtly Different Immunohistochemistry Profiles

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Abstract

Tubo-ovarian transitional cell carcinoma (TCC) is grouped with high-grade serous carcinoma (HGSC) in the current World Health Organization classification. TCC is associated with BRCA mutations and a better prognosis compared to HGSC. Previous papers examining the immunohistochemical features of TCC have studied limited numbers of samples. No marker reflecting the biological difference between TCC and HGSC is known. We collected a large cohort of TCC to determine whether TCC and HGSC could be distinguished by immunohistochemistry. A tissue microarray was built from 89 TCC and a control cohort of 232 conventional HGSC. Immunohistochemistry was performed, scored and statistically analyzed for routine markers of HGSC and urothelial tumors: PAX8, WT1, p53, p16, ER, p63 and GATA3. Using scoring cutoffs commonly employed in clinical practice, the immunohistochemical profile of TCC was indistinguishable from HGSC for all markers. However, more detailed scoring criteria revealed statistically significant differences between the two groups of tumors with respect to ER, PAX8 and WT1. HGSC showed more diffuse and intense staining for PAX8 (P=0.004 and P=0.001, respectively) and WT1 (P=0.002 and P=0.002, respectively); conversely, TCC showed more intense staining for ER (P=0.007). TCC and HGSC therefore show subtle differences in their immunohistochemical profiles which might reflect underlying (epi)genetic differences. Further studies using proteomic analysis will focus on the identification of differentially expressed proteins that might serve as markers of transitional cell carcinoma-like differentiation, which could

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help explain biological differences between TCC and HGSC and might identify other cases of HGSC with a better prognosis.

Keywords

Ovarian cancer; transitional cell carcinoma; high-grade serous carcinoma; immunohistochemistry; biomarkers

Introduction

While transitional cell carcinoma of the ovary (TCC) was listed as a separate ovarian carcinoma histotype in the 2003 World Health Organization (WHO) classification, it is currently defined as a variant of high grade serous carcinoma of the ovary (HGSC) in the 2014 WHO classification system (1). Silva *et al.* observed components of TCC-like morphology in approximately 10% of ovarian carcinomas (2). The true incidence of TCC remains unclear due to its rarity and because it was subsumed under HGSC, although estimates suggest it represents less than 2% of all ovarian tumors (3).

Previous studies of TCC hypothesized that it was a distinct histopathological entity with similar morphological characteristics to transitional cell carcinoma (i.e. infiltrative urothelial carcinoma and non-invasive papillary urothelial carcinomas) and distinct from Brenner tumor (4, 5). However, immunohistochemical evidence has shown that TCC is a Müllerian-type carcinoma rather than a *bona fide* urothelial neoplasm. And while TCC usually shows a similar immunohistochemical and molecular profile to HGSC (6-10), which is useful to classify TCC as a variant of HGSC, to our knowledge, no one has identified immunohistochemical variations between TCC and conventional HGSC that correlate with and potentially explain their distinct morphologies and, possibly, clinical behaviors.

There is conflicting evidence on the prognostic significance of TCC. Some studies report a better prognosis than conventional HGSC (2, 11-13), whereas others do not (14-16), possibly because of different definitions of TCC and recent progress in the histotyping of the ovarian carcinomas to which TCC was compared (17).

No marker reflecting the biological difference between TCC and HGSC is known. Therefore, the aim of this study was to determine whether TCC and HGSC could be distinguished with a panel of established diagnostic immunohistochemical markers applied to a large cohort.

Materials and Methods

Case Selection

This study was done under the University of British Columbia Research Ethics Review Board protocol H02-61375 (renewed March 30, 2017). Eighty-nine cases of TCC were studied, defined as carcinomas having TCC-like morphology in more than 50% of the tumor (60 cases with pure TCC-like morphology, and 29 cases with TCC-like morphology mixed with conventional HGSC), and 232 cases of conventional HGSC. TCC-like morphology was

defined as architecture resembling high-grade urothelial carcinoma, including plump macropapillae, large nests, or thick undulating epithelial bands lining cystic spaces, composed of cytologically high-grade (nuclear grade 2 or 3) stratified urothelial-like or microcystic epithelium. All cases of mixed TCC showed regions with features of conventional HGSC, including slit-like spaces, papillary, glandular, cribriform and solid areas. Tumors with TCC-like morphology were excluded from the designation of TCC if they contained any of the following: less than 50% TCC-like morphology, benign Brenner tumor, or endometrioid carcinoma.

The slides and blocks of formalin-fixed paraffin-embedded tissues for these tumors were retrieved from the Departments of Pathology at Vancouver General Hospital (Vancouver, BC), Memorial Sloan Kettering Cancer Center (New York, NY), from referral center series (Halle, Germany: S.H.; Mannheim, Germany: F.K.), or from the AGO study group (tumor samples from tissues banks created during the OVAR3 (18) and OVAR11 (19) trials; S.K.). All slides were centrally reviewed by a gynecologic pathologist (F.K.) and diagnosed as per the 2014 WHO classification system (1). A tissue microarray (TMA) was constructed containing 60 pure TCC, 29 mixed TCC (coring both the TCC-like and conventional HGSC components from each case) and 16 cases of conventional HGSC. An additional 216 conventional HGSC were sourced from a pre-existing TMA at Vancouver General Hospital.

Immunohistochemistry (IHC)

A panel of antibodies (PAX8, WT1, p53, p16, ER, p63 and GATA3) was used for immunohistochemical staining of each TMA. The details of the primary antibodies used are shown in Table 1. IHC was performed on 4-μm-thick formalin-fixed paraffin-embedded sections of tissue microarrays in the Genetic Pathology Evaluation Centre of Vancouver General Hospital using the Ventana Discovery XT and the Ventana Benchmark XT automated system (Ventana Medical Systems, Tucson, Arizona). Sections were mounted on charged glass slides and baked at 60 °C for 15 min. Heat-induced antigen retrieval was performed in Cell Conditioning solution CC1 (Ventana) for all immunohistochemical markers.

IHC Scoring

TMAs were scored by two pathologists (FK, BTC). For clinically employed scoring criteria, a cut-off of 1% of cells with nuclear staining was used to differentiate between positive (≥1% of cells) and negative (<1%) scores for WT1, PAX8 and ER (20-22). A cut-off of 10% of cells with nuclear staining was used to differentiate between positive (≥10% of cells) and negative (<10%) for GATA3 (23) and p63 (24). p16 was scored as negative (<1% of cells), weak/variable (1-80%) or strong/diffuse staining (>80%) (20). p53 was scored on a previously established 3-tiered system: <1% cells staining or a ‘‘null” or “complete loss” pattern corresponding to deleterious mutations in the *TP53* gene; 1% to 80% or a ‘‘normal’’ pattern corresponding to wild-type *TP53*; >80% or an ‘‘overexpression’’ pattern corresponding to missense mutations in the *TP53* gene (25, 26). TMA cores were only scored as null/complete loss in the presence of internal positive control staining of stromal cells. To provide more detail, PAX8, ER, WT1, p63 and GATA3 were also scored using more comprehensive assessments of percentage and intensity. The percentage of cells was

scored using the following criteria: 0-1% (score 0), 2-25% (score 1), 26-50% (score 2), 51-75% (score 3), and >75% (score 4). The staining intensity was scored as follows: negative (score 0), weak (score 1), moderate (score 2), and strong (score 3). Statistical comparisons of categorical data were carried out using a test of Proportions and using Fisher's exact test for independence with the expanded categories. All tests were two-sided, and *P*-values were adjusted for multiple comparisons using the False discover rate method. An adjusted *P*-value of <0.05 was considered statistically significant.

Results

Histologic Findings

The TCC samples collected for this study were high grade tumours predominantly composed of a macropapillary pattern, consisting of large plump stromal papillae covered by a multilayered urothelial-like solid or microcystic epithelium, or thick undulating epithelial bands lining cystic spaces comprising multiple layers of malignant epithelium resembling non-invasive papillary urothelial carcinoma. A nested architectural pattern with pushing borders was also observed. All cases of mixed TCC had areas of conventional HGSC comprising <50% of the tumor (Fig. 1).

IHC – Clinical Criteria

Using scoring criteria commonly employed in clinical practice, we did not identify any statistically significant differences in the expression of IHC markers between TCC vs. conventional HGSC. Furthermore, there was no difference in the expression of IHC markers in the two components of mixed TCC. The results of the immunostaining for TCC vs. HGSC are shown in Table 2 and Figure 2. In our cohort of TCC (n = 89, consisting of both pure TCC and the TCC-like component of mixed-type TCC) and HGSC (n = 232), PAX8 was expressed in 81 and 221 cases respectively, (92% and 97%, $P = 0.545$), WT1 in 79 and 217 (90% and 94%, $P = 0.545$), ER in 83 and 220 (94% and 95%, $P = 1$), p63 in 1 and 4 (1% and 2%, $P = 1$), and GATA3 in 2 and 1 (2% and 1%, $P = 0.666$). p16 showed strong-diffuse expression in 56 and 123 cases (63% and 54%, $P = 0.545$). p53 showed mutant-type pattern (complete loss or overexpression) in 80 TCC and 196 HGSC cases (94% and 91%, $P = 0.755$), of which 24 TCC cases (30%) displayed a null type pattern and 56 cases (70%) showed p53 overexpression pattern, while 65 HGSC cases (33%) displayed a null pattern type and 131 cases (67%) showed p53 overexpression pattern. The differences in the proportions of each of the markers between the two groups were not statistically significant after accounting for multiple comparisons (all comparisons have $P > 0.5$).

Analysis of mixed-type TCC using standard HGSC immunomarkers was performed to interrogate any potential dissimilarity between the TCC-like and conventional HGSC components from each mixed-type TCC (n=29). The result of this comparison is shown in Table 2 and Figure 3. In our cohort, TCC-like and HGSC components expressed PAX8 in all 29/29 and 28/28 cases, respectively, WT1 in 26/29 and 26/28 (90% and 93%, $P = 1$), ER in 27/28 and 27/28 (96% and 96%, $P = 1$), and neither component expressed p63. GATA3 was only expressed in the HGSC component in one case (1/28, 4%, $P = 1$). p16 showed strong-diffuse expression in 17/29 and 19/28 (59% and 68%, $P = 1$). p53 showed mutant-type

pattern in 25/26 TCC-like and 22/24 HGSC components (96% and 92%, $P = 1$), of which 7/26 TCC-like components (28%) displayed a null type pattern and 18/26 (72%) showed p53 overexpression pattern, while 4/24 HGSC components (18%) displayed a null pattern type and 18/24 (82%) showed p53 overexpression pattern. The differences in the proportions for each of the markers between the two groups were not statistically significant after accounting for multiple comparisons (all comparisons have $P > 0.5$).

IHC – Semi-quantitative Criteria

In order to provide a more detailed analysis of the expression of the immunomarkers, PAX8, ER, WT1, p63 and GATA3 were also scored using semi-quantitative assessment of the percentage (quartiles) and intensity (weak, moderate, strong) for each marker. The results of the scores for TCC vs. HGSC are shown in Table 3 and Figure 4. HGSC showed more diffuse and intense staining for PAX8 and WT1. For PAX8, 85% of HGSC vs. 63% of TCC showed staining in >75% of cells (percentage score 4, P=0.004), and 70% of HGSC vs. 51% of TCC stained with strongly (intensity score 3, P=0.001). For WT1, 82% of HGSC vs. 60% of TCC showed staining in >75% of cells (percentage score 4, P=0.002), and 67% of HGSC vs. 41% of TCC stained with strongly (intensity score 3, P=0.002). Conversely, TCC showed more intense staining for ER than HGSC (64% of TCC vs. 42% of HGSC with strong staining, intensity score 3, P=0.007). No statistically significant differences were observed between TCC and HGSC in the staining of p63 and GATA3.

Discussion

TCC is currently grouped with HGSC in the 2014 WHO classification system based on their similar immunohistochemical and molecular profiles. Past studies have utilized markers including WT1, p53, ER and p16 to compare the immunoprofile of TCC with urothelial tumors, Brenner tumors and HGSC (6-10). In this study, we investigated a series of 7 established immunohistochemical markers (WT1, p53, ER, p16, p63, PAX8, GATA3) in the largest immunohistochemical cohort of TCC reported to date.

No statistically significant differences between TCC and HGSC were identified using scoring criteria employed in clinical practice. Most cases of TCC stained positively for WT1 (90%), ER (94%), showed strong diffuse p16 expression (63%), and displayed abnormal p53 immunoexpression indicating a mutation (94%). This is in agreement with previous studies of HGSC, which showed that TCC is indistinguishable from HGSC. The morphology of TCC resembles urothelial carcinoma, however the TCC cases were overwhelmingly negative for cytokeratin 20 and the urothelial markers thrombomodulin and uroplakin III (8, 9, 27). The vast majority of TCC in the current study were negative for p63 (99%) and GATA3 (98%), markers that have been studied in urothelial carcinomas (28-32) but not in TCC. These results support the conclusion that although TCC resembles urothelial neoplasms architecturally, they are molecularly distinct. Similarly, the immunoprofile of TCC differs from malignant Brenner tumors, the majority of which are positive for urothelial markers (8), negative for HGSC markers (6-8, 10) and have a wild type immunostaining pattern for p53 (6, 10). This is further supported by our findings that most pure TCC were positive for

PAX8 (92%), an established marker of ovarian epithelial tumors with Mullerian differentiation, which is not expressed in urothelial carcinomas (24, 33).

While TCC was indistinguishable from HGSC using conventional scoring criteria, we observed statistically significant differences between these tumors using more detailed immunoreactive scoring methods for PAX8, WT1 and ER. PAX8 and WT1 both showed more diffuse and intense expression in HGSC compared to TCC, whereas TCC showed more intense expression of ER compared to HGSC. To our knowledge, this is the first report of immunophenotypic differences between TCC and HGSC. The reciprocal staining trends for PAX8/WT and ER in each tumour type suggests the staining differences between TCC and HGSC are reflections of biological differences, not pre-analytical variables (such as different fixation times between HGSC and TCC cases). The lower and weaker PAX8 and WT1 expression in TCC suggests that it has a less Mullerian phenotype than HGSC; the significance of the differences in ER staining intensity is unclear. It is not known at this time whether the subtle immunophenotypic differences we observed correlate with differences in underlying genetics or clinical behavior.

Silva and colleagues were the first to demonstrate TCC had a superior prognosis to conventional HGSC (2, 11, 13). Subsequently, Kommoss et al. also noted that TCC had a better prognosis compared to other ovarian carcinomas, including HGSC, after standard chemotherapy with platinum and paclitaxel (12). In contrast, three other studies (two primary analyses and one meta-analysis) observed no association between the TCC-like pattern and survival (14-16).

A definitive conclusion about the prognostic significance of tumors designated as TCC is hampered by small sample sizes, differences in diagnostic criteria for TCC between studies, and important changes in diagnostic criteria for ovarian cancers. For example, three studies which showed that TCC conferred a better prognosis included tumors that had minor component of endometrioid carcinoma as part of their definition of TCC (2, 11, 12), and one study did not formally exclude endometrioid carcinoma as part of the definition of TCC (13) as we have done in the current study. Endometrioid carcinomas with transitional-like morphology may have a better prognosis than conventional HGSC (34), so the inclusion of tumors showing a minor endometrioid component is a potential source of bias that could suggest a beneficial prognostic effect of TCC. The study by Hollingsworth and colleagues showed no prognostic significance of TCC compared to ‘serous carcinomas’ (15). However, 24% of the serous carcinomas in that study had grade 1 nuclear features (i.e. were low-grade serous carcinomas), and 36% were grade 2 and most like represented a mix of LGSC and HGSC (35). The inclusion of a significant percentage of LGSC in the ‘serous carcinoma’ cohort could potentially mask a beneficial survival effect of TCC compared to HGSC. Furthermore, important changes in histotyping criteria for ovarian carcinomas have occurred since these survival studies were published that have resulted in significant changes in diagnoses (17), which further complicates the interpretation of the results from these studies.

We set out to only study TCC-like tumors that most resemble HGSC. For mixed TCC, we excluded transitional tumors with a component of endometrioid carcinoma or Brenner tumor. For pure TCC, our IHC results support the fact that we successfully excluded most

malignant Brenner tumors and endometrioid carcinomas with TCC-like morphology (34). Of 9/88 (10%) tumors that were WT1 negative, 3 showed a wild type p53 pattern; therefore, only 3/88 (3%) of tumors in our study are unlikely to represent TCCs that resemble HGSC. The remaining 6 WT1-negative tumors with mutant-pattern p53 could either represent WT1-negative HGSC or high-grade endometrioid carcinomas. One of these tumors was weakly positive for p63 but strongly positive for ER, arguing against a malignant Brenner tumor (6).

The explanation for the putative beneficial prognosis associated with TCC is likely multifactorial. Robey et al. demonstrated TCC had better outcomes regardless of residual disease after surgery due to a favourable response to chemotherapy (94% complete response and 83% with no evidence of disease for TCC patients vs. 37% and 11.4% respectively for non-TCC vs. 39% and 0% for serous carcinoma) (13). Kommoss et al. suggested that a propensity for micronodular rather than macronodular extraovarian spread and better surgical resectability due to a lesser degree of diffuse infiltrative growth may contribute to a survival benefit in TCC (12). HGSC bearing germline or somatic *BRCA1* mutations or *BRCA1* promoter methylation were significantly associated with solid, pseudoendometrioid, and TCC-like morphology ('SET' features) (36, 37) and a pushing (vs. infiltrative) pattern of omental implants (38); the pushing pattern was associated with improved outcome (39). Mutations in genes involved in homologous recombination such as *BRCA1* and *BRCA2* are associated with improved 5 year survival (40, 41) and increased tumor infiltrating lymphocytes, in particular cytotoxic T cells (42), which are independent predictors of prolonged survival in HGSC (43, 44). The putative beneficial prognosis of TCC may therefore be due to underlying genetics (i.e. homologous recombination defects) that lead to a combination of enhanced chemosensitivity, improved resectability, and favourable immune infiltrates. We are currently sequencing a cohort of TCC to determine whether these tumours show genetic differences from HGSC (e.g. more *BRCA1* or *BRCA2* mutations).

In conclusion, this is the largest and most comprehensive comparison of the immunohistochemical features of TCC and conventional HGSC. Our results confirm that TCC – as defined in this study – and HGSC have similar immunohistochemical profiles, and we have identified novel and subtle immunohistochemical differences between these tumours using currently available immunomarkers. This type of rigorous histological and immunohistochemical analysis, combined with an analysis of tumor genetics, will help select a uniform group of TCC patients – i.e. patients with HGSC-like tumors with predominantly or purely TCC-like morphology – to determine with greater certainty whether TCC has a better prognosis than conventional HGSC. If so, it will be important to determine whether the genetic, immune, and proteomic correlates of the improved prognosis of TCC may be useful as prognostic biomarkers for patients with conventional HGSC.

Conflicts of Interest and Source of Funding:

The authors declare no conflict of interest and no funding support for this research.

References

1. WHO Classification of Tumours of Female Reproductive Organs. Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. Lyon: International Agency for Research on Cancer; 2014.

2. Silva EG, Robey-Cafferty SS, Smith TL, Gershenson DM. Ovarian carcinomas with transitional cell carcinoma pattern. Am J Clin Pathol. 1990;93(4):457–65. [PubMed: 2321577]

3. Ichigo S, Takagi H, Matsunami K, Murase T, Ikeda T, Imai A. Transitional cell carcinoma of the ovary (Review). Oncol Lett. 2012;3(1):3–6. [PubMed: 22740845]

4. Austin RM, Norris HJ. Malignant Brenner tumor and transitional cell carcinoma of the ovary: a comparison. Int J Gynecol Pathol. 1987;6(1):29–39. [PubMed: 3570630]

5. Eichhorn JH, Young RH. Transitional cell carcinoma of the ovary: a morphologic study of 100 cases with emphasis on differential diagnosis. Am J Surg Pathol. 2004;28(4):453–63. [PubMed: 15087664]

6. Ali RH, Seidman JD, Luk M, Kalloger S, Gilks CB. Transitional cell carcinoma of the ovary is related to high-grade serous carcinoma and is distinct from malignant brenner tumor. Int J Gynecol Pathol. 2012;31(6):499–506. [PubMed: 23018212]

7. Cuatrecasas M, Catasus L, Palacios J, Prat J. Transitional cell tumors of the ovary: a comparative clinicopathologic, immunohistochemical, and molecular genetic analysis of Brenner tumors and transitional cell carcinomas. Am J Surg Pathol. 2009;33(4):556–67. [PubMed: 19033864]

8. Logani S, Oliva E, Amin MB, Folpe AL, Cohen C, Young RH. Immunoprofile of ovarian tumors with putative transitional cell (urothelial) differentiation using novel urothelial markers: histogenetic and diagnostic implications. Am J Surg Pathol. 2003;27(11):1434–41. [PubMed: 14576476]

9. Riedel I, Czernobilsky B, Lifschitz-Mercer B, Roth LM, Wu XR, Sun TT, et al. Brenner tumors but not transitional cell carcinomas of the ovary show urothelial differentiation: immunohistochemical staining of urothelial markers, including cytokeratins and uroplakins. Virchows Arch. 2001;438(2):181–91. [PubMed: 11253121]

10. Takeuchi T, Ohishi Y, Imamura H, Aman M, Shida K, Kobayashi H, et al. Ovarian transitional cell carcinoma represents a poorly differentiated form of high-grade serous or endometrioid adenocarcinoma. Am J Surg Pathol. 2013;37(7):1091–9. [PubMed: 23681072]

11. Gershenson DM, Silva EG, Mitchell MF, Atkinson EN, Wharton JT. Transitional cell carcinoma of the ovary: a matched control study of advanced-stage patients treated with cisplatin-based chemotherapy. Am J Obstet Gynecol. 1993;168(4):1178–85; discussion 85-7. [PubMed: 8475964]

12. Kommoss F, Kommoss S, Schmidt D, Trunk MJ, Pfisterer J, du Bois A. Survival benefit for patients with advanced-stage transitional cell carcinomas vs. other subtypes of ovarian carcinoma after chemotherapy with platinum and paclitaxel. Gynecol Oncol. 2005;97(1):195–9. [PubMed: 15790458]

13. Robey SS, Silva EG, Gershenson DM, McLemore D, el-Naggar A, Ordonez NG. Transitional cell carcinoma in high-grade high-stage ovarian carcinoma. An indicator of favorable response to chemotherapy. Cancer. 1989;63(5):839–47. [PubMed: 2644014]

14. Costa MJ, Hansen C, Dickerman A, Scudder SA. Clinicopathologic significance of transitional cell carcinoma pattern in nonlocalized ovarian epithelial tumors (stages 2-4). Am J Clin Pathol. 1998;109(2):173–80. [PubMed: 9583889]

15. Hollingsworth HC, Steinberg SM, Silverberg SG, Merino MJ. Advanced stage transitional cell carcinoma of the ovary. Hum Pathol. 1996;27(12):1267–72. [PubMed: 8958296]

16. Mackay HJ, Brady MF, Oza AM, Reuss A, Pujade-Lauraine E, Swart AM, et al. Prognostic relevance of uncommon ovarian histology in women with stage III/IV epithelial ovarian cancer. Int J Gynecol Cancer. 2010;20(6):945–52. [PubMed: 20683400]

17. Kommoss S, Gilks CB, du Bois A, Kommoss F. Ovarian carcinoma diagnosis: the clinical impact of 15 years of change. Br J Cancer. 2016;115(8):993–9. [PubMed: 27632374]

18. du Bois A, Luck HJ, Meier W, Adams HP, Mobus V, Costa S, et al. A randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst. 2003;95(17):1320–9. [PubMed: 12953086]

19. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26):2484–96. [PubMed: 22204725]

20. Kalloger SE, Kobel M, Leung S, Mehl E, Gao D, Marcon KM, et al. Calculator for ovarian carcinoma subtype prediction. Mod Pathol. 2011;24(4):512–21. [PubMed: 21131918]

21. Nonaka D, Chiriboga L, Soslow RA. Expression of pax8 as a useful marker in distinguishing ovarian carcinomas from mammary carcinomas. Am J Surg Pathol. 2008;32(10):1566–71. [PubMed: 18724243]
22. Sieh W, Kobel M, Longacre TA, Bowtell DD, deFazio A, Goodman MT, et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. Lancet Oncol. 2013;14(9):853–62. [PubMed: 23845225]
23. Gonzalez RS, Wang J, Kraus T, Sullivan H, Adams AL, Cohen C. GATA-3 expression in male and female breast cancers: comparison of clinicopathologic parameters and prognostic relevance. Hum Pathol. 2013;44(6):1065–70. [PubMed: 23266442]
24. Carvalho JC, Thomas DG, McHugh JB, Shah RB, Kunju LP. p63, CK7, PAX8 and INI-1: an optimal immunohistochemical panel to distinguish poorly differentiated urothelial cell carcinoma from high-grade tumours of the renal collecting system. Histopathology. 2012;60(4):597–608. [PubMed: 22260386]
25. Kobel M, Piskorz AM, Lee S, Lui S, LePage C, Marass F, et al. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. J Pathol Clin Res. 2016;2(4):247–58. [PubMed: 27840695]
26. Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih Ie M, et al. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. Mod Pathol. 2011;24(9):1248–53. [PubMed: 21552211]
27. Soslow RA, Rouse RV, Hendrickson MR, Silva EG, Longacre TA. Transitional cell neoplasms of the ovary and urinary bladder: a comparative immunohistochemical analysis. Int J Gynecol Pathol. 1996;15(3):257–65. [PubMed: 8811388]
28. Chang A, Amin A, Gabrielson E, Illei P, Roden RB, Sharma R, et al. Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus, and lung. Am J Surg Pathol. 2012;36(10):1472–6. [PubMed: 22982890]
29. Gulmann C, Paner GP, Parakh RS, Hansel DE, Shen SS, Ro JY, et al. Immunohistochemical profile to distinguish urothelial from squamous differentiation in carcinomas of urothelial tract. Hum Pathol. 2013;44(2):164–72. [PubMed: 22995333]
30. Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, et al. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol. 2014;38(1):13–22. [PubMed: 24145643]
31. Buza N, Cohen PJ, Pei H, Parkash V. Inverse p16 and p63 expression in small cell carcinoma and high-grade urothelial cell carcinoma of the urinary bladder. Int J Surg Pathol. 2010;18(2):94–102. [PubMed: 20164052]
32. Kunju LP, Mehra R, Snyder M, Shah RB. Prostate-specific antigen, high-molecular-weight cytokeratin (clone 34betaE12), and/or p63: an optimal immunohistochemical panel to distinguish poorly differentiated prostate adenocarcinoma from urothelial carcinoma. Am J Clin Pathol. 2006;125(5):675–81. [PubMed: 16707367]
33. Laury AR, Perets R, Piao H, Krane JF, Barletta JA, French C, et al. A comprehensive analysis of PAX8 expression in human epithelial tumors. Am J Surg Pathol. 2011;35(6):816–26. [PubMed: 21552115]
34. Karnezis AN, Aysal A, Zaloudek CJ, Rabban JT. Transitional cell-like morphology in ovarian endometrioid carcinoma: morphologic, immunohistochemical, and behavioral features distinguishing it from high-grade serous carcinoma. Am J Surg Pathol. 2013;37(1):24–37. [PubMed: 23108017]
35. Malpica A, Deavers MT, Lu K, Bodurka DC, Atkinson EN, Gershenson DM, et al. Grading ovarian serous carcinoma using a two-tier system. Am J Surg Pathol. 2004;28(4):496–504. [PubMed: 15087669]
36. Howitt BE, Hanamornroongruang S, Lin DI, Conner JE, Schulte S, Horowitz N, et al. Evidence for a dualistic model of high-grade serous carcinoma: BRCA mutation status, histology, and tubal intraepithelial carcinoma. Am J Surg Pathol. 2015;39(3):287–93. [PubMed: 25581732]

37. Soslow RA, Han G, Park KJ, Garg K, Olvera N, Spriggs DR, et al. Morphologic patterns associated with BRCA1 and BRCA2 genotype in ovarian carcinoma. Mod Pathol. 2012;25(4):625–36. [PubMed: 22193042]

38. Reyes MC, Arnold AG, Kauff ND, Levine DA, Soslow RA. Invasion patterns of metastatic high-grade serous carcinoma of ovary or fallopian tube associated with BRCA deficiency. Mod Pathol. 2014;27(10):1405–11. [PubMed: 24577588]

39. Hussein YR, Ducie JA, Arnold AG, Kauff ND, Vargas-Alvarez HA, Sala E, et al. Invasion Patterns of Metastatic Extrauterine High-grade Serous Carcinoma With BRCA Germline Mutation and Correlation With Clinical Outcomes. Am J Surg Pathol. 2016;40(3):404–9. [PubMed: 26574845]

40. Bolton KL, Chenevix-Trench G, Goh C, Sadetzki S, Ramus SJ, Karlan BY, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307(4):382–90. [PubMed: 22274685]

41. Garsed DW, Alsop K, Fereday S, Emmanuel C, Kennedy C, Etemadmoghadam D, et al. Homologous Recombination DNA Repair Pathway Disruption and Retinoblastoma Protein Loss are Associated with Exceptional Survival in High-Grade Serous Ovarian Cancer. Clin Cancer Res. 2017.

42. McAlpine JN, Porter H, Kobel M, Nelson BH, Prentice LM, Kalloger SE, et al. BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. Mod Pathol. 2012;25(5):740–50. [PubMed: 22282309]

43. Hwang WT, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol. 2012;124(2):192–8. [PubMed: 22040834]

44. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203–13. [PubMed: 12529460]

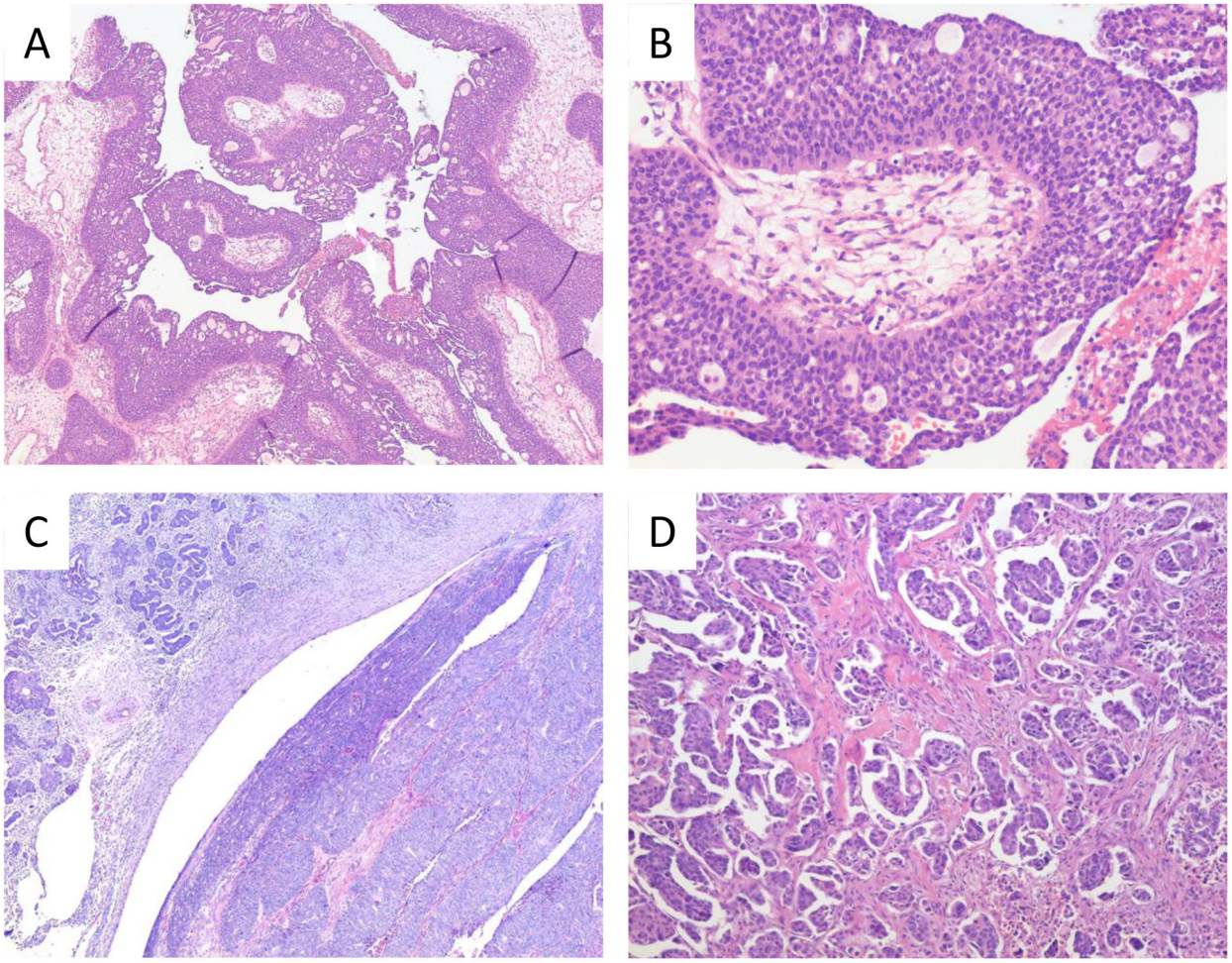

Figure 1:

Histomorphology of TCC. (A) TCC showing a macropapillary pattern and a cystic space lined by an undulating band of urothelial-like cells. (B) TCC with multiple layers of epithelium and focal microcysts. (C) Mixed TCC, with thick undulating epithelial bands (lower right) next to an area of conventional HGSC (upper left). (D) Conventional HGSC component of a mixed TCC, consisting of small irregular papillae and micropapillae with an infiltrative pattern and desmoplastic stromal reaction.

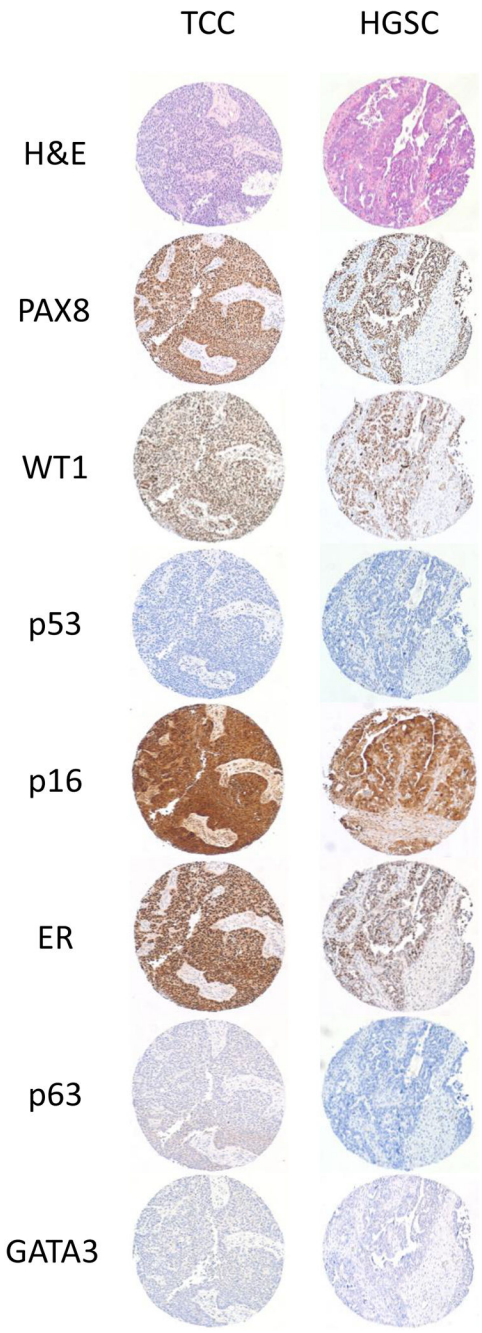

Figure 2:
Immunohistochemical comparison of TCC and conventional HGSC. Pure TCC (left panels) and conventional HGSC (right panels).

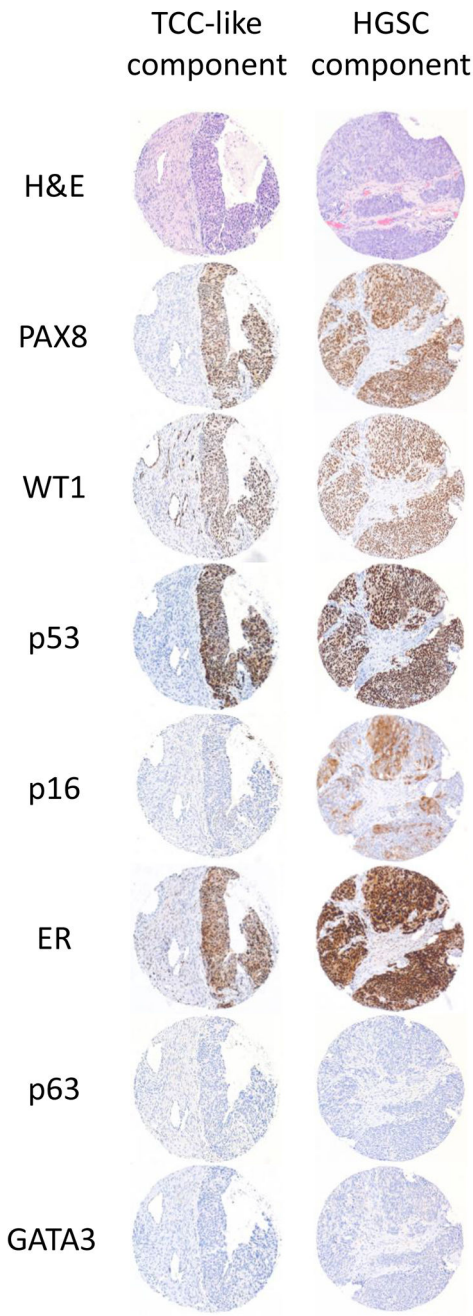

Figure 3:
Immunohistochemical comparison of the two components of mixed TCC. TCC-like component (left panels) and HGSC component (right panels).

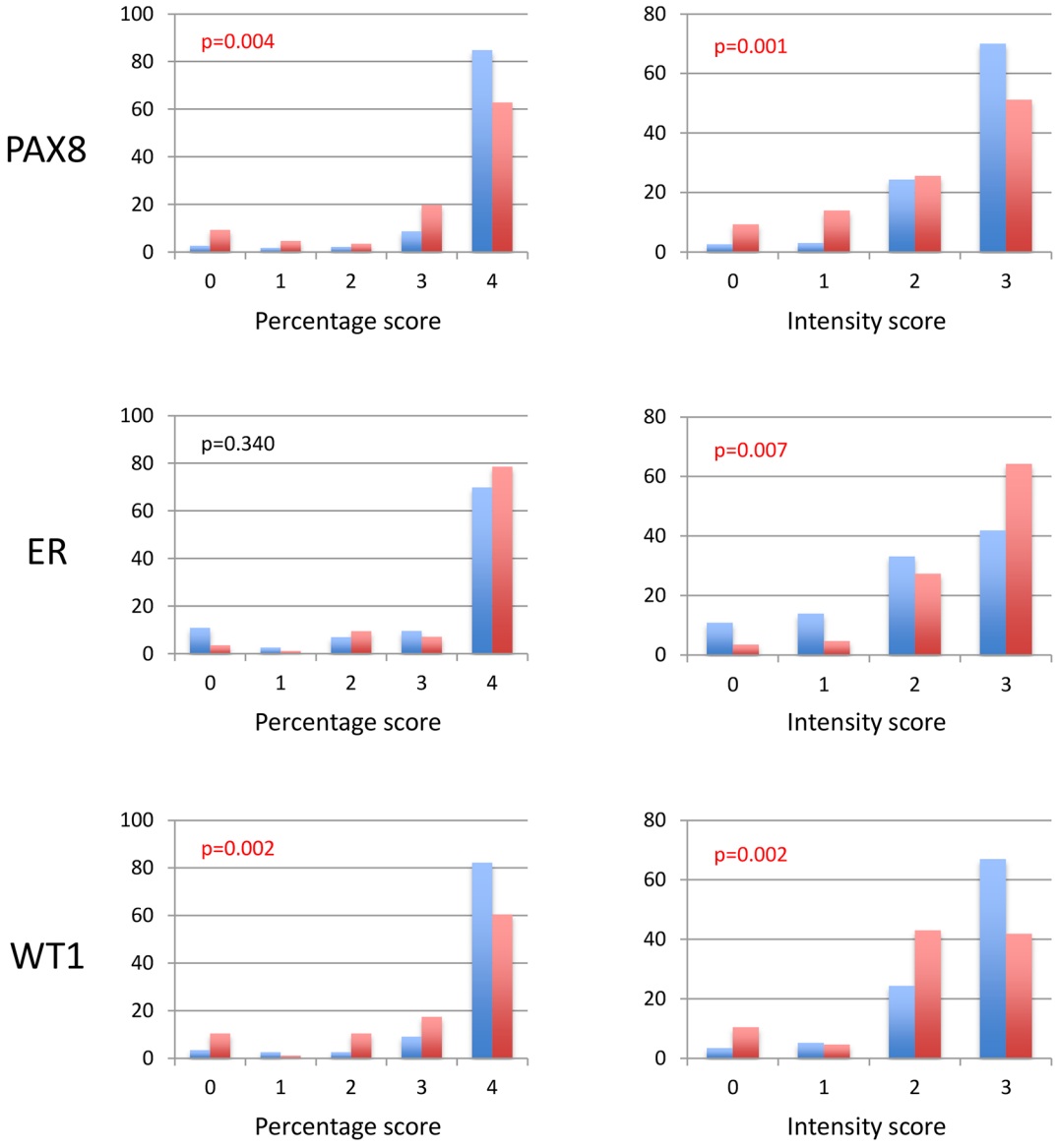

Figure 4.

Immunohistochemical comparison between TCC and conventional HGSC for PAX8, ER and WT1. Y axis: percent of cases (data from Table 3). Blue bars: conventional HGSC. Red bars: TCC. See Methods for scoring criteria.

Table 1:

Antibody information and scoring criteria.

Antibody	Clone	Dilution	Source	Antigen Retrieval method	Scoring Cutoff (localization)
PAX8	Rabbit polyclonal	1:200	BIOCARE	Heat; CC1 for 32 mins	Negative = <1% Positive = ≥1% (nuclear)
WT1	Mouse monoclonal, 6F-H2	1:100	DAKO	Heat; CC1 for 32 mins	Negative = <1% Positive = ≥1% (nuclear)
p53	Mouse monoclonal, DO-7	1:800	DAKO	Heat; CC1 for 32 mins	Complete loss/null mutation = <1% Normal/wild-type = 1-80% Overexpression/missense mutation = >80% (nuclear)
p16	Mouse monoclonal, E6H4	1:3	CINtec	Heat; CC1 for 32 mins	Negative = <1% Weak/Variable = 1-80% Strong/Diffuse = >80% (nuclear + cytoplasmic)
ER	Rabbit monoclonal, EPR5701	1:2000	Epitomics	Heat; CC1 for 32 mins	Negative = <1% Positive = ≥1% (nuclear)
p63	Rabbit monoclonal, SP1	1:50	Thermo-Fisher	Heat; CC1 for 32 mins	Negative = <10% Positive = ≥10% (nuclear)
GATA3	Mouse monoclonal, L50-823	1:200	CellMarque	Heat; CC1 for 32 mins	Negative = <10% Positive = ≥10% (nuclear)

Table 2:

Immunohistochemistry results in TCC (all cases) vs. conventional HGSC and in each morphologic component of cases of mixed TCC using clinical scoring criteria.

			Conventional HGSC vs. TCC		Mixed TCC	
Antibody		**Score**	**Conventional HGSC**	**TCC (all cases)**	**TCC-like component**	**HGSC component**
PAX8		+	221/228 (97%)	81/88 (92%)	29/29 (100%)	28/28 (100%)
		−	7/228 (3%)	7/88 (8%)	0/29 (0%)	0/28 (0%)
WT1		+	217/230 (94%)	79/88 (90%)	26/29 (90%)	26/28 (93%)
		−	13/230 (6%)	9/88 (10%)	3/29 (10%)	2/28 (7%)
ER		+	220/232 (95%)	83/88 (94%)	27/28 (96%)	27/28 (96%)
		−	12/232 (5%)	5/88 (6%)	1/28 (4%)	1/28 (4%)
p63		+	4/232 (2%)	1/87 (1%)	0/28 (0%)	0/28 (0%)
		−	228/232 (98%)	86/87 (99%)	28/28 (100%)	28/28 (100%)
GATA3		+	1/232 (1%)	2/88 (2%)	0/28 (0%)	1/28 (4%)
		−	231/232 (99%)	86/88 (98%)	28/28 (100%)	27/28 (96%)
p53	Mut	Null	65/215 (30%)	24/85 (28%)	7/26 (27%)	4/24 (17%)
	WT	Normal	19/215 (9%)	5/85 (6%)	1/26 (4%)	2/24 (8%)
	Mut	Overex-pression	131/215 (61%)	56/85 (66%)	18/26 (69%)	18/24 (75%)
	All mutants (null + overexpr)		196/215 (91%)	80/85 (94%)	25/26 (96%)	22/24 (92%)
p16	−	Negative	3/228 (1%)	4/89 (5%)	1/29 (3%)	0/28 (0%)
	−	Weak/Variable	102/228 (45%)	29/89 (33%)	11/29 (38%)	9/28 (32%)
	+	Strong/Diffuse	123/228 (54%)	56/89 (63%)	17/29 (59%)	19/28 (68%)
	All “–“ cases (neg and weak)		105/228 (46%)	33/89 (37%)	12/29 (41%)	9/28 (32%)

“+” = positive score, “−“ = negative score, Mut = mutant pattern, WT = wild type pattern.

Table 3.

Immunohistochemistry results in TCC (all cases) vs. conventional HGSC using percentage and intensity score

		PAX8		ER		WT1		p63		GATA3	
Percentage	**Score**	**HGSC**	**TCC**	**HGSC**	**TCC**	**HGSC**	**TCC**	**HGSC**	**TCC**	**HGSC**	**TCC**
0-1	0	6 (3%)	8 (9%)	25 (11%)	3 (4%)	8 (4%)	9 (10%)	214 (92%)	85 (98%)	220 (94%)	81 (92%)
2-25	1	4 (2%)	4 (5%)	6 (3%)	1 (1%)	6 (3%)	1 (1%)	17 (7%)	1 (1%)	12 (5%)	4 (5%)
26-50	2	5 (2%)	3 (3%)	16 (7%)	8 (10%)	6 (3%)	9 (10%)	2 (1%)	1 (1%)	1 (0.5%)	2 (2%)
51-75	3	20 (9%)	17 (20%)	22 (10%)	6 (7%)	21 (9%)	15 (17%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)
76-100	4	195 (85%)	54 (63%)	160 (70%)	66 (79%)	189 (82%)	52 (60%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
P-value		0.004		0.340		0.002		0.155		0.279	

		PAX8		ER		WT1		p63		GATA3	
Intensity	**Score**	**HGSC**	**TCC**	**HGSC**	**TCC**	**HGSC**	**TCC**	**HGSC**	**TCC**	**HGSC**	**TCC**
Negative	0	6 (3%)	8 (9%)	25 (11%)	3 (4%)	8 (3%)	9 (10%)	214 (92%)	87 (98%)	220 (94%)	81 (92%)
Weak	1	7 (3%)	12 (14%)	32 (14%)	4 (5%)	12 (5%)	4 (5%)	6 (3%)	0 (0%)	11 (5%)	5 (6%)
Moderate	2	56 (24%)	22 (26%)	76 (33%)	23 (27%)	56 (24%)	37 (43%)	8 (3%)	0 (0%)	2 (1%)	2 (2%)
Strong	3	161 (70%)	44 (51%)	96 (42%)	54 (64%)	154 (67%)	36 (42%)	5 (2%)	2 (2%)	0 (0%)	0 (0%)
P-value		0.001		0.007		0.002		0.243		0.656	