Immunoexpression of PAX-8 as a Useful Marker in Distinguishing Gynecological Malignancy from Colorectal Carcinomas: a Tissue Microarray-Based Approach

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Abstract: Introduction: PAX 8 is a transcription factor that belongs to PAX gene family. The data on the diagnostic applications of PAX-8 is limited. In this study, the expression of PAX-8 in colorectal, endometrial and ovarian carcinomas is evaluated. **Material and methods:** Tissue microarrays were prepared from archival of colorectal carcinomas (n: 133), endometrial carcinomas (n: 79) and ovarian carcinomas (75) obtained from the Department of Pathology at King Abdulaziz University Jeddah, Saudi Arabia. Tissue sections were immunostained using monoclonal antibodies to PAX-8. The immunohistochemical stains were scored semiquantitatively from 0 to 4+. **Results:** PAX-8 immunoexpression was detected in 132/ 154 (83%) of the Mullerian carcinomas (93 and 43% for non-mucinous and mucinous carcinomas, respectively). PAX-8 expression was found in all serous carcinomas from ovarian and endometrial origin. PAX-8 was not detected in any of the colorectal carcinoma. **Conclusion:** PAX-8 is a sensitive marker for non-mucinous carcinomas of Mullerian origin and it is a useful marker in differentiating endometrial and ovarian carcinomas from colorectal carcinomas.
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Key words: PAX-8, immunoexpression, colorectal, endometrial carcinoma, ovarian carcinoma, metastasis.

1. Introduction:

PAX genes are a family of transcription factors that play critical roles during organogenesis. They are regulatory proteins expressed in embryonic or neoplastic cells of the same lineage. PAX-8 is crucial for organogenesis of the Müllerian system, kidney and thyroid gland. Few studies have demonstrated expression of PAX 8 in neoplasms including thyroid follicular neoplasm (1-4), renal cell carcinoma (5), nephrogenic adenoma (6-12), Wilms' tumors (13), and ovarian carcinoma (14-17). Gynecological malignancies (endometrial and ovarian) and colorectal carcinomas are among the common malignancies in women. Distinction between metastasis from gynecological malignancies and colorectal carcinomas is important as their prognoses differ significantly. It is important to differentiate metastatic Mullerian tumors from metastatic colorectal carcinoma in liver, lung or peritoneum. It is also important to differentiate primary Mullerian tumors from metastatic colorectal cancer to the ovaries. The data on the expression of PAX-8 in colorectal and gynecological malignancies is limited. We applied PAX-8 immunostain on a large number of ovarian, endometrial and colorectal carcinomas to evaluate the diagnostic significance.

2. Patients and Methods

The study included paraffin wax blocks of colorectal carcinomas (n: 133), endometrial (n: 79) and ovarian (75). Tumor specimens represent the surgical treatment of patients with no prior chemotherapy or radiotherapy given. Blocks were retrieved from the archives of the Department of Pathology at King Abdulaziz University, Jeddah, Saudi Arabia. The study was approved by the Research Committee of the Biomedical Ethics Unit, Faculty of Medicine, King Abdulaziz University.

Tissue Microarray:

Tissue microarrays (TMA) were designed and constructed as previously described (18). Briefly, hematoxylin and eosin-stained sections of endometrial carcinomas, ovarian carcinomas, normal colorectal mucosae, colorectal adenomas, and primary tumors and nodal metastasis were reviewed by an experienced pathologist. Areas of interest were chosen from the original blocks and were marked on the slides. Necrotic, autolytic areas and areas containing predominantly the stromal tissue were avoided. Tissue cores each 1.5 mm in diameter were punched from donor block (s) in an automated TMA instrument (TMA Master 1.14 SP3 from 3D Histech Ltd. Budapest, Hungary) and inserted into a recipient paraffin block. Placenta was used for orientation. Slides were cut from TMA block and stained with

hematoxylin and eosin for initial morphological assessment of accuracy of construction.

Immunohistochemistry:

Paraffin blocks of constructed TMAs were cut at 4 μm, and mounted on positive-charged slides (Leica Microsystems Plus Slides). Sections were deparaffinised in xylene and rehydrated in an automated immunostainer (BenchMark XT, Ventana® Medical systems Inc., Tucson, AZ, USA). Pre-treatment was done using CC1 (prediluted cell conditioning solution) for 60 minutes. Anti PAX-8 antibody. Immunohistochemical studies were performed on all the above-mentioned tissues using anti-Pax-8 (mouse monoclonal, Ventana) was incubated at 37°C for 16 minutes. Ventana® Ultraview Universal DAB detection kit was used according to kit manufacturer instructions. Subsequently, slides were washed, counterstained with Mayer's hematoxylin and mounted. Negative control (substitution of the primary antibody with Tris-buffered saline) and positive control slides were included.

Interpretation of PAX-8-1mmunostaining:

Sections were evaluated independently without knowledge of the clinicopathological characteristics of patients by pathologist (JM). The extent of nuclear staining was graded as follows: 1+, 1% to 25%; 2+, 25% to 50%; 3+, 50% to 75%; 4+, >75%.

3. Results:

Colorectal carcinomas include 33 well-differentiated, 83 moderately-differentiated, 17 poorly-differentiated. Endometrial adenocarcinomas include 69 endometrioid and 10 serous. Ovarian carcinomas include 46 serous carcinomas, 23 mucinous carcinomas and 6 endometrioid carcinomas. PAX-8 immunoexpression was detected in 132/ 154 (83%) of the Mullerian carcinomas (ovarian and endometrial). The vast majority of the positive cases reveal high expression level (4+ or 3+). PAX-8 immunoexpression was found in all serous carcinomas from ovarian and endometrial origin; 56/56 (100%), in 61/69 (88.4%) endometrial endometrioid carcinomas, 5/6 (83.3%) ovarian endometrioid carcinomas and in 10/23 (43%) ovarian mucinous carcinomas. PAX-8 was not detected in any of the colorectal carcinoma.

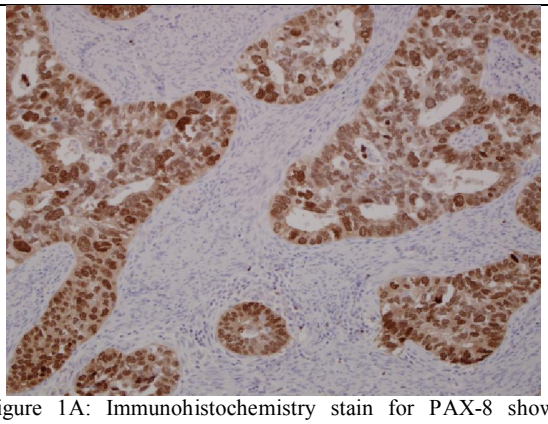

Figure 1A: Immunohistochemistry stain for PAX-8 show positive staining (4+) in endometrial carcinoma, endometrioid type (original magnification X200).

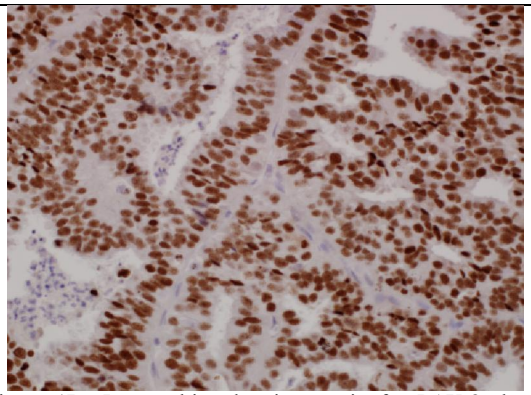

Figure 1B: Immunohistochemistry stain for PAX-8 show positive staining (4+) in endometrial carcinoma, another case of endometrioid type (original magnification X400).

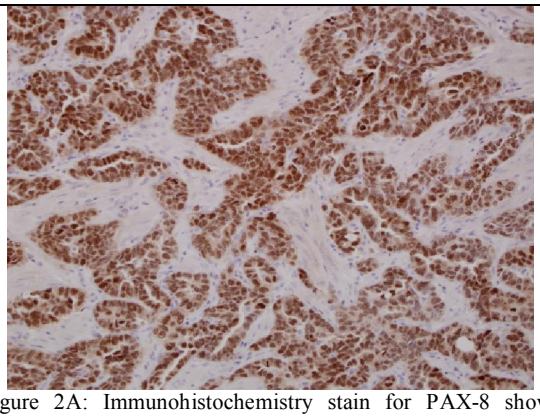

Figure 2A: Immunohistochemistry stain for PAX-8 show positive staining (4+) in ovarian carcinoma, serous type (original magnification X200).

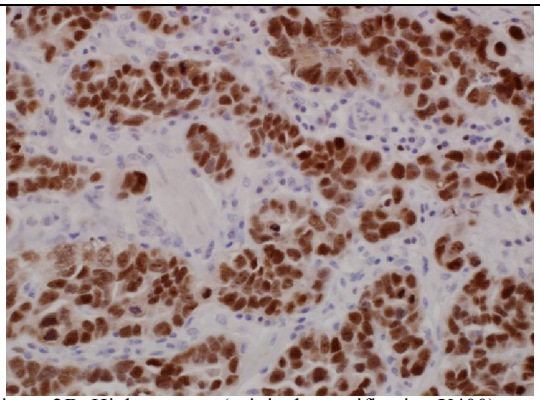

Figure 2B: Higher power (original magnification X400).

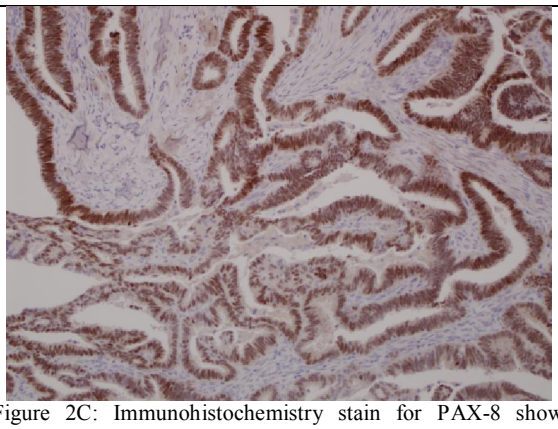

Figure 2C: Immunohistochemistry stain for PAX-8 show positive staining (4+) in ovarian carcinoma, endometrioid type (original magnification X200).

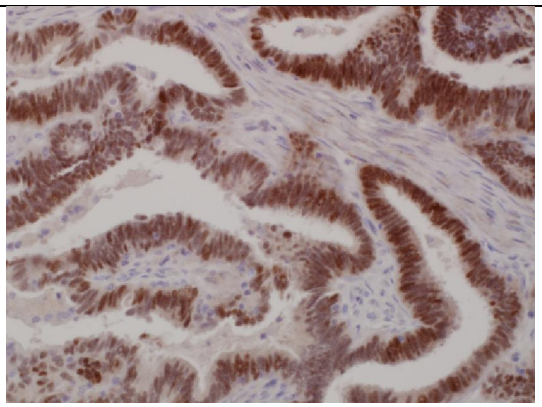

Figure 2D: Higher power (original magnification X400).

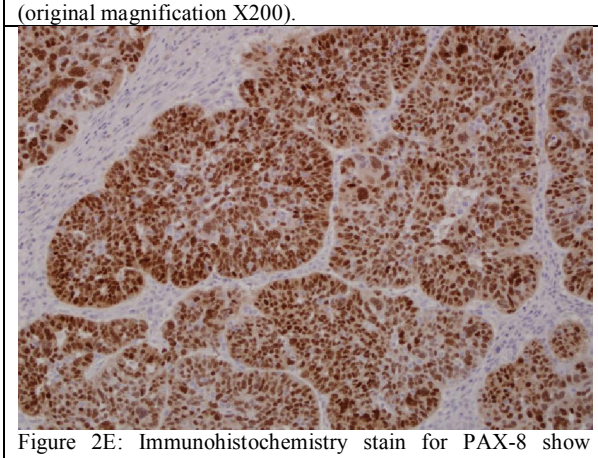

Figure 2E: Immunohistochemistry stain for PAX-8 show positive staining (4+) in poorly differentiated ovarian carcinoma, endometrioid type (original magnification X200).

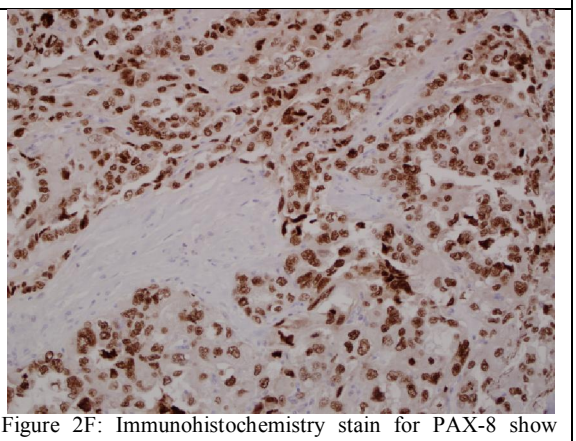

Figure 2F: Immunohistochemistry stain for PAX-8 show positive staining (4+) in ovarian carcinoma with poorly differentiated area, (original magnification X400).

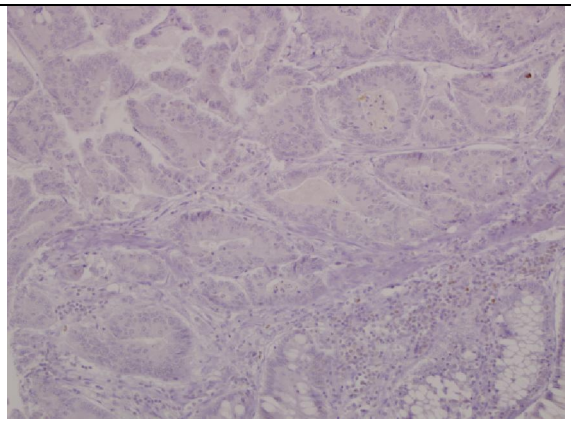

Figure 3A: Immunohistochemistry stain for PAX-8 show negative staining in well differentiated colonic carcinoma (original magnification X400).

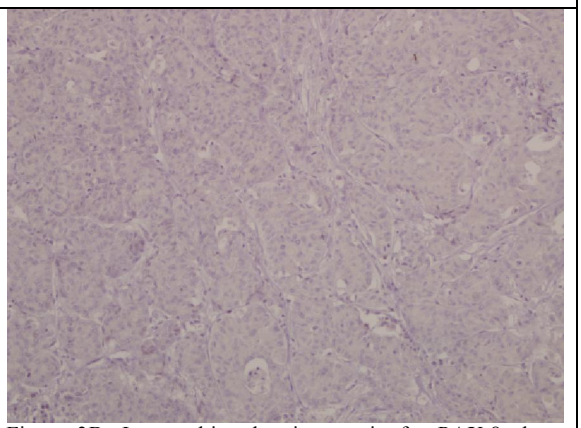

Figure 3B: Immunohistochemistry stain for PAX-8 show negative staining in poorly differentiated colonic carcinoma (original magnification X400).

4. Discussion:

PAX-8 is a transcription factor that belongs to the paired box gene family consisting of nine members (PAX1 through PAX9). They have a crucial role in the formation of tissues and organs during embryonic development (19;20). As a transcription factor, PAX-8 is known to control the development and organogenesis of the kidney, thyroid gland, central nervous system, organs deriving from the mesonephric (Wolffian) duct, and those related to the Mullerian duct (13;21-27).

Colorectal carcinoma and Gynecological malignancies (endometrial and ovarian) are considered among the most common malignancy in women. Distinguishing primary ovarian carcinoma, particularly endometrioid subtype, from metastatic colorectal carcinoma to the ovary is sometime difficult on histological examination alone. The differentiation in positive body fluid is also difficult area. Differentiating metastatic colorectal cancer to ovary, from primary ovarian tumor or from endometrial carcinoma metastatic to the ovary is an important issue. The appropriate tumor staging and management depend on clear determination of the primary and metastatic type of the neoplasm.

Few specific IHC markers have been identified to recognize the carcinomas of Mullerian origin. Markers such as CDX-2, CA-125, WT-1, ER, and PR are often used in combination with other IHC markers to determine the possible Mullerian origin and the primary type of the tumors, (28-30) but they have low sensitivity and specificity.

In this study, we found that PAX-8 is useful in the differentiation between gynecological (ovarian and endometrial) and colorectal carcinomas. The vast majority of ovarian and endometrial carcinomas were positive for PAX-8, while all colorectal primaries and lymph node metastases were negative. It is concluded that PAX-8 antibody is a useful tool in the distinction between colorectal and gynecological malignancies. It can be used for distinction in case of metastases where the routine histological appearance may be ambiguous. Our data is consistent with the recent data that support the utility of PAX-8 factor in determining Mullerian tumors (28-31). Our results are in keeping with the study of Ozcan *et al.* who demonstrated that PAX-8 immunoexpression was not detected in gastrointestinal tract adenocarcinoma 0/25 (0%) or metastatic tumor of colon cancer 0/39 (0%). They found that 90% of metastatic tumors of Mullerian origin were positive for PAX-8 (100, 100, 95, and 50% for endometrioid, clear cell, serous, and undifferentiated tumors, respectively) (32;33).

It has been demonstrated that PAX-8 plays an important role in the tumorigenic phenotype of ovarian cancer cells and identified PAX-8 as a potential new target for the treatment of ovarian cancer (34). McKnight et al concluded that PAX-8-positive, calretinin-negative staining appears to be highly specific and sensitive for detecting metastatic ovarian serous carcinoma in cytologic preparations and can be useful in distinguishing it from mesothelial cells in fluid cytology (35). Nonaka *et al.* found that PAX-8 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 types of nonmucinous ovarian carcinomas (17). Waters et al found that PAX-2 and PAX-8 are valuable diagnostic markers for metastatic Mullerian carcinomas and RCCs in effusion cytology (36).

In some study PAX-8 expression was found to be prognostic factor as well. Overexpression of PAX-8 protein by endometrial cancer was found to be associated with poor disease outcomes (37). It was suggested that inhibition of PAX-8 may be a very attractive targeted therapy for selective patients.

Tacha *et al.* found that PAX-8 expression in only 1% of lung cancer and in none of cancers of the colon, breast, prostate, liver, testicular, stomach, esophagus, melanoma, gastrointestinal stromal tumors, leiomyosarcoma, and pheochromocytoma. They also concluded that PAX-8 is a specific and sensitive marker for renal cell and ovarian carcinomas and should be a valuable addition to the histopathology laboratory (38).

PAX-8 has been identified as a potential immunohistochemical marker of pancreatic neuroendocrine tumors (39-41). It was reported that among well differentiated neuroendocrine tumors, only tumors from the pancreas were PAX8-positive (14/25, 56%) whereas no cases of pulmonary, ileal, duodenal, rectal or ovarian well differentiated neuroendocrine tumors were positive for PAX-8 (39).

Nonaka *et al.* found that PAX-8 would serve as a useful marker in differentiating carcinomas in the lung where primary lung (usually PAX-8 negative) and metastasis from the thyroid gland (usually PAX-8 positive). It can also help in the neck tumor where the differential diagnoses include primary thyroid carcinoma and metastatic carcinoma from the lung (3).

We concluded that PAX-8 is a useful marker in histopathology. It is helpful in differentiating ovarian and endometrial carcinoma from colorectal carcinomas. PAX-8 is a sensitive marker for diagnosing carcinomas of Mullerian origin and recommended to be added to the immunohistochemistry panel to identify metastasis of unknown primary or to support the diagnosis of primary Mullerian neoplasm against colorectal metastasis.

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