Original Article

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Pathology Section

Immunohistochemical Expression of Paired Box 2 (PAX2) in Normal Endometrium, Endometrial Hyperplasia, Endometrial Adenocarcinoma and its Association with Clinicopathological Parameters: A Cross-sectional Study

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ABSTRACT

Introduction: Endometrial Carcinoma (EC) is an increasingly problematic gynaecological cancer. Endometrial Hyperplasia (EH), particularly with atypia, is known to precede endometrial adenocarcinoma. Paired box 2 (PAX2) is a member of the paired box gene family that is involved in transcriptional regulation during embryogenesis and has been found to mutate early during endometrial carcinogenesis. Studies regarding PAX2 expression in endometrial lesions are limited.

Aim: To determine the expression of PAX2 in proliferative endometrium, non atypical hyperplasia, atypical EH, and EC, and its association with other clinicopathological parameters.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology at Ramaiah Medical College and Hospital, Bengaluru, Karnataka, India, on endometrial specimens received for routine histopathological evaluation from the Department of Obstetrics and Gynaecology (OBG) between March 2021 and October 2022. A total of 78 endometrial biopsy specimens and 30 total abdominal hysterectomy specimens were received. All specimens underwent Haematoxylin and Eosin staining (H&E) and Immunohistochemistry (IHC) with a PAX2 antibody. All H&E-stained slides were screened for histological type; in the case of carcinoma, slides were also screened for tumour grade, stage, myometrial invasion, angiolymphatic invasion, lymph node metastasis, and parametrial involvement. Data were analysed using Statistical Packages of Social Sciences (SPSS) version 22.0 (IBM SPSS Statistics, Somers, NY, USA) software. The Chi-square test, Fisher's-exact test, and Analysis of Variance (ANOVA) test were primarily applied. A p-value of <0.05 was considered statistically significant.

Results: A statistically significant difference was found between histological diagnosis and PAX2 intensity ($p<0.001$). During progression from normal to malignancy, PAX2 expression decreases in both distribution and intensity of staining. There was no association between PAX2 expression and clinicopathological parameters like tumour size, grade, angiolymphatic invasion, and lymph node metastasis, except for the stage of carcinoma ($p=0.04$).

Conclusion: PAX2 staining is lost with the progression of lesions to atypical hyperplasia and endometrioid adenocarcinoma. Follow-up studies correlating PAX2 expression with cancer-specific 5-year survival statistics need to be conducted to assess the prognostic value of PAX2 in EC.

Keywords: Endometrial biopsy, Myometrial invasion, Tumour suppressor gene

INTRODUCTION

The EC is the second most common and the fourth leading cause of death due to gynaecological cancer among women worldwide in 2020. According to Global Cancer Observatory (GLOBOCAN) cancer statistics, there were an estimated 417,367 EC cases and 97,370 deaths due to EC worldwide in 2020. EC is the sixth most frequently diagnosed cancer in the world in 2020 [1]. According to the International Agency for Research on Cancer, the frequency of EC cases is estimated to rise by more than 50% worldwide by 2040 [2]. EH is characterised by the proliferation of endometrial glands with an increased gland-to-stroma ratio.

Endometrial adenocarcinoma is preceded by atypical EH and hence has significant clinical implications. However, pathologists and clinicians have always faced challenges in distinguishing between innocuous EH and its harmful counterparts. An accurate diagnosis of precancerous conditions of the endometrium and the exclusion of co-existing EC are essential for the optimal management of patients.

The PAX (Paired Box) factors are a highly conserved family of transcription factors that include nine members, which play key roles in cell fate, early patterning, and organogenesis. PAX2 (Paired Box 2) belongs to this family, and its altered expression has been described as an essential driver of cancer initiation and progression [3]. The role of PAX proteins in carcinogenesis is not fully understood, but it has been shown that PAX genes are proficient of acting as proto-oncogenes by transactivating promoters of target genes involved in cellular transformation, cell growth regulation, self-sufficiency, and apoptosis. PAX2 is generally expressed during the development of mesenchymal and ductal components of the urogenital system [4]. Some researchers have found that PAX2 is expressed extensively in prostatic cancer and metastatic renal cancer. The proliferation of prostatic cancer cells is inhibited by the down-regulation of PAX2. PAX2 has been recognised as a strong marker for metastatic renal cell carcinoma owing to its higher immunoreactivity and strong nuclear immunoreactivity. In terms of downstream target genes of PAX2, only glial cell-derived neurotrophic factor, secreted frizzled-related protein 2 gene, and Wilms' tumour suppressor

gene have been reported [3]. Oestrogen and tamoxifen-induced hypomethylation of the PAX2 promoter has been demonstrated in endometrial carcinogenesis [5]. Aberrant overexpression of PAX2 in renal tumours, including Wilms' tumour, nephrogenic adenomas, and renal cell carcinomas, has been observed. More recently, PAX2 has been suggested as a potential therapeutic target gene in renal cancer [4].

Studies regarding the expression of PAX2 in endometrial epithelial cells in hyperplastic and malignant tissues are minimal [4,6-10]. The present study aimed to evaluate PAX2 expression in hyperplastic and malignant endometrial tissue in comparison to non pathological endometrial samples, which would help determine the utility of PAX2 as a marker for differentiating between these conditions.

MATERIALS AND METHODS

The cross-sectional study was conducted in the Department of Pathology at Ramaiah Medical College and Hospital, Bengaluru, Karnataka, India, on endometrial specimens received for routine histopathological evaluation from the Department of OBG between March 2021 and October 2022. Approval for the study was granted by the Ethical Committee of Ramaiah Medical College and Hospital (IEC No: MSRMC/EC/PG-04/01-2021, dated 25/01/2021). During the 19-month study period, 78 endometrial biopsy and 30 total abdominal hysterectomy specimens were received in the Department of Pathology.

Inclusion criteria: Endometrial biopsies and total hysterectomy specimens from patients aged 18 years and above, with a histopathological diagnosis of proliferative endometrium, non atypical EH, atypical EH, Endometrial Intraepithelial Neoplasia (EIN), or endometrial adenocarcinoma, were included in the study.

Exclusion criteria: Cases where the curettage specimen had scant endometrial tissue, deemed inadequate for evaluation and the cases where there was extensive tumour necrosis without sufficient viable tumour for accurate evaluation of histopathological features or PAX2 expression were excluded from the study.

Study Procedure

The endometrial biopsies and hysterectomy specimens were received in the Pathology Department in 10% formalin. After obtaining informed consent, detailed clinical histories and results of relevant investigations were collected from the patients' case files. In every case, the standard protocol for the surgical grossing of uterine specimens was followed. Tumour size was categorised as <2 cm and >2 cm [11]. After conventional processing and embedding in paraffin wax, sections of 5 µm thickness were cut and stained using H&E for histopathological study. In addition, 4 µm sections were cut from a paraffin block of endometrial/tumour tissue and placed on a glass slide coated with adhesive poly-L-lysine for IHC to evaluate PAX2 expression. The technique for IHC included antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with a primary mouse monoclonal antibody against PAX2 protein (BioGenex mouse monoclonal antibody PAX2, clone PAX2/1105), linking with a rabbit anti-mouse secondary antibody, enzyme labelling with streptavidin-horseradish peroxidase, developing the chromogen with Diaminobenzidine (DAB), and counterstaining with haematoxylin. Positive and negative controls were run with each batch of slides [12]. The H&E-stained slides were examined for endometrial/tumour histology, and the standard reporting protocol was followed in cases of malignancy.

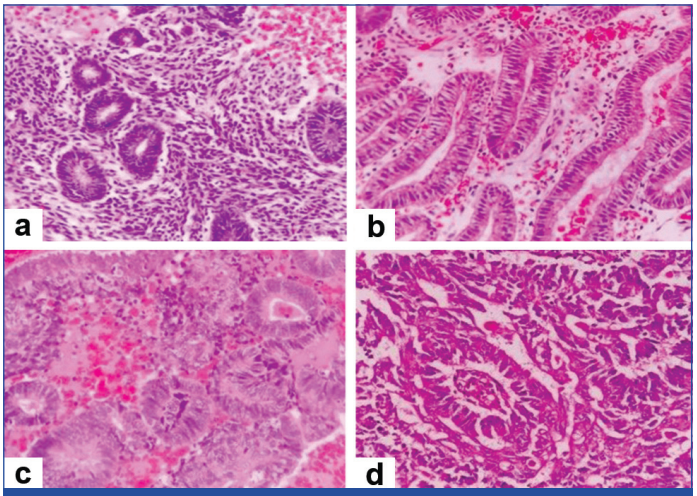
Evaluation of immunohistochemical staining: The immunostained slides were examined for nuclear staining with the anti-PAX2 antibody. The percentage of positive staining for PAX2 was recorded in the endometrial epithelial cells in each case and graded as follows: 0 for no staining; 1 for 1-11%; 2 for 12-33%; 3 for 34-66%; and 4 for 67-100% nuclear staining. The intensity of staining was graded as 0 for negative staining, 1 for weak, 2 for moderate, and 3 for strong staining [6]. The expression of PAX2 in endometrial adenocarcinoma was compared with clinicopathological parameters such as age, tumour size, grade, extent (myometrial invasion), angiolymphatic invasion, lymph node metastasis, parametrial involvement, and stage.

STATISTICAL ANALYSIS

All data were analysed using the Statistical Package for the Social Sciences (SPSS) software, version 22.0. Continuous data were represented as mean and standard deviation, while categorical data were represented in the form of frequencies and percentages. For tests of significance, Chi-square or Fisher's-exact test was used. ANOVA was used as a test of significance to identify the mean difference between more than two qualitative variables. A p-value of <0.05 was considered statistically significant.

RESULTS

Out of 78 biopsy specimens, 30 were histologically diagnosed as proliferative endometrium, 30 as non atypical hyperplasia, and 18 as atypical EH. Thirty hysterectomy specimens were diagnosed as endometrioid adenocarcinoma [Table/Fig-1]. The mean age of patients within the diagnostic categories was 47.6 years (range 37-63 years) for proliferative endometrium, 47.8 years (range 31-67 years) for non atypical EH, 45.2 years (range 29-56 years) for atypical EH, and 54.6 years (range 38-79) for endometrial adenocarcinoma [Table/Fig-2]. PAX2 was observed in the epithelial cells of the endometrial glands. A statistically significant difference was found between histological diagnosis and the extent of staining of the PAX2 antibody, with a p-value of <0.001 [Table/Fig-3]. Proliferative endometrium and non atypical EH exhibited a high proportion of cells with PAX2 staining. Loss of PAX2 expression was found in atypical EH and endometrioid adenocarcinoma. A statistically significant difference was also observed between histological diagnosis and the intensity of PAX2 antibody staining, with a p-value of <0.001 [Table/Fig-4]. Proliferative endometrium showed significantly stronger staining for PAX2 compared to endometrioid adenocarcinoma [Table/Fig-5]. No statistical significance was found between PAX2 expression levels and other clinicopathological parameters like age [Table/Fig-6], tumour size, grade, angiolymphatic



[Table/Fig-1]: Photomicrographs showing: a) Proliferative endometrium (H&E,40X); b) Non atypical endometrial hyperplasia (H&E,40X); c) Atypical endometrial hyperplasia, (H&E,40X); and d) Endometrioid adenocarcinoma (H&E,40X).

Diagnostic groups	Proliferative endometrium	Non atypical endometrial hyperplasia	Atypical endometrial hyperplasia	Endometrioid adenocarcinoma
N	30	30	18	30
Mean age in years (range)	47.6 (37-63)	47.8 (31-67)	45.2 (29-56)	54.6 (38-79)

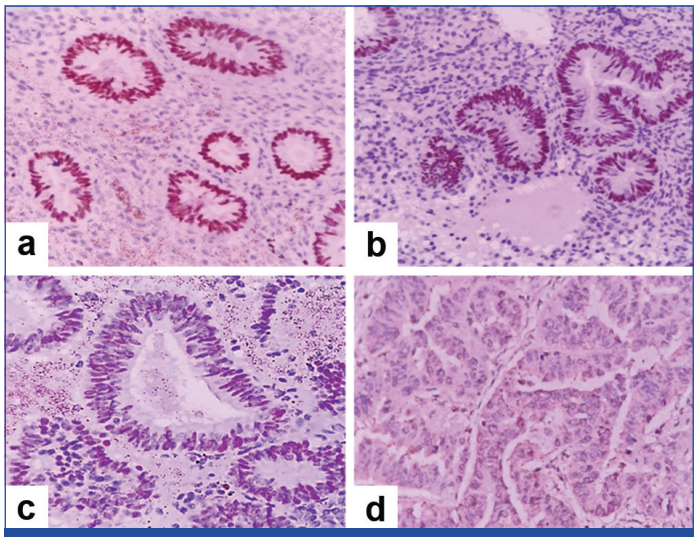
[Table/Fig-2]: Age distribution of proliferative, non atypical hyperplasia, atypical hyperplasia and adenocarcinoma cases.

Diagnostic groups	Grade 1		Grade 2		Grade 3		Grade 4		p-value
	N	%	N	%	N	%	N	%	
Proliferative endometrium	0	0	3	10.0	5	16.7	22	73.33	<0.001, statistically significant
Non atypical endometrial hyperplasia	1	3.3	4	13.3	6	20.0	19	63.3	
Atypical endometrial hyperplasia	8	44.4	6	33.3	3	16.7	1	5.6	
Endometrioid adenocarcinoma	28	93.3	2	6.7	0	0	0	0	

[Table/Fig-3]: Distribution of groups according to staining extent of the PAX2 antibody.

Diagnostic groups	Weak	Moderate	Strong	p-value
	n (%)	n (%)	n (%)	
Proliferative endometrium	1 (3.3)	10 (33.3)	19 (63.3)	<0.001, statistically significant
Non atypical endometrial hyperplasia	5 (16.7)	11 (36.7)	14 (46.7)	
Atypical endometrial hyperplasia	8 (44.4)	6 (33.3)	4 (22.2)	
Endometrioid adenocarcinoma	27 (90.0)	3 (10.0)	0	

[Table/Fig-4]: Distribution of groups according to staining intensity of the PAX2 antibody.



[Table/Fig-5]: PAX2 expressions in: a) Proliferative endometrium with strong nuclear expression (G3) (IHC,40X); b) Non atypical endometrial hyperplasia with strong nuclear expression (G3) (IHC,40X); c) Atypical endometrial hyperplasia with moderate expression (G2) (IHC,40X); and d) Endometrioid adenocarcinoma with mild expression (G1) (IHC,40X).

PAX2 staining intensity	Age (years) Mean±SD	p-value
Weak	50.90±10.751	0.234
Moderate	47.00±8.116	
Strong	49.14±8.8766	

[Table/Fig-6]: PAX2 staining intensity with respect to mean age.

invasion, lymph node metastasis, and myometrial invasion (p>0.05). There was a statistical significance was noted between PAX2 expression and tumour stage, with a p-value of 0.04. Due to the low sample size, no definite conclusion could be drawn. No statistical analysis was conducted between PAX2 expression and parametrial involvement, as all cases showed the parametrium to be free from tumour [Table/Fig-7].

Features		PAX2 staining intensity			p-value
		0-1	2	3	
Tumour size	<2 cm	2	0	-	1.00
	>2 cm	25	3	-	
Stage	1	23	1	-	0.04
	2	-	-	-	
	3	4	1	-	
	4	1	0	-	
Histological grade	G1	10	2	-	0.207
	G2	14	-	-	
	G3	3	1	-	
Myometrial invasion	<50%	21	2	-	1.00
	>50%	6	1	-	
Angiolymphatic invasion	Absent	22	2	-	0.501
	Present	5	1	-	
Lymph node metastasis	Absent	23	2	-	0.43
	Present	4	1	-	
Parametrial involvement	Absent	28	2	-	No statistic is computed
	Present	-	-	-	

[Table/Fig-7]: Distribution of PAX2 antibody staining intensities in relation to clinicopathological parameters.

DISCUSSION

The EH is characterised by an increased gland-to-stroma ratio and glandular architectural irregularity and complexity. Over the years, there have been several classifications of EH; however, the current one in use is the one put forward by the World Health Organisation (WHO) in 2014, which categorises EH as either without atypia or atypical hyperplasia/EIN [13]. EC mainly affects elderly women, specifically those in the postmenopausal age group, and its incidence rate varies worldwide, being highest in western populations [14].

The PAX2 is associated with transcriptional regulation during embryogenesis [7]. While the role of PAX2 in the development of Müllerian-derived organs is well known, there are few studies that evaluate its expression in various pathological processes. Initial reports established its expression in the fallopian tubes, ovaries, endometrium, cervix, and in malignancies of the genital tract. In proliferating and regenerating epithelial cells of the endometrium, PAX2 behaves as a tumour suppressor [15]. During development, PAX2 assists in the formation of the mid-hindbrain and regulates the fate determination of precursor neurons. Its expression is retained in mature cells [16].

The presence of PAX2 identifies the entire population of Gamma-Aminobutyric Acid (GABA)ergic interneurons in the deep cerebellar nuclei and cerebellar cortex [17]. In prostate cancer cells, PAX2 overexpression promotes the development of a metastatic state by upregulating the expression of cell membrane proteins [18].

In a study by Kahraman K et al., proliferative endometrium showed statistically significantly stronger staining for PAX2 when compared with endometrioid adenocarcinoma (p<0.001). Loss of PAX2 expression was detected in precursors and carcinoma. PAX2 expression in endometrioid carcinoma was similar to that in atypical hyperplasia (p>0.05) [4]. The present study also demonstrated a significant statistical difference between histological diagnosis and PAX2 expression (p<0.001).

Allison KH et al., reported that complete loss of PAX2 increased with the increasing severity of hyperplasia. Partial loss of PAX2 expression was observed in normal endometrium (17.9%); however, this occurred in small proportions of tissue and was less frequent than in simple hyperplasia (47.8%) (p=0.001), complex hyperplasia (32.5%) (p<0.01), atypical hyperplasia (22.2%) (p<0.01), and FIGO grade 1 carcinomas (20.0%) (p<0.01) [7]. A study by Madakshira MG and Ranjan P reported that proliferative endometrium retained expression of PAX2, while complete loss of PAX2 was found in endometrioid adenocarcinoma [8]. In the study by Bedi D et al., the average percentage loss of PAX2 was 23.92±15.24% in EH and 69.71±17.50% in cases of EIN, with results found to be highly

significant (p<0.001) [9]. A study by Rewcastle E et al., showed a progressive decrease in PAX2 expression from proliferative endometrium to EIN to endometrioid adenocarcinoma [10]. Similarly, in the present study, proliferative endometrium and non atypical hyperplasia showed a high proportion of cells with PAX2 staining (p<0.001). Loss of PAX2 expression was found in atypical hyperplasia and endometrioid adenocarcinoma.

In the study by Kahraman K et al., the mean percentage of PAX2-staining cells was 80.8%, 88.6%, 92.7%, and 99.2% for proliferative endometrium, complex hyperplasia, complex atypical hyperplasia, and adenocarcinoma, respectively, which is contrary to the findings of our study [4]. The present study indicated that the loss of PAX2 expression is associated with progression from normal endometrium to non atypical hyperplasia, to atypical hyperplasia, and finally to endometrial adenocarcinoma.

Kahraman K et al., found no association between PAX2 expression levels and the stage, histological grade, myometrial invasion, or lymph node status in cancer cases [4,6]. In the present study, no statistical significance was found between PAX2 expression and tumour size, histological grade, myometrial invasion, angiolymphatic invasion, or lymph node status. However, a statistical significance was found between tumour stage and PAX2 expression (p=0.04), in contrast to the findings of Kahraman K et al., (p=0.317) [4]. Nevertheless, due to the low sample size, no definite conclusion could be drawn.

Limitation(s)

The limitations of the study include a small sample size (30 cases of endometrial adenocarcinoma out of 108 total cases) and a short duration (19 months) during which all sample collection, histopathological reporting, IHC, data collection, and analysis were conducted. In some aspects, such as tumour stage, definitive conclusions could not be drawn due to the small sample size.

CONCLUSION(S)

In conclusion, authors suggested that the absence of PAX2 expression is associated with the progression from normal endometrium to hyperplasia to EC. Follow-up studies that correlate PAX2 expression with cancer-specific 5-year survival statistics need to be conducted for a definitive assessment of the prognostic value of PAX2 in EC.

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REFERENCES

[1] Agarwal S, Melgandi W, Sonkar DR, Ansari FA, Arora S, Rathi AK, et al. Epidemiological characteristics of endometrial cancer patients treated at a tertiary health center in National Capital Territory of India. J Cancer Res Ther. 2023;19(2):452-56.
[2] Zhang S, Gong TT, Liu FH, Jiang YT, Sun H, Ma XX, et al. Global, Regional, and National Burden of Endometrial Cancer, 1990-2017: Results from the Global Burden of Disease Study, 2017. Front Oncol. 2019;9:1440.
[3] Wang J, Jia N, Lyv T, Wang C, Tao X, Wong K, et al. Paired box 2 promotes progression of endometrial cancer via regulating cell cycle pathway. J Cancer. 2018;9(20):3743-54.
[4] Kahraman K, Kiremitci S, Taskin S, Kankaya D, Sertcelik A, Ortac F. Expression pattern of PAX2 in hyperplastic and malignant endometrium. Arch Gynecol Obstet. 2012;286(1):173-78.
[5] Wu H, Chen Y, Liang J, Shi B, Wu G, Zhang Y, et al. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. Nature. 2005;438(7070):981-87.
[6] Karakuş E, Erdem Ö, Taşkıran Ç, Onan A, Karakuş R. PTEN, PAX2 and PINCH protein expression in normal endometrium, endometrial hyperplasia, and endometrioid adenocarcinomas. J Clin Anal Med. 2018;9(2):133-37.
[7] Allison KH, Upson K, Reed SD, Jordan CD, Newton KM, Doherty J, et al. PAX2 loss by immunohistochemistry occurs early and often in endometrial hyperplasia. Int J Gynecol Pathol. 2012;31(2):151-59.
[8] Madakshira MG, Ranjan P. Expression of PAX2 and PTEN in oestrogen-driven endometrial hyperplasia and neoplasia. Indian J Gynecol Oncolog. 2019;17(73):01-10.
[9] Bedi D, Kataria AS, Sharma S, Verma V, Paul S. Evaluation of loss of Paired Box 2 gene expression in endometrial hyperplasia for detecting endometrial intraepithelial neoplasia. National Journal of Laboratory Medicine. 2018;7(3):23-28.
[10] Rewcastle E, Varhaugvik AE, Gudlaugsson E, Steinbakk A, Skaland I, van Diermen B, et al. Assessing the prognostic value of PAX2 and PTEN in endometrial carcinogenesis. Endocr Relat Cancer. 2018;25(12):981-91.
[11] Jin X, Shen C, Yang X, Yu Y, Wang J, Che X. Association of tumor size with myometrial invasion, lymphovascular space invasion, lymph node metastasis, and recurrence in endometrial cancer: A meta-analysis of 40 studies with 53,276 patients. Front Oncol. 2022;12:881850.
[12] Taylor CR, Shi SR, Barr NJ. Techniques of immunohistochemistry: Principles, Pitfalls and Standardization. In: Dabbs DJ. Diagnostic Immunohistochemistry: Theranostic and Genomic Applications. 3rd ed. Philadelphia: Saunders Elsevier. 2010. p01-36.
[13] Emons G, Beckmann MW, Schmidt D, Mallmann P; Uterus commission of the Gynecological Oncology Working Group (AGO). New WHO Classification of Endometrial Hyperplasias. Geburtshilfe Frauenheilkd. 2015;75(2):135-36.
[14] Purdie DM, Green AC. Epidemiology of endometrial cancer. Best Pract Res Clin Obstet Gynaecol. 2001;15(3):341-54.
[15] Fadhil ZA, Mirza SA, Ali SA. PAX2 Expression in Endometrial Intraepithelial Neoplasia. Indian Journal of Forensic Medicine and Toxicology. 2021;15(3):4410-18.
[16] Lv N, Wang Y, Zhao M, Dong L, Wei H. The role of PAX2 in neurodevelopment and disease. Neuropsychiatr Dis Treat. 2021;17:3559-67.
[17] Maricich SM, Herrup K. Pax-2 expression defines a subset of GABAergic interneurons and their precursors in the developing murine cerebellum. J Neurobiol. 1999;41(2):281-94.
[18] Ueda T, Ito S, Shiraishi T, Kulkarni P, Ueno A, Nakagawa H, et al. Hyper-expression of PAX2 in human metastatic prostate tumors and its role as a cancer promoter in an in vitro invasion model. Prostate. 2013;73(13):1403-12.

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