

## Original Article

# Expression and clinical significance of nuclear progesterone receptor subtype A and B in ovarian serous cystadenocarcinoma

Hong Zheng<sup>1</sup>, Yong-Sheng Huang<sup>1</sup>, Zheng Zheng<sup>2</sup>, Jia-Jia Huang<sup>1</sup>, Na Tan<sup>1</sup>, Kai Wang<sup>1</sup>

<sup>1</sup>Department of Pathology, The Affiliated Hospital of Zunyi Medical College, Zunyi 563003, China; <sup>2</sup>West China College of Stomatology, Sichuan University, Sichuan, China

Received October 22, 2015; Accepted January 16, 2016; Epub February 15, 2016; Published February 29, 2016

**Abstract:** Objective: To investigate the role of nuclear progesterone receptor subtype A and B in occurrence and development of ovarian serous cystadenocarcinoma (OSC). Methods: Immunohistochemistry Elivision method was used to detect PR-A and PR-B expression in 52 OSC subjects, 22 ovarian borderline serous cystadenoma (OBSC) subjects and 22 umbrellas of normal fallopian (UNF) subjects. Results: The positive rates of PR-A were 94.5%, 94.5% and 68.38% in OSC, OBSC and UNF, respectively, with significant difference ( $P < 0.05$ ) among the three groups. The positive rates of PR-B were 100%, 77.27% and 40.38% in OSC, OBSC and UNF, respectively, with significant difference ( $P < 0.05$ ) among the three groups. There was significant difference ( $P < 0.05$ ) among the ratio of PR-A/PR-B in three groups. Expression of PR-A and PR-B was reduced gradually. The differences were significant ( $P < 0.05$ ) for comparisons of stage I/II versus III/IV and grade I versus grade II. The difference was significant ( $P < 0.05$ ) for comparison of the PR-A/PR-B ratios in class I versus class II. The expression of PR-B in OSC with metastasis group and without metastasis group was significantly different ( $P < 0.05$ ). PR-A and PR-B expression was positively correlated in OSC tissues ( $P < 0.05$ ). Conclusion: Accompanied by OSC malignant transformation and development, expression of PR-A and PR-B was down-regulated, while the change of PR-B was more significant, which marked the malignant transformation of ovarian tissues. The increase of PR-A/PR-B ratio might predict poor ovarian cancer differentiation. The relative high expression of PR-B facilitated the inhibition of OSC lymph node metastasis.

**Keywords:** Ovarian cancer, PR-A, PR-B, immunohistochemistry

## Introduction

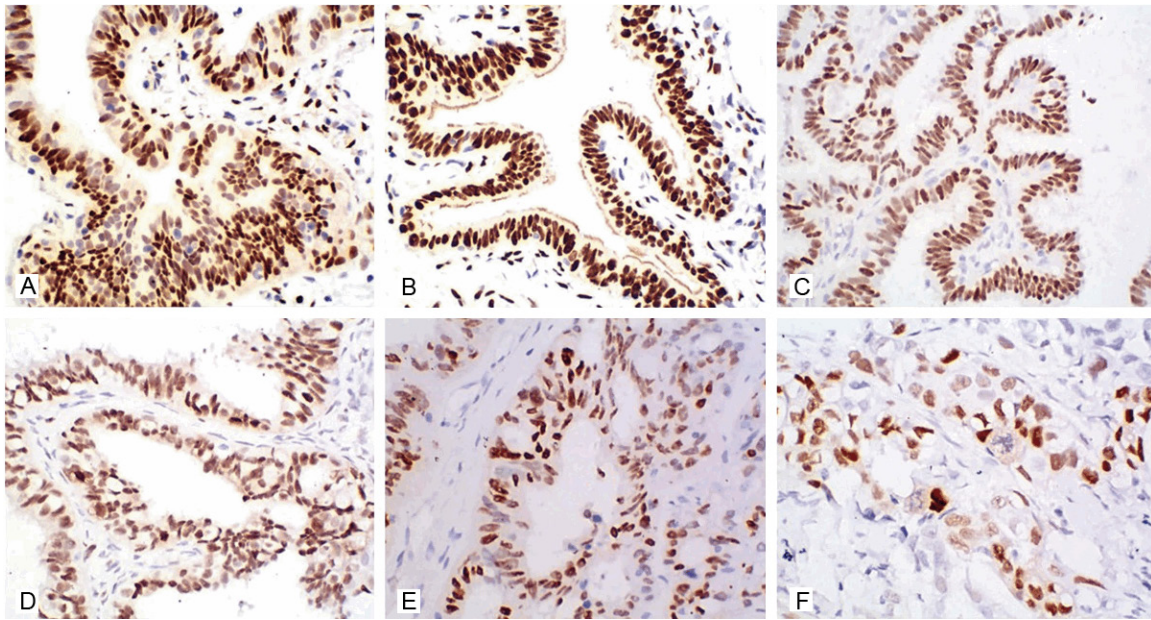
Ovarian cancer is one of the three common malignant tumors in female reproductive system. As the occurrence is vague, most clinical diagnoses are not confirmed until it progresses to late stage. With a five-year survival of approximately 30%, ovarian cancer is the most common lethal factor in gynecologic malignancies and severely jeopardizes female health. The incidence has been rising in recent years while the mechanism of pathogenesis remains unclear. Jaeyon et al. [1] demonstrated that ovarian high grade serous carcinoma was originated from fallopian tube interstitium by constructing mouse models. Study of Nik et al. [2] also considered that most ovarian high grade serous carcinoma was originated from fallopian epithelium but not from ovarian surface epithelia. It has been demonstrated that Cyclin E1 dysregulation drives the malignant transformation of fallopian secretary cells to the origin of ovar-

ian high grade serous carcinoma [3]. It has been reported that PR-A and PR-V play different regulatory roles in occurrence and development of breast cancers, affecting the biological behavior of breast cancer cells such as adherence, movement, growth and migration [4]. Therefore, receptor expression directly influences biological function of progesterone. This study detected the expression of PR-A and PR-B in umbrellas of normal fallopian (UNF), ovarian borderline serous cystadenoma (OBSC) and ovarian serous cystadenocarcinoma (OSC) and investigated the relationship with OSC clinical pathological characteristics.

## Materials and methods

### Sample sources

Fifty two OSC samples were collected from Department of Pathology in Zunyi Medical University Affiliated Hospital, which were diag-



**Figure 1.** A: PR-A protein positive in the cell nucleus of umbrella of normal fallopian tissue (EliVision method); B: PR-B protein positive in the cell nucleus of umbrella of normal fallopian tissue (EliVision method); C: PR-A protein positive in the cell nucleus of obsc tissue (EliVision method); D: PR-B protein positive in the cell nucleus of obsc tissue (EliVision method); E: PR-A Protein positive in the cell nucleus of OSC tissue (EliVision method); F: PR-B protein positive in the cell nucleus of OSC tissue (EliVision method).

nosed between January 2012 and December 2013. All samples were fixed using 10% neutral formalin, embedded in paraffin, HE stained and re-evaluated to confirm diagnosis. According to 2014 WHO classification criteria, there were 19 cases of grade I and 33 cases of grade II. Based on 1988 FIGO staging criteria, there were 3 cases of stage I, 13 cases of stage II, 28 cases of stage III and 8 cases of stage IV. 33 subjects underwent pelvic lymph node dissection, including 12 subjects with pelvic lymph node metastasis. Patients aged from 38 to 72 years, with a mean age of 55 years (23 subjects <50 years and 29 subjects ≥50 years). None of the patients experienced radiotherapy or chemotherapy prior to surgery. 22 tissue samples were randomly selected from OBSC and UNF group respectively, which were used as control group.

#### Reagents

Mouse anti-human PR-A and anti-human PR-B monoclonal antibodies were both purchased from Shanghai Lianshuo Co. EliVision kit, DAB chromogenic reagent and other reagents were all purchased from Fuzhou Maixin Co. The working concentration of PR-A and PR-B monoclonal antibodies was 1:200 and 1:100, respectively.

#### Methods

The documented OSC, OBSC and UNF tissues were embedded with paraffin, and sectioned continuously into slides with a thickness of 3 μm for HE staining and immunohistochemistry staining by EliVision method. All procedures were followed strictly with kit instruction. Slides were then stained with DAB, re-stained with hematoxylin, dehydrated, transparently sealed and observed under the microscope. The positive slide purchased from Shanghai Lianshuo Co. was used as positive control, while PBS was used to replace primary antibody in negative control. Tissue antigen was treated with high pressure repair.

#### Result analysis

The positive signal of PR-A and PR-B protein was both brown yellow or dark brown, located in the nucleus. The number of positive cells and chromogenic intensity were classified into three categories: number of positive cells <10% was weak positive (+), with chromogenic intensity of light yellow or only individual yellow to brown yellow cells; number of positive cells >60% was strong positive (+++), with most cells in yellow or brown yellow; positive cell number and intensity between the above two was (++).

## The expression clinical significance of cystadenocarcinoma

**Table 1.** Ratio of PR-A/PR-B and expression of PR-A and PR-B in UNF, OBSC and OSC tissues

Group	n	PR-A				PR-B				PR-A/PR-B		
		-	+	Positive ratio (%)	P value	-	+	Positive ratio (%)	P value	≤1	>1	P value
UNF	22	1	21	95.45	<0.05 <sup>&amp;</sup>	0	22	100	<0.05 <sup>&amp;Δ</sup>	15	7	<0.05 <sup>&amp;</sup>
OBSC	22	1	21	95.45	<0.05 <sup>*</sup>	5	17	77.27	<0.05 <sup>*</sup>	14	8	<0.05 <sup>*</sup>
OSC	52	18	34	35.28	<0.05 <sup>#</sup>	31	21	40.38	<0.05 <sup>#</sup>	19	33	<0.05 <sup>#</sup>

<sup>Δ</sup>Comparison between UNF and OBSC; <sup>\*</sup>Comparison between OBSC and OSC; <sup>#</sup>Comparison between UNF and OSC; <sup>&</sup>Comparison among the three groups.

**Table 2.** Relationship of PR-A/PR-B ratio, PR-A and PR-B protein expression and OSC clinical pathological characteristics

Pathological Characteristics	n	PR-A			PR-B			PR-A/PR-B		
		-	+	P value	-	+	P value	≤1	>1	P value
Surgical Staging I+II	16	2	14	<0.05	6	10	<0.05	3	13	
III+IV	36	16	20		25	11		16	20	
Pathological Grading I	19	3	16		6	13		3	16	
II	33	15	18	<0.05	25	8	<0.05	16	17	<0.05
Lymph Node Metastasis Present	12	5	7		9	3	<0.05	5	7	
Absent	21	5	16		7	14		8	13	

### Image analysis

The mean optical density (MOD) of image of slides was analyzed by Image-Pro Plus Version 6.0 (IPP 6.0), developed by US Media Cybernetics Co. PR-A/PR-B ratio was obtained by the MOD value of PR-A and PR-B expression, and classified into ≤1 and >1 two categories.

### Statistical analysis

All data were analyzed using SPSS 18.0 software. X table  $\chi^2$ -test, paired fourfold table  $\chi^2$ -test, Fisher exact test and Spearman correlation analysis were used for statistical analysis.

### Results

#### Expression of PR-A in OSC, OBSC and UNF tissues

In UNF, OBSC and OSC groups, progesterone nuclear receptor subtype PR-A and PR-B were mainly located in the nucleus, exhibiting as yellow or brown-yellow granules, while obvious staining was not observed in cell membrane or cytoplasm (**Figure 1A-F**). The positive ratio of PR-A was 94.5% (21/22) in both UNF and OBS groups, significantly higher than that in OSC group (68.38%, 34/52). The expression of PR-A was significantly different among three groups ( $\chi^2=14.899$ ,  $P<0.05$ ). There was no significant

difference for comparison of UNF versus OBC. The difference was significant for comparison of UNF versus OSC ( $P<0.05$ ) and OBSC versus OSC ( $P<0.05$ ).

#### PR-A/PR-B ratio and PR-B protein expression in OSC, OBSC and UNF tissues

The positive ratio of PR-B protein in UNF tissues was 100% (22/22), higher than that in OBSC group (77.27%, 17/22) and OSC group (40.38%, 21/52). The expression of PR-B was significantly different among three types of tissues ( $\chi^2=26.100$ ,  $P<0.05$ ). The differences was significant for comparison of UNF group versus OBSC group ( $P<0.05$ ), UNF group versus OSC group ( $P<0.05$ ) and OBSC versus OSC group ( $P<0.05$ ). In the three types of tissues, the numbers of PR-A/PR-B ≤1 and PR-A/PR-B >1 were significantly different ( $\chi^2=8.315$ ,  $P<0.05$ ). There was no significant difference for comparison of UNF group and OBSC group. The differences were significant for comparison of UNF versus OSC group ( $P<0.05$ ), and OBSC versus OSB group ( $P<0.05$ , **Table 1**).

#### Relationship of PR-A/PR-B ratio, PR-A and PR-B protein expression and OSC clinical pathological characteristics

PR-A and PR-B protein levels were decreased with the surgery stage of OSC elevated, with significant differences between I+II stage and



**Table 3.** Correlation of PR-A and PR-B expression in OSC tissues

PR-B	PR-A		<i>r</i> value	<i>P</i> value
	-	+		
-	15	3	0.332	<0.05
+	16	8		

III+IV stage ( $P<0.05$ ). There were no significant differences between the number of PR-A/PR-B ratio  $\leq 1$  and number of PR-A/PR-B ratio  $>1$  in two groups. The positive rates of PR-A and PR-B were gradually decreased as the histology grade of OSC increased and the differences were significant for comparison of grade I and grade II ( $P<0.05$ ). The number of PR-A/PR-B ratio  $\leq 1$  and number of PR-A/PR-B ratio  $>1$  in histology grade I were significantly different in histology grade I and grade II ( $P<0.05$ ). There was no significant difference in other inter-group comparison. The difference of PR-B expression in OSC with and without lymph node metastasis was significant ( $P<0.05$ ). The higher the PR-B protein expression rate, the lower the number of subjects with lymph node metastasis (Table 2).

#### *Correlation analysis of PR-A and PR-B expression in OSC tissues*

Expression of PR-A and PR-B in OSC tissues were positively correlated ( $\chi^2=6.432$ ,  $r=0.332$ ,  $P<0.05$ , Table 3).

#### **Discussion**

Progesterone nuclear receptor is a canonical type of nuclear receptor, mainly located in the nucleus and cytoplasm. Two main subtypes, A and B, are coded by the gene but with different start codon for translation [5]. Therefore, with different structures and functions, there is cell specificity in PR-A and PR-B mediated physiological functions. In recent years, some truncated progesterone receptor subtypes were found: PR-C, PR-M, PR-S, PR-T, etc. As a transcription factor, PR is able to bind to the hormone response element on the genome after binding with progesterone, and change the speed of gene transcription and translation, exerting a biological influence. The research of PR-A and PR-B mainly focuses on endometrial cancer and breast cancer, while their functions in ovarian cancer are less reported.

By analyzing breast cancer tissues, Junhui Huang et al. [6] showed that PR-A/PR-B ratio was positively correlated with axillary lymph node metastasis, while negatively correlated with disease free survival. The disease free survival of patients with PR-A/PR-B ratio  $>1$  was significantly lower than that of patients with PR-A/PR-B ratio  $\leq 1$ , suggesting the different roles of PR-A and PR-B subtypes in tumors. Patients with PR-A dominant expression were more prone to lymph node metastasis, with a poor prognosis. Dapeng Li et al. [7] also reported that the ratio of subjects only expressing PR-A or dominantly expressing PR-A was significantly increased, and that PR-B expression deficiency happened prior to PR-A. In endometrial cancer group, PR-A and PR-B protein expression were both negatively correlated with tumor differentiation. PR-A and PR-B co-express in normal tissues, but lose the co-expression in cancerous tissues. PR subtype expression deficiency at various degrees, imbalanced ratio, and especially PR-B protein expression deficiency reduced the anti-proliferation effect of progesterone and led to tumorigenesis. Zafran et al. [8] demonstrated that endocrine therapy achieved a better efficacy in endometrial cancer only expressing PR-B or dominantly expressing PR-B protein. Through multiple factor analysis, Miriam et al. [9] demonstrated that PR-B protein was the independent prognosis marker for ovarian cancer patients. All these results suggested a protective role of progesterone nuclear receptor PR-B on tissues.

Results of the present study showed that progesterone nuclear subtype PR-A and PR-B protein expression were both down-regulated in UNF, OBSC and OSC, with the down-regulation of PR-B more obvious, and the difference was significant. This suggested that with the occurrence and development of ovarian tissue malignant transformation, nuclear progesterone receptor subtype PR-A and PR-B were gradually lost, while PR-B was lost faster, decreasing the protective effect on ovarian tissues and facilitating tissue malignant transformation process. The ratio of PR-A/PR-B was significantly changed among the three groups. The ratio of patients with PR-A/PR-B ratio  $>1$  in OSC was significantly increased. The difference between OSC and the other groups was significant, suggesting that during the process of ovarian tis-

sue malignant transformation, the number of patients with ratio of PR-A/PR-B >1 significantly increased, which might be a predictive signal for ovarian tissue malignant transformation. It can be seen that the process of PR-A and PR-B proteins co-expression, expression deficiency and ratio imbalance, was just the process of tissue malignant transformation [6], consistent with the results of Junhui Huang et al. [6] and Dapang Li [7]. Comparing PR-A and PR-B protein with OSC clinical pathological characteristics, PR-A and PR-B expression decreased with increasing surgical stage and histology grade, with significant differences. With further development of ovarian cancer tissues, nuclear progesterone receptor PR-A and PR-B protein were gradually down-regulated, with PR-B down-regulated faster. The difference of PR-B protein in OSC with and without lymph node metastasis was significant: the higher PR-B protein relative expression rate, the lower possibility of lymph node in ovarian cancer, which was consistent with the results of Dapeng Li et al [7]. Study of Miriam et al. [9] demonstrated that PR-B protein was an independent prognosis signal for ovarian cancer patients through multiple factor analysis. This meant that as the protective receptor of ovarian cancer, gradual down-regulation of PR-B expression might be the factor leading to further development of cancerous tissues. The differences of PR-A/PR-B ratio in different OSC histology grades were significant. In more poorly differentiated ovarian cancer tissues, PR-A/PR-B ratio might be higher, or the number of patients with PR-A/PR-B ratio >1 might be higher, suggesting that PR-A/PR-B ratio might be the marker to indicate the extent of ovarian cancer differentiation.

With staging and differentiation of OSC, expression of nuclear progesterone receptor PR-A and PR-B protein was gradually down-regulated, and the ratio of PR-A/PR-B was increased. These results were caused not only by single PR-B protein deficiency. Actually during this process, PR-A and PR-B expression was positively correlated in OSC tissues. PR-A was down-regulated together with PR-B down-regulation, but the PR-B down-regulation was more obvious. It was this change that made cells with down-regulated PR-B or deficient in PR-B more invasive and reduced intercellular adhesion, facilitating tumor cells metastasis and invasion, and reducing the proliferation inhibition and apoptotic effects of progesterone on cancer cells. These results explained the two

sided effect and dose-dependent manner of progesterone on ovarian cancer, raised by Fauvet et al [10]. Low dosage of progesterone was able to stimulate cancer cell proliferation, while only high dosage of progesterone was able to exert the anti-cancer effect. Because of the dominant expression of PR-A in ovarian cancer, or only PR-A expression in certain tumors, low dosage of progesterone would bind with PR-A receptor first and stimulate proliferation. As dosage increased, remaining progesterone would bind with PR-B protein, inhibit tumor cell proliferation and promote apoptosis. Therefore, detection of PR-A and PR-B protein in ovarian cancer tissues and calculation of PR-A/PR-B ratio would better describe the degree of tumor malignance, and predict the efficacy of progesterone combination treatment, providing new insights for clinical therapy. However, so far there is no definitive conclusion whether it was PR-B reduced level that led to increased malignancy, or increased malignancy caused PR-B protein reduction, providing new thought for our subsequent experiments.

In summary, with the occurrence and development of OSC, PR-A and PR-B expression was gradually down-regulated. The down-regulation of PR-B expression was more obvious and might be the mark of ovarian tissue malignant transformation. Increased PR-A/PR-B ratio possibly predicted the poor differentiation of ovarian cancer. Relative high expression of PR-B facilitated the inhibition of OSC metastasis to lymph node.

## Acknowledgements

This work was supported by the Department of Science and Technology, Gui Zhou Province [2007] 2128.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Hong Zheng, Department of Pathology, The Affiliated Hospital of Zunyi Medical College, Zunyi City Dalian Road 149, Zunyi 563003, China. Tel: +86-18608520929; Fax: +86-18608520929; E-mail: zhhongdr@163.com

## References

- [1] Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM and Matzuk MM. High-grade serous ovarian cancer arises from fallopian tube in a

## The expression clinical significance of cystadenocarcinoma

- mouse model. *Proc Natl Acad Sci U S A* 2012; 109: 3921-3926.
- [2] Nik NN, Vang R, Shih IeM and Kurman RJ. Origin and pathogenesis of pelvic (ovarian, tubal, and primary peritoneal) serous carcinoma. *Annu Rev Pathol* 2014; 9: 27-45.
  - [3] Karst AM, Jones PM, Vena N, Ligon AH, Liu JF, Hirsch MS, Etemadmoghadam D, Bowtell DD and Drapkin R. Cyclin E1 deregulation occurs early in secretory cell transformation to promote formation of fallopian tube-derived high-grade serous ovarian cancers. *Cancer Res* 2014; 74: 1141-1152.
  - [4] Kariagina A, Aupperlee MD and Haslam SZ. Progesterone receptor isoforms and proliferation in the rat mammary gland during development. *Endocrinology* 2007; 148: 2723-2736.
  - [5] Boonyaratanakornkit V, Bi Y, Rudd M and Edwards DP. The role and mechanism of progesterone receptor activation of extra-nuclear signaling pathways in regulating gene transcription and cell cycle progression. *Steroids* 2008; 73: 922-928.
  - [6] Huang JH, Guo XH, Shen ZT, Jiang Q, He X and Zhang Y. Expression of progesterone isoforms in breast carcinomas and its significance. *Journal of Practical Oncology* 2008; 23: 8-12.
  - [7] Li DP, Li C and Sheng XG. Clinical significance of progesterone receptors A and B expressions in endometrial carcinoma. *Shandong Medical Journal* 2011; 51: 23-26.
  - [8] Zafran N, Levin A, Goldman S and Shalev E. Progesterone receptor's profile and the effect of the hormone and its derivatives on invasiveness and MMP2 secretion in endometrial carcinoma cell lines. *Harefuah* 2009; 148: 416-419, 477.
  - [9] Lenhard M, Tereza L, Heublein S, Ditsch N, Himsl I, Mayr D, Friese K and Jeschke U. Steroid hormone receptor expression in ovarian cancer: progesterone receptor B as prognostic marker for patient survival. *BMC Cancer* 2012; 12: 553.
  - [10] Fauvet R, Dufournet Etienne C, Poncelet C, Bringuier AF, Feldmann G and Daraï E. Effects of progesterone and anti-progestin ( mifepristone) treatment on proliferation and apoptosis of the human ovarian cancer cell line, OVCAR-3. *Oncol Rep* 2011; 15: 743-748.