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Expression and Clinico pathological correlation of Rab 25 Protein in Human Ovarian cancers.

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Abstract

Biomarkers have contributed to the management of ovarian cancers by monitoring response to treatment, recurrence distinguishing benign and malignant pelvic masses as well as attempting to detect disease at an earlier stage. In line with this perspective Rab25 protein is incorporates also as the biomarker of early detection of ovarian cancer however details reports are lacking in the pattern of its distribution in different subtypes of ovarian cancers. This was a retrospective study, of a raw data from Biomax .US with Identification Number**: OV2085:** including Ovary cancer survey tissue array (1 of 5), including TNM, clinical stage and pathology grade, 104 cases; cores. Where a total of 94 patients suffering from ovarian cancers were enrolled and we carry immunohistochemistry quantification for assessing the expression and distribution of Rab25 protein and clinicopathological parameters. Of note, 10 cases of normal ovarian tissues were used as control from healthy subject. Our results show that Rab25 is highly expressed in all type of ovarian cancer; we also conducted the assessment of the association of Rab25 protein with clinicopathological factors including age, staging, grading, and metastasis, which were analyzed using the Pearson's $\chi 2$ test. The results indicated that Rab25 protein expression was significantly associated with age, stage grading, and metastasis. Our reports showed that Rab25 protein expression and the prevalence of the ovarian cancers were cell type-and age-dependent. Rab 25 protein is abundantly expressed in the germ cell tumor, sex cord stroma, then less in the epithelial cells' tumor. Suggesting that Rab25 protein could be a novel diagnostic biomarker for the germ cells ovarian tumor.

Keywords: Ovarian cancer, Rab25, prevalence of ovarian cancer clinicopathologic parameters.

INTRODUCTION

Ovarian cancer (OC) is one among the occurring gynecological malignancy and the leading cause of death among the entire women worldwide [1]. Annually worldwide reported to have an estimated number of 239,000 of newly diagnosed cases and mortality was reported to have 152,000 number of cases [2]. Globally the overall 5-year survival ranges between 30%-40% and has seen relatively moderate for its increases since 1995 [3]. The incidence of OC manifests worldwide in a different geographical location [4. In the year 2015, [4] in USA the number of the new cases is reported to be 21,290 with the mortality case of 14,180. [5] There is an association between decreased survival rates and advancing stage of the diseases. According to the cohort study conducted in Hong Kong data , the results showed that a 5-year survival rate of OC was 90.2% stage I, 68.3% stage II, 32.96% stage III, and 16.1% Stage IV respectively [6]. Ovarian cancers are basically classified into three subdivisions: epithelial ovarian carcinoma, sex cord stromal, and germ cell tumors on the basis of the histogenesis and direction of their differentiation. Meanwhile, each of these three subtypes also includes a series of subtypes. Carcinoma of ovarian tissues is considered as a disease of heterogeneous group, which included primary invasive epithelial, borderline epithelial and non-epithelial ovarian neoplasm. [7] 'A number of genes and proteins have been reported to be involved in the pathogenesis of ovarian cancers. Of them, Ras-related protein

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25 (Rab25) is suggested to be linked to the increased risk of ovarian cancer development[8] Rab25, an intracellular transport protein, belongs to the Rab small GTPase family and regulates various aspects of internalized membrane protein recycling and trafficking within the cells to the plasma membrane. It is known that Rab25 is involved in promoting cell proliferation and preventing apoptosis and invasion in the ovarian cancer. Imbalance of Rab gene expression, especially Rab25, may induce the aggressiveness of human cancers, as observed in the ovarian and breast cancers, which is associated with the increased Rab 25 levels [8] Prostate tumor [9] , transitional cell carcinoma of the bladder [10], and invasive breast cancer cells [11] also provided a pathological role of Rab25 in the evolution of progression of neoplasm in the different kinds of epithelial lineages. However, the molecular mechanism by which Rab25 mediates its functions remains idiopathic.

Moreover, there was few data on Rab25 expression and sub-classification of the ovarian cancer. In this study, we investigated the pattern of distribution Rab25 in the different subtypes of the ovarian cancer. Many studies suggested that Rab25 is insinuated in the pathological process of ovarian cancer [12]. Few studies have investigated the expression of Rab25 in patients with epithelial ovarian cancers [8, 13, 14], while there is also lack of enough data on the remaining forms of ovarian.

MATERIALS AND METHODS

Research design

This was a retrospective study designed relaying on secondary data. The tissue microarray slides containing malignant and normal ovarian tissues where obtained from Biomax.US Inc. cancer tissue bank collection (US Biomax, ov2085) with Identification Number: OV2085: Ovary cancer survey tissue array (1 of 5), including TNM, clinical stage and pathology grade, 104 cases.

Study population

A total of 104 women were enrolled in this study, among them 10 cases served as the control group. 94 cases were diagnosed to have ovarian cancer which were categorized base on the subtype of cancer as follow: 65 cases as serous adenocarcinoma, 6 cases mucinous adenocarcinoma, 2 case endometroid adenocarcinoma, 1 case endodermal sinus carcinoma, 7 cases granulosa cell tumor, 5 cases clear cells carcinoma, 4 cases dysgerminoma, 2 cases malignant teratoma, 1 case Sertoli -leyding cell tumor and 1 case mixed germ cells tumor.

Immunohistochemistry

This was conduct according to the manufacture protocol VECTASTAIN ABC Systems. Briefly, by deparaffinize and dry array slide as referred to in protocol of deparaffinization. Rinse array slide twice with PBS for 5 min each. The endogenous peroxidase activity is blocked at room temperature by a 5~10 min incubation in the final 3% H 2O 2 in distilled water. Rinse array slide in PBS for 5 min. Then Antigen retrieval. Rinse array slide in PBS for 5 min. apply the blocking antibody, incubate for 20 min at room temperature, and throw off residual fluid. Apply the primary antibody anti human Rab25 monoclonal antibody 60 min at regular temperature (RT) or 4 ℃. Rinse array slide twice for 5 min each. Incubate array slide with a biotin-conjugated secondary antibody at 20~37°C for 20 min. Rinse array slide twice for 5min each. Incubate array slide with SABC reagent at 37°C for 20 min. Rinse array slide 4 times for 5 min each. Proceed with chromogen of final developmental DAB . Wash array slide in distilled water. Stain and differentiate array slide in hematoxylin. Dehydration and transparency of array slide. Mount array slides. Picture are taken the 200 X objectives.

Immunohistochemical quantification

2 images from serous, 6 from mucinous, 2 from endometroid, 6 from granulosa, 4 from clear cell 4 from dysgerminoma, and 2 from teratoma were analyzed; Rab25 protein expression was quantified as described in Soslow RA etal 2000 based on the combination of staining intensity of immunohistochemical images and the percentage of positive cells. Briefly, no staining is scored as 0, 1-10% of positive cells stained is scored as 1, 11-50% as 2, 51-81% as 3, 81- 100 as 4. Staining intensity is rated on the scale of 0 to 3, with 0= negative, 1= weak, 2= moderate,3= strong. The raw data were converted by multiplying the quantity and staining intensity score.

Statistical analysis

Data from the quantification of immunohistochemistry staining was analyzed using GraphPad Prism 6.05 for Windows statistical software (Graph-Pad Software, La Jolla California USA. Data were expressed as mean ± SEM and analyzed Statistical difference was determined by one-way ANOVA procedure followed by Student-Newman-Keuls post hoc test with 95% confidence. A level of $p < 0.05$ was accepted as statistically significant.

Ethical consideration

This study was approved by ethical committees of Huazhong University of sciences and Technology, Tondji Medical College Wuhan china.

RESULTS

Characteristics of study population

The clinico pathologic parameters of 91 cases of ovarian cancer are summarized in the table1. Age is strongly associated the ovarian cancer being higher in high age group..Among the different ovarian cancer subtype the serous subtype prevalence dominates followed by granulosa and mucinous types Fig1

Table 1: Ratio of different type of ovarian cancers (N= 94)

Type	Cases	Ratio	Metastasis cases	Cases in stage 1-4	Case in Grade 1-3
Serous adenocarcinoma	65	69.14%	12	49	44

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Mucinous adenocarcinoma	06	6.38%	00	06	05
Endometroid adenocarcinoma	02	2.13%	00	02	02
Embryonal sinus carcinoma	01	1.06%	00	01	00
Granulosa	07	7.45%	00	07	00
Clear cell	05	5.32%	00	5	00
Dysgerminoma	04	4.25%	00	4	00
Malignant teratoma	02	2.13%	00	2	00
Sertoli- lye dig cell tumor	01	1.06%	00	01	00
Mixed germ cell	01	1.06%	00	01	00
Interstitial cell	01	1.06%	00	01	01
Total of all subtypes	94	100%	94	75	50
Normal ovaries tissue	10	9.6%	00	00	00

Association of rab25 with variable pathological parameter

To further characterize our population of this study we categorized the subtype of ovarian cancer in association with positive staining and negative cells staining regarding Rab 25 expression. Our results showed that Rab25 is highly expressed statistically in all subtypes of cancer subtypes, but also there is a statistically significant association with the metastasis stage and grading, while age is associated significantly with Rab25 expression (Table 2 figure2).

Table2: Chi square test: Associations between Rab25 expression and clinico pathological variables**:** (N=94)

Variables	Total (n)	Rab25 staining		P value
		Mean Positives	Mean Negatives	
Age (years)				P<0.0001
>48	39	352	145	
25-47	32	366	125	
<25	4	350	135	
Histopathological type				
Serous adenocarcinoma	65	304	154	P<0.05
Mucinous Adenocarcinoma	6	105	280	P<0.0001
Endometroid	2	623	32	P<0.0001
Granulosa	7	400	482	P<0.0001
Clear cell carcinoma	5	330	81	P<0.0001
Dysgerminoma	4	487	117	P<0.0001
Malignant Teratoma	2	501	170	P<0.001
Cumulative average of ovarian cancers subtypes	91	401	192	
M stage				P<0.0001
Mo	88	312	162	
M1	6	404	107	
T stage				P<0.0001
T1,2	54	310	160	
T3,4	21	57	380	
Histological grade				P< 0.0001
G1,2	21	419	116	
G3	29	406	109	

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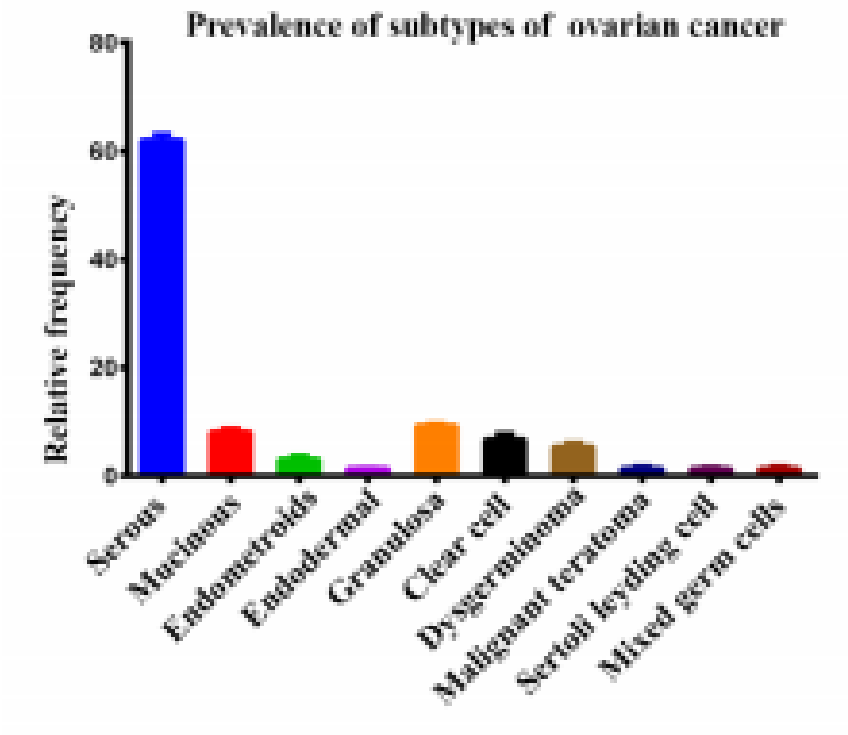

Figure1: Prevalence and distribution of subtypes of ovarian cancers

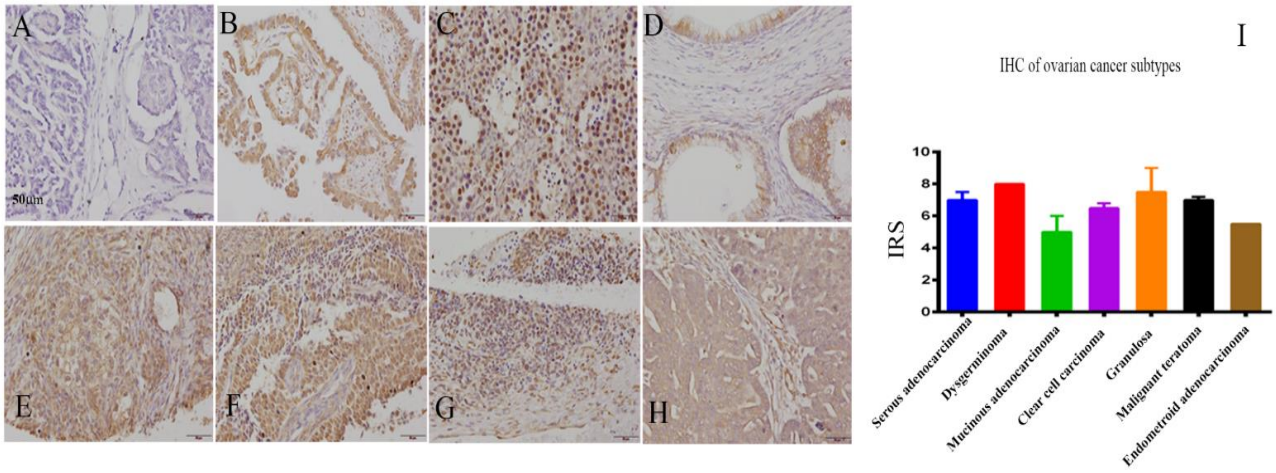

Figure 2: Immunohistochemistry staining and IRS Quantification of Rab25 in different subtypes of ovarian cancer: *Rab25 were highly abundantly in Germ cell ovarian tumors, dysgerminoma, and sex cord stromal, granulosa cell tumor. A= normal control; B= serous adenocarcinomas= dysgerminoma;D=mucinous adenocarcinoma;E= clear cells carcinomas; F= granulosa;G= malignant teratoma; H= endometroid adenocarcinoma*

DISCUSSION

Ovarian cancer is the seventh commonly diagnosed cancer among women in Worldwide, according to the World Cancer Report of 2014 the serous subtype adenocarcinoma is the most common. The incidence of OCs can occur since very young age, and increase with age after the age of 40 until reaching its peak at the age group of 55–59 years old. Similar results are observed in our current study. It has documented by many research reports that the most common histological type of ovarian cancer is serous carcinoma, followed by mucinous carcinoma and endometrioid carcinoma, similar pattern is observed in this study except for the endometroid which displayed a low prevalence. It was reported that pathogenesis of ovarian cancer has been linked to Rab 25, which is associated with increased risk of ovarian cancer development [8]by its implication in cell proliferation and preventing apoptosis and invasion in ovarian cancer [23]. Although some studies suggested that Rab25 mRNA is highly expressed in the ovarian cancers, detailed clinical studies in

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Rab25 protein level are limited [8, 15]. So far, only two studies have investigated the expression of Rab25 in patients with epithelial ovarian cancers[8, 13]. A very limited number of clinical studies have reported the distribution of Rab25 expression in the different classes of ovarian cancers. In line with other previously reported studies [13, 16]our data strongly showed that Rab25 protein is highly expressed in epithelial ovarian tumor, including serous, mucinous, endometroid, and clear cells (Fig.2 and Table 2). Similar results was also reported by Zhao.M et al [17]. Additionally in this study, Rab25 protein is also highly expressed in the granulosa and dysgerminoma cell type (Fig.2) and moderately expressed in the malignant teratoma which is in contrast to Sheach's study [13]. Sheach et al reported that the highest expression of Rab25 protein occurred in the endometrioid tumors, but our data showed opposite result in the endometroid cell type with less expression of Rab25 protein. However, we found higher expression of Rab25 in dysgerminoma, granulosa serous, and malignant teratoma respectively. Our data also showed an important Rab25 expression increase in the granulosa ovarian cancer (Fig.2), but opposite result was found in the study conducted by Zhao.M etal [17] where they reported no increase in Rab25 expression in ovarian granulosa cell tumor. Therefore they postulated that the action of Rab25 gene on the ovarian cancers may be cancer cell types specific.

To further characterize relationship between Rab 25 expression and ovarian cancer subtype, we investigated its association with variables clinicopathological parameters Since previous study have showed that BAX expression negatively correlates with RAB25 expression in ovarian cancer cells and Suppressing RAB25 by means of RNAi or RFP14 inhibitory hydrocarbon-stapled peptide sensitizes ovarian cancer cells to chemotherapy as well as RAB25-mediated proliferation, invasion and migration; suggesting that RAB25 is a potential therapeutic target for ovarian cancer[18]. Our result revealed that Rab25 protein in general is significantly associated with all mentioned clinicopathological parameters, including grading, stage metastasis, and age. However, a large sample study stipulated that Rab25 over-expression has no correlation with epithelial ovarian cancer concerning Staging as parameter [13]. But the other study also implied the same rule applied for the metastasis this is legitimated by the fact Rab25 mutation is not identified in any ovarian cancer [17, 19]while opposite result is obtained in this study. Therefore, it should interest to assess these parameters using large sample Also is age factor is strongly associated with ovarian cancers subtypes and this is in line with many previous. Reports

CONCLUSION

The present study clearly demonstrated that Rab25 protein expression is predominantly increased in the certain ovarian cancer subtypes including dysgerminoma, granulosa serous, and malignant teratoma, and highly associated with clinical factors including age, metastasis, stages, and grading. With the limitation of the sample size and our population in this study and lack of enough data for some subtypes of ovarian cancer, our data showed that Rab25 is not only highly expressed in epithelial ovarian cancers but also highly expressed in some of germ cell tumors. Furthermore, our data showed that Rab25 is involved in the pathogenesis of ovarian cancers on the basis of its pattern of distribution in this subtypes of ovarian cancer, which suggested that Rab25 could be a therapeutic target as well as biomarker for ovarian cancers.

DECLARATIONS

Ethical Approval and Consent to participate

The tissue microarray slides containing malignant and normal ovarian tissues (n = 94 Ovarian cancer and n = 10 as control) were obtained from US Biomax Inc. cancer tissue bank collection (US Biomax, ov2085). The Ethics Committee of the Huazhong University of Science and Technology; Tongji Medical college Wuhan china approved the study documents and the use of archived cancer tissues

Consent for publication

The Ethics Committee of the Huazhong University of Science and Technology; Tongji Medical college Wuhan china where this study has been conducted approved the study documents and the use of archived cancer tissues

Availability of data and materials

The availability of data and materials can be founds online at WWW.US Biomax, (ov2085). And attached under section supplementary materials of the research article

Competing interests

All authors declare no competing interests in both financial and non-financial in nature.

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Authors' contributions

Dr Zainab Daud; Prof Wumingfu; Dr M.T.M Salissou designed the research analyzed the data; and. wrote the paper.

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