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RESEARCH ARTICLE

Better Predictive Value of Cancer Antigen125 (CA125) as Biomarker in Ovary and Breast Tumors and its Correlation with the Histopathological Type/Grade of the Disease

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Abstract: ***Background*:** Both ovarian/breast cancers are the most prevalent hormone-associated gynecological-cancers, where uncontrolled cellular proliferations/genetic-errors are noticed. The cancer-antigen125 (CA125), which we assessed presently is an important biomarker in the early detection/monitoring of this disease-pathogenesis.

***Methods*:** Serum/tissue CA125 was measured by solid-phase-Enzyme-linked-immunosorbent-assay in women with ovarian/breast tumors of different types (epithelial/non-epithelial; benign/ borderline/ malignant)/stages. Breast-tumor tissues were employed for histoarchitecture/DNA-stability (comet) assay. Extensive protein-protein(CA125) interactions were studied by the STRING (Search-Tool-for-the-Retrieval-of-Interacting Genes/Proteins) Bioinformatics-software.

***Results*:** Serum CA125 levels were <35 U/ml in 94% of benign epithelial-cases, 35-65 U/ml in 100% of borderline-tumors and >100 U/ml in the 41.17% of patients with malignant-tumors. The malignant epithelial tumor showed significantly higher (>100U/ml) CA125 than the non-epithelial malignant-tumor (<35-65 U/ml). Highly enhanced cellularity/histo-architectural impairment/un-stable-DNA-materials/CA125 was found in advanced breast-cancers. The CA125 is highly metabolically interactive, especially with mesothelin impairing cell-cell adhesion and enhancing tissue anti-establishment.

***Conclusion*:** CA125 is a predictive-marker in ovarian/breast carcinoma depending on disease nature/stages. CA125, in combination with other tests like mesothelin/estradiol can be of great use in the better diagnosis of this disease. Not only as a detection-marker, has the CA125 played an interactive role in the disease processes. The Bioinformatics study revealed an important role of mesothelin and other mucin like proteins. Early detection and proper diagnosis are important determinants for the greater possibility of the relief from the disease like cancers. Selection of suitable biomarker combinations may increase the better diagnosis of the types and severity of this disease. Our present result has an impact on these aspects.

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INTRODUCTION

Ovarian cancer and the Breast cancer are the second most common and the most lethal gynecologic malignancy. The incidence of this cancer varies widely in frequency among different geographic regions and ethnic groups, with high incidences observed in Scandinavia, Western Europe and North America [1]. All cell types of the human ovary may undergo neoplastic transformation. The vast majority (80–90%) of the benign and malignant tumors are derived from the Ovarian Surface Epithelium (OSE) and its cystic derivatives [2]. Generally, CA125 is elevated in the serum of most of the women with ovarian cancer, but the pre-operative serum CA125 levels are below the conventional cut off of 35 U/ml in approximately 50% of the clinically detected stage I cases [3]. The estimated normal reference range is 0–35 U/ml, and its level increases in about 90% of

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women with advanced ovarian epithelial cancer, and in about 50% of patients in the initial stages, particularly in the cases of tumors of serous nature [4]. There have been reports of elevated soluble CA125 levels in a number of other malignant conditions such as breast cancer [5, 6]. The CA125 levels have also been found elevated in benign conditions [7, 8] such as endometriosis [9], pregnancy ovulatory cycles [10], liver diseases and congestive heart failure [11], and in infectious disease *i.e.*, tuberculosis [12].

CA125 is a mucin-type *O*-linked glycoprotein expressed as a membrane-bound protein on the surface of cells that undergo metaplastic differentiation [13] or released in soluble form in the body fluids. The CA125 gene was named as MUC16, after the mucin-like nature of CA125 protein, which is conserved through evolution [14]. This feature includes a high serine, threonine, and proline content in an N-terminal region of nine partially conserved tandem repeats (156 amino acids each) and a C-terminal region non-tandem repeat sequence containing a possible trans-membrane region and a potential tyrosine phosphorylation site [14, 15]. The attachment of the oligosaccharide to the CA125 suggests a role of this marker in cell-mediated immune response [16]. In vitro cytotoxicity inhibition study reveals that the CA125 acts as a suppressor of the anti-tumor immune response in the disease condition [17]. CA125 also binds to mesothelinn [18,19], a 40 kDa protein. The interaction between mesothelin and CA125 results in a peritoneal-implantation of ovarian tumor cells that constitutively expresses a membrane-bound form of mesothelin [20].

Estrogens have been reportedly suspected as a causative agent of various types of breast and ovarian cancer. Estrogen levels were at least 100-fold higher in the ovarian tissue than the circulating levels and much higher in the follicular fluid of ovulatory follicles [21]. Eventually, the ovarian surface epithelium is likely to be exposed to a high level of estrogens. Estrogen receptor (ER)-mediated responses for growth stimulation of normal and malignant OSE cells by estradiol-17β and estrone have been reported [22]. Estradiol and human chorionic gonadotropin (HCG) regulate cellular growth responses of ovarian cancer cells through the induction of insulin like growth factor (IGF-1) and epidermal growth factor (EGF) [23]. It is evident that estrogen as a stimulator of genes involved in the growth and development of cancer, along with its genotoxic nature [24]. Logically, in certain instances it can be speculated that estrogen-induced genomic regulation in ovarian surface epithelial cells can be accused for the transformation of normal cells to cancerous one. Peripheral estrogen formation and oxidative stress induction also play as promotional factors for the breast and ovarian cancer progression [25].

Epidemiological evidence indicates that prolonged lifetime exposure to estrogen is associated with an elevated breast cancer risk in women. Oxidative stress and estrogen receptor-associated proliferative changes are suggested to play important roles in estrogen-induced breast carcinogenesis [26, 27, 28]. Ninety percent of the ovarian cancer patients are carriers of mutated Breast Cancer Gene 1 (BRCA1) and/or BRCA2 genes, which are also implicated in hereditary breast cancer [29]. Increased CA125 was also found in breast cancer patients and that was associated with the metastasis in or near the pleura [30]. As noticed in the present study, CA125 levels were also elevated in breast cancer patients with lung metastasis [31]. CA125 was significantly higher in serum and nipple discharge of cancer patients than that of the control group. It gradually increased with disease severity and was much higher in recurrent groups [32, 33]. Malignancies other than ovarian carcinomas are associated with elevated serum levels of CA125 as well as several inflammatory conditions and its markers. An immunohistochemistry study showed the presence of CA125 in all normal tissue samples and was located on apical surfaces, ductal contents, and cytoplasmic granules, and on the membrane in a significant number of breast carcinoma cases [34].

Our aim of this study was to study the level of CA125 in different types of ovarian cancer and in different grades of breast cancers. We were intended to find out the clinical efficiency of CA125 in early diagnosis of ovarian and breast carcinoma.

MATERIALS AND METHODS

Ovarian and Breast Cancer Sample

The current study is a hospital based cross-sectional study. The study was carried out with a sample size of sixty ovarian cancers at the Department of Gynecology and Obstetrics, Burdwan Medical College, a tertiary health care center with a proper ethical clearance. The breast tumor samples were obtained from the Midnapore Medical College and Hospital and the studies were carried out in this hospital and in the Cell and Molecular Therapeutics Laboratory, Oriental Institute of Science and technology after the completion of proper regulatory affairs and permission. Both ovarian and breast tumors were suspected clinically, sent for radiological confirmation and the blood samples were taken for CA125 estimation. After operation ovarian specimens were sent for histological typing and grading in the department of pathology. Breast cancers were classified on the basis of TNM (Tumor, Nodes and Metastases) staging. Samples were collected and preserved at -20 ℃ by the registered health professionals, clinicians and surgeons.

Inclusion and Exclusion Criteria

Blood samples included or used for CA125 assay was collected from those patients who have been radiologically diagnosed to have a tumor and have not received any chemotherapy prior to blood collection (except couple of cases of breast cancer). Patients suffering from endometriosis, pelvic inflammatory diseases, tuberculosis, liver diseases, endometrial carcinoma, pancreas carcinoma, colon carcinoma, lung carcinoma, pregnancy, menstruation were excluded.

Sample Processing and Cytosol Preparation

Serum was separated from the collected blood samples of all cancer patients. A small amount of breast tumor tissues (~150 mg) and same amount of non-tumor tissues (control) from the surrounding regions were collected from the corresponding patients and homogenized in ice-cold phosphate buffered saline (pH 7.4). The homogenate was

centrifuged at 10000 x g for 30 minutes in a cold centrifuge. The supernatant was preserved in aliquots at -20 °C for CA125 assay.

CA125 Assay

CA125 in the serum was measured by ELISA method. The ELISA kit used was based on the principle of a solid phase enzyme-linked immunosorbent assay (KIT- reference). Absorbance was measured spectrophotometrically at 450 nm.

Histoarchitechture Studies of Cancerous Tissues

Cancerous growth in breast tissue and its surrounding tissues were embedded in paraffin, serially sectioned at 5 μM by an automated cryostat slicing machine (Leica Biosystems), stained with eosin and hematoxylin (Harris), and observed under a microscope (Nikon, Eclipse LV100, magnification 20X) to study the tissue histoarchitecture.

Comet Assay

The alkaline Comet assay was performed according to the Singh and colleagues' method with some minor modifications [35]. A total of 75µl of low melting point agarose (0.6%) in PBS at 37 °C was added to a 25µl of cell suspension (10^5 cells). The mixture was then dropped onto a microscope slide precoated with 1% agarose, and a coverslip was placed on top. After the agarose had solidified the coverslips were removed and the slides were immersed in ice cold lysis buffer (2.5 mM NaCl, 85 mM EDTA, 10 mM Trizma base, 1% Triton X-100, 10% DMSO and 1% sodium lauryl sarcosinate, adjusted to pH 10) for 1 hour at 4 °C. After lysis the slides were washed three times in PBS at room temperature. Next, 50 ml of buffer (control) or T4 endo V (Epicentre) (4 U/slide) in buffer was transferred to the slides. Coverslips were put on and the slides were incubated at 37◦C for 45 min. The coverslips were then removed and the slides were washed in water twice more to remove any excess salt. Slides were then placed in a submarine gel electrophoresis chamber (Bio-Rad, USA) filled with alkaline electrophoresis buffer (0.3 M NaOH and 1 mM EDTA) for 25 min. Following this incubation, electrophoresis was performed for 30 min at 25 V and the current was adjusted to 300 mA by raising the buffer level. Slides were then neutralized with PBS and stained with a solution of 10 mg/ml ethidium bromide for 5 min. Excess stain was removed by washing in water. Slides were read using a fluorescence microscope (Nikon, Eclipse LV100 POL), with the VisComet (Impuls Bildanalyse) software. A total of 100 comets/ slide were read for each experiment.

Possible Interactions of CA125 with Other Proteins

STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*) is a biological database and web resource of known and predicted protein-protein interactions [36, 37]. String is a precomputed database derived from experimental data, literature mining, analysis of coexpressed genes *etc.* String applies a unique scoring method based on the different types of associations against a common reference set and produces a single confidence score per prediction [38]. Evidence based interactive or interrelated pattern has been deduced by this online resource/analytical software (http://string-db.org/)

Statistical Analysis

The statistical analyses were done by using the SPSS for Windows statistical software package (SPSS Inc., Chicago, IL, USA, 2010). Normally distributed data were tested by Kolmogorov-Smirnov test. Baseline variables and outcome measures are compared with the Students t' test for continuous variables and the chi-square test for categorical variables.

RESULTS

Among the 60 selected ovarian tumor patients, 49 were in premenopausal age and 11 were in the postmenopausal age group. Histopathological examination confirmed that 37 (61.6%) cases were benign ovarian tumor, 6 (10%) cases were borderline ovarian tumor and 17 (28.3 %) cases were malignant ovarian tumor.

CA125 Levels in Benign, Borderline and Malignant Ovarian Tumor

In the present study, majority of patients with benign tumor (35/37) 94.59% have their serum CA125 value <35 U/ml and the majority of patients with borderline & malignant tumors (18/23) 78.2% have their serum CA125 value >35 U/ml (χ^2 = 59.837, p<0.001). Again, (7/17) 41.17% of patients with malignant tumor have their serum CA125 value > 100 U/ml. 100% of patient with borderline tumors have their serum CA125 value in between 35-65 U/ml (χ^2 = 12.64, p<0.001) (Fig. **1a**). CA125 levels are presented in different grades of the disease (χ^2 = 16.913; p<0.01). Irrespective to the CA125 value the distribution of patients in different disease grade is noticed to be significant (χ^2 = 11.12; p<0.001) (Fig. **1b**).

CA125 Levels in Epithelial and Non-Epithelial Types of Benign and Malignant Ovarian Tumors

Benign epithelial and non epithelial tumor: 94.44% of Benign epithelial tumor had their serum CA125 values <35U/ml, whereas 5.55% had >35U/ml but below 65U/ml. 94.73% Benign non epithelial tumor had their serum CA125 values <35U/ml, whereas only 5.2% of them had CA125 values >35 but below 65 U/ml. (Fig **1c**). CA125 levels present in a number of patients which are found to be significantly different (χ^2 = 14.18, p<0.01) between borderline/malignant epithelial tumor and borderline/malignant nonepithelial tumor (Fig. **1d**).

In FIGO Staging and CA 125 Levels

While considering the FIGO staging of all borderline & malignant ovarian tumor in association with levels of serum CA125, the study revealed (3/23), 13.04 % cases of stage I tumor and (3/23) 13.04% cases of stage III tumor had their serum CA125 level >100U/ml. And (5/23) 21.7%, (8/23) 34.7% & (2/23) 8.69 % cases of stage I had their serum CA125 values <35 U/ml, between 35-65U/ml & between 66-100U/ml respectively. There is no statistically significant difference in serum CA125 level in ascending stage of any histopathological type of ovarian tumors.

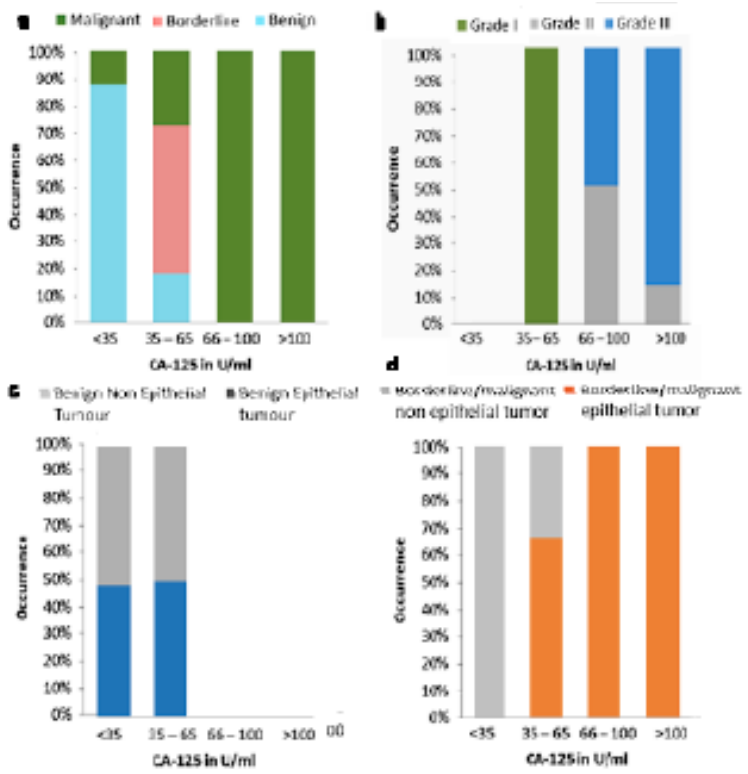

Fig. (1). a. CA125 levels are presented in benign, borderline and malignant ovarian tumor. This distribution pattern is found to be highly significant (χ^2 = 59.837, p<0.001). Irrespective to the CA125 value the distribution of patients with benign and borderline/malignant tumor is highly significant (χ^2 = 12.64, p<0.001).
b. CA125 levels are presented in different grades of the disease (χ^2 = 16.913; p<0.01). Irrespective to the CA125 value the distribution of patients in different disease grade is noticed to be significant (χ^2 = 11.12; p<0.001).
c. CA125 levels are presented in epithelial and non epithelial types of benign ovarian tumors.
d. CA125 levels are presented in number of patients with borderline/malignant epithelial tumor versus borderline/malignant nonepithelial tumor (χ^2 = 14.18, p<0.01).

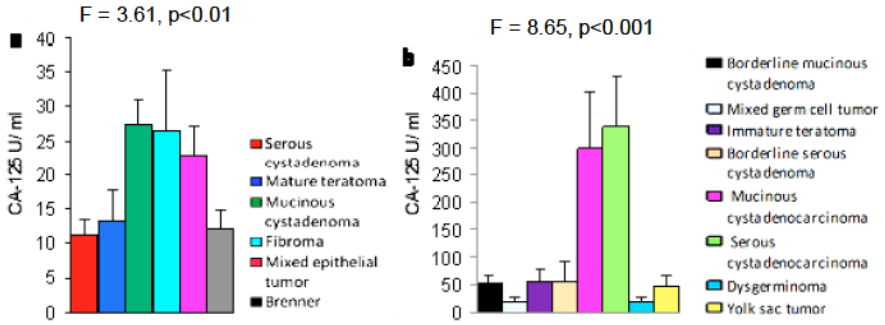

Fig. (2). a. Mean CA125 levels of histopathological types of benign tumor (multiple comparison ANOVA, F = 3.61, p<0.01).
b. Mean CA125 levels of histopathological types of borderline/ malignant tumor (multiple comparison ANOVA, F = 8.65, p<0.001).

CA125 Values in Different Histopathological Types of Ovarian Tumor

CA125 was also measurued in different histopathological types of Benign, borderline and malignant tumor, all the benign types of ovarian tumor had there serum CA125 values <35 U/ml. CA125 value in benign serous cystadenoma was 11.127 U/ml which rises to 54.075 U/ml in borderline serous cystadenoma and was enormously high in Malignant serous cystadenocarcinoma, 339.103 U/ml. In the same way CA125 value in benign mucinous cystadenoma was 27.48 U/ml which rises to 53.81 U/ml in borderline mucinous cystadenoma and further increased to 299.773 U/ml in malignant mucinous cystadenocarcinoma. CA125 value in benign mature teratoma was 13.139 U/ml and was 56.145 U/ml in advanced immature teratoma. The serum CA125 value was 26.65 U/ml in benign fibroma tumor and 22.83 U/ml in benign type of mixed epithelial tumor and was 12.22 U/ml in benign Brenner. Individuals with malignant Yolk sac tumor had there serum CA125 values 47.15 U/ml and malignant tumors mixed germ cell and Dysgerminoma had CA125 values as 18.41 and 19.225 U/ml which is less than the normal cutoff of <35 U/ml (Fig. **2a,2b**).

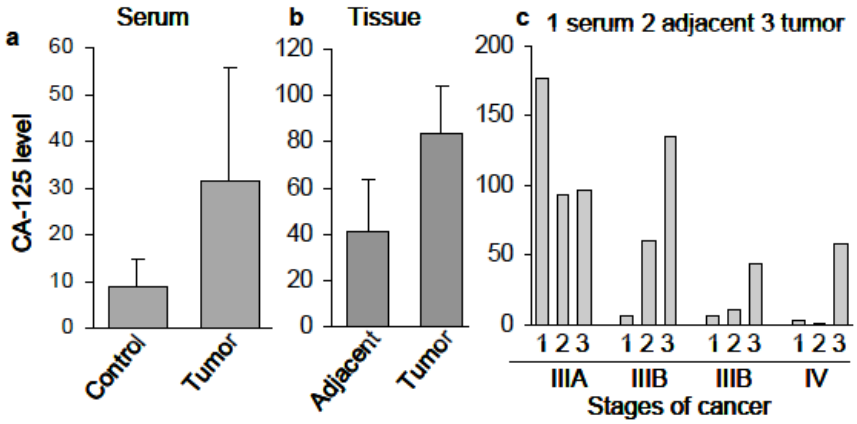

Fig. (3). CA125 levels are presented in 10 breast cancer patients. Serum values are compared with the control values. Though the percentage of the differences found to be high in these groups no significant changes were noticed due to the higher inter-individual variability (a). CA125 levels from the tumor tissues are compared with the adjacent control tissues from the corresponding tissues (b). Comparisons have been made within CA125 levels of serum, adjacent or tumor tissues from different individuals (c).

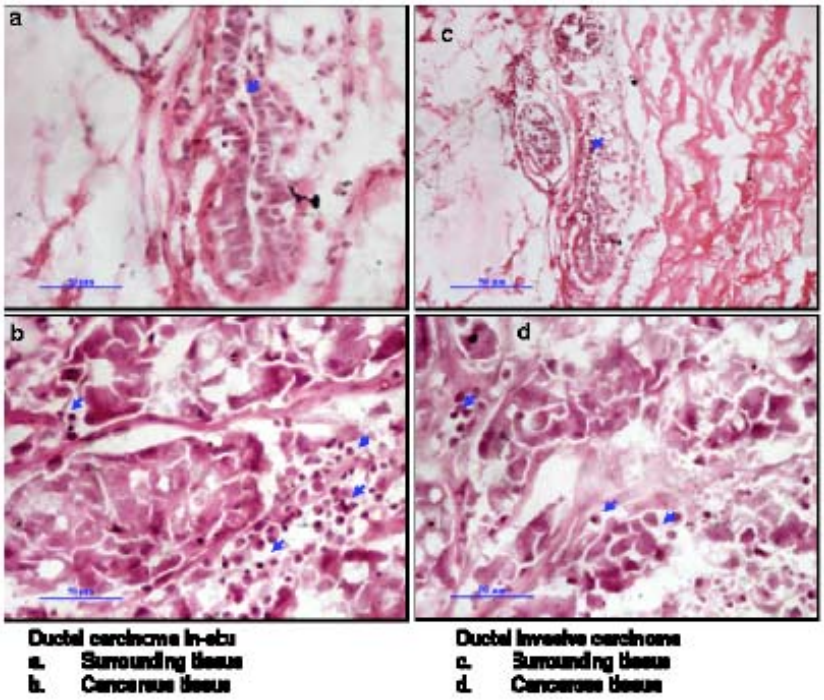

Fig. (4). Histological studies of Ductal Breast Carcinoma of different stages grade III. The snapshot of a and c are the histoarchitecture of the surrounding tissues of the corresponding carcinomas growth b and d respectively. A representative picture is shown.

CA125 Levels in Breast Cancer

In case of breast cancer, the average Serum CA125 levels was 9 U/ml in control women and 31.42 U/ml in breast cancer patients of two different grades, IIIA and IIIB of disease outcome (Fig. **3a**). The average CA125 value was 83.5 U/ml in breast tumor tissue and 41.58 U/ml in the corresponding surrounding tissue (Fig. **3b**). CA125 levels in the tumor tissue of stage IIIB (undergone no chemotherapy) was 136U/ml and in its corresponding surrounding tissue it was 61U/ml, whereas the CA125 level in another IIIB tumor (undergone 2 cycles of chemotherapy) was 4U/ml and in its corresponding surrounding it was 10.35 U/ml. CA125 levels in the tumor tissue of stage IV (undergone several cycles of chemotherapy) was 58U/ml and in its corresponding surrounding tissue it was 1U/ml. The CA125 value was 96U/ml in the tumor tissue of cancer stage IIIA (no chemotherapy) and in its corresponding surrounding tissue it was 94U/ml (Fig. **3c**).

Histopathology Outcome

Histological studies of ductal breast carcinoma of different stages grade III are presented. The ductal carcinoma in-situ and the ductal carcinoma (invasive) are shown and compared with the histoarchitecture of the corresponding surrounding control tissues. The fibrocystic changes are evident from the pictures. Normal duct channels, blood vessels and lymphatic channels are disorganized in the invasive carcinomas (Fig. **4**).

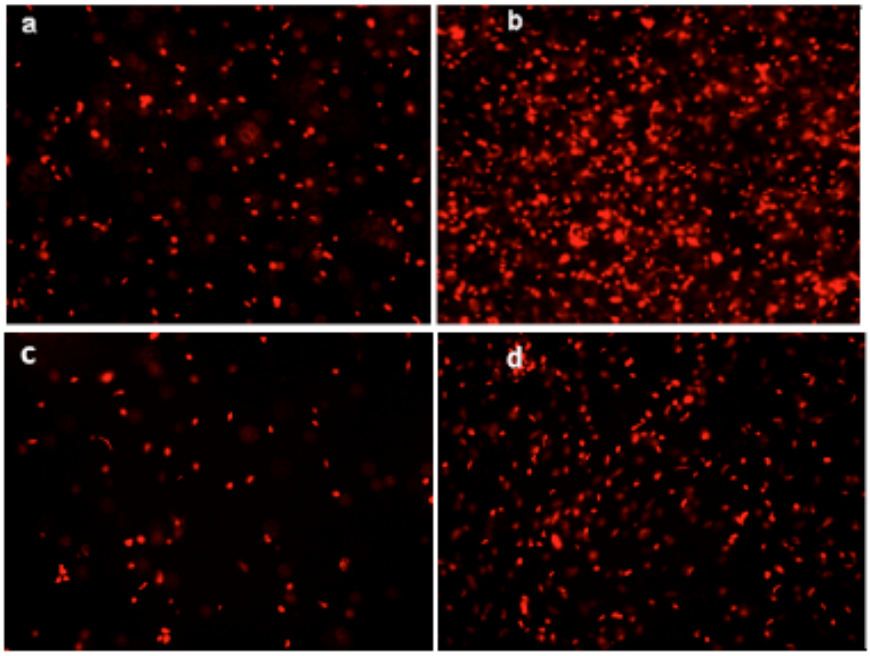

Fig. (5). Increased cellularity in grade III and grade IV ductal breast carcinoma is shown in right panel of the picture (b) & (d) with compare to its surrounding (a) & (c). Their higher damaged state of cellular DNA explained the invasive nature of cell growth with their apoptotic death. The unstable DNA materials are clearly visualized.

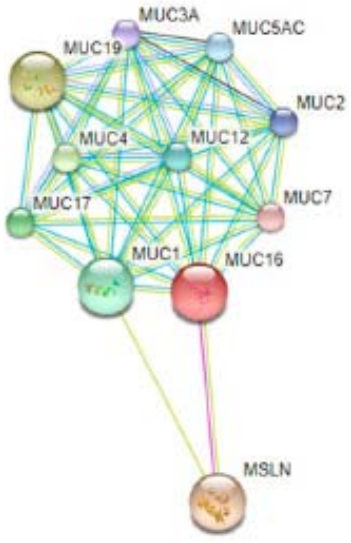

Fig. (6). Interactive role of CA125 or MUC16 is represented. The STRING-10, efficient protein interacting software was used to prepare this diagram (a). The Mesothelin (MSLN, 630 aa) is an important protein member which exerts direct influence with CA125 which is clearly discussed in the text. Most of the other proteins are of mucin in nature and associated with MUC16 for cell-cell interactions (a). Brief descriptions of all interacting proteins are explained in the lower panel screenshot (b) of the picture. Neighborhood, gene-fusion, concurrence, co-expressions, experiments, databases, test-mining and homology modeling were the default option in the software to generate this interactive diagram.

Comet Assay Results

The present comet assay result reveals a higher and unorganized cellularity in the breast tumor tissues (Fig. **5b**, **5d**) as compared to the respective surrounding tissue (Fig. **5a**, **5c**). Both the tumor and the surrounding tissue shows integrated DNA, but the tumor tissue show some larger/ expanded DNA material.

Bioinformatics Results

Our present bioinformatics result suggests that CA125 interacts with several proteins as described in Fig. (**5b**), showing a strongest interacting score with mesothelin (MSLN) *i.e.*, a combined score of 0.977 (Fig. **6a**, **6b**) based on experimental and biochemical data and text mining. The predicted specific action was a strong binding between MSLN and CA125 (Muc16) with a binding score of 0.800 evidenced from an in vitro experiment [42]. It concluded that mesothelin is a novel CA125-binding protein and that CA125 might contribute to the metastasis of ovarian cancer to the peritoneum by initiating cell attachment to the mesothelial epithelium *via* binding to mesothelin

DISCUSSION

In our study, CA125 levels were found to be highly elevated in cases of borderline/malignant epithelial tumors but not in non epithelial malignant tumors, which abides by the existing literature that its level increases in advanced ovarian epithelial cancer [4]. The sensitivity of CA125 towards epithelial tumor indicates a correlation between them. The level of CA125 rises as an early marker of this disease [39]. But, eventually it causes the tumor cell to bind to the epithelium through its strong mesothelin binding property [20], suggesting its prominent role in the cell-cell interaction, other than playing as a simple marker molecule. The binding of CA125 to mesothelin is involved in cellular adhesion [20]. The CA125 is overexpressed both at mRNA and protein levels in ovarian carcinoma [40], specifically in most of the epithelial ovarian tumors where a significant

level of mesothelin is also noticed. Whereas, the entire germ cells exception to the teratomas were found to be consistently negative for the mesothelin immunostaining. The main finding of our study that CA125 is very low in malignant germ cell tumors like dysgerminomas, mixed germ cell tumor and yolk sack tumor and extremely high in malignant epithelial tumors. This suggests that the parity in the expression patterns of CA125 and mesothelin might be mechanistically inter-dependant. Silencing of the mesothelin gene with anti-mesothelin microRNA caused a significant loss of viability and invasiveness of the ovarian cancer cells [41]. Taken together, the higher expression of mesothilin and CA125 might be specifically involved in causing epithelial tumors to be originated followed by becoming more pathogenic. And that is helpful for a firm establishment of a metastasized cell to some other locations of the body [20]. The independent and highly interactive role of mesothelin with CA125 and other mucin like proteins is clearly evident from the protein-protein interaction data generated by the STRING software in the present Bioinformatics study. Where, mesothilin (MSLN) had the highest score of 0.977 (Fig. **6b**) based on experimental evidence which concluded that mesothelin is a novel CA125-binding protein and that CA125 might contribute to the metastasis of ovarian cancer to the peritoneum by initiating cell attachment to the mesothelial epithelium *via* binding to mesothelin [42]. The CA125 might have a significant role in the pathogenesis of epithelial ovarian cancer as in our study; we found a high level of CA125 level in the epithelial malignant tumors and very low CA125 level in non epithelial tumor though being malignant. Evidence suggests that CA125 protects tumor cells from the immune system by suppressing the response of natural killer cells (NK cells) [43] reminiscent of CA125 binds to NK cells of pregnant women [44] during the normal proliferatives phase of the human endometrium. The CA125 gene expresses highly just before the expression of the NK cell specific genes in this tissue [45]. NK cells are well involved in the maintenance of the pregnancy [46] suggesting a potential immunosuppressive role of CA125 and indirect prevention of immunological rejection of the fetus. On the other hand, prevention from rejection of a tumor cells (extra-cellular growth with uncontrolled proliferation) is established and finally recognized and accepted by the physiological system. And the growth of the new cell mass is unrecognized or overlooked by the host immune system with the help of CA125 mediated suppression of the NK cells and other immune competent cells [47].

The report demonstrates that estradiol is synthesized by the epithelial tumor tissue. In addition, 3β-Hydroxysteroid dehydrogenase (3β-HSD) activity is localized in the stroma of the epithelial ovarian tumors [48]. Estradiol may have a significant role in epithelial ovarian cancer beginning from its benign till malignant stages appear. The malignant dysgerminomas and mixed germ cell tumors were found to be hormonally inert in its initial stages, but the estradiol level is elevated at the time of malignant transformation [49]. A report infers that the estradiol signals exert malignant transformation of immature ovarian teratoma through a non-genomic regulations [50], suggesting estrogen being functionally responsible for the malignant transformation of tumors. Hormone replacement therapy (HRT) with estradiol showed persistently high values of CA125 which returned to the normal level after discontinuation of the therapy [51]. This suggests that estrogen may regulate CA125. Thus, both the ovarian epithelial tumors and non epithelial tumors were found to have high estrogen at their malignant stages. In our study CA125 was found to be extremely high only in malignant epithelial ovarian tumors (Fig. **2a**, **2b**). This needs further investigation for a more conclusive remark.

Ovaries give rise to germ cell, stromal and epithelial ovarian tumors depending on the constitutive cell types. The 90% of all the ovarian cancers are epithelial ovarian carcinoma and are derived from the celomic epithelium [52]. Epithelial tissue possesses the most efficient cell proliferative capacity due to its stem cell like property which may support its sensitivity for tumor generation on certain dysregulations of its normal function [53, 54]. Cancer stem cells, like somatic stem cells, are thought to be capable of self-renewal or unlimited proliferation [55]. This suggests that the self- renewal property and multi-lineage potential of epithelial tissue could be responsible for tumor development and differentiation of more mature epithelial ovarian cells attributing to tumorogenesis. The elevated levels of testosterone and 17β-estradiol induce neoplastic transformation in the stem cell-generated prostatic tissues [56]. Thus, non-reproductive function of E2 may partly relate to the stem cell proliferation and its malignant transformation resulting in E2-related cancers and may induce the ovarian epithelial tissue to raise its stem cell like property and malignant transformation. Taking into account the CA125 binding to cell surface adhesins, mesothelin is inducing in cellular transformation, it may be suggested that the E2-associated epithelial stem cell transformation and tumerogenesis may have some interdependent signaling with CA125 associated disease pathogenesis.

The literature consistently provides evidences that CA125 is a gold standard tumor-marker in ovarian carcinoma [57]. A statistically significant high-risk of ovarian cancer and breast cancer was observed in women with elevated CA125, with poor prognosis in stage IV breast cancers [58]. Evidence suggests that CA125 along with vascular endothelial growth factor (VEGF), CA15-3, ER and progesterone receptor (PR) can guide both diagnosis and treatment for breast cancer [59]. Our findings suggest that CA125 can be considered as a disease marker which can detect the intermediate degree of the disease severity and is least efficient in diagnosing the disease at its benign stage. So, CA125 alone cannot be an ideal biomarker for all the different cancers with such inconsistencies in its expression in benign, borderline and malignant epithelial and non-epithelial tumors. To increase its clinical performance, in terms of better sensitivity and specificity in relation to the tumors' epithelial/non epithelial nature (germ cell tumors and yolk sac tumors), benign/borderline/malignant stages, CA125 may be used in combination with some other biomolecules as early disease markers.

In our study the CA125 levels were higher in breast tumor tissues as compared to its adjacent control tissues. Estrogen is associated with breast cancer risk in women. Oxidative stress and estrogen receptor-associated

proliferative changes are suggested to play important roles in estrogen-induced breast carcinogenesis [26-28]. Studies have targeted estrogen and estrogen metabolizing enzymes as the therapeutic target. One of them is estrogen sulfotransferase (EST or human SULT1E1) which can be redox regulated [60]. It metabolizes estradiol by sulfoconjugation forming estradiol sulfate, which is immediately excreted from the body [61] Estradiol sulfate becomes unable to bind to estrogen receptors and to mediate both genomic and non genomic functions [61]. In breast cancer cases, estradiol and CA125 may have some interdependency as in our study low estradiol is found to be associated with low CA125 in the adjacent normal tissue with comparison to high estradiol level with high CA125 in the concerned tumor tissues (data not shown). Estrogen sulfotransferase (EST) mRNA expression in ovarian surface epithelium was found to be higher than in epithelial ovarian cancer with abundant E1 to E2 conversion and minimal sulfo-conjugation [62]. This explains that EST is down-regulated in the cancerous tissue as compared to the normal tissue and may be the cause of E2 abundance in the cancer tissue. This needs further investigation.

Among the three different breast cancer stages in our study it was found that CA125 was highest in the lowest cancer stage that is IIIA gradually decreased in a case of stage IIIB, whereas, in another IIIB tumor, the tumor tissue had extremely high CA125 than that of the surrounding tissue (Fig. **3c**). CA125 can be suggested as a good marker for disease prognosis [63, 64]. During downstaging of the disease, CA125 levels fluctuates significantly and an increase in CA125 levels within individuals undergoing remission is a strong predictor of the recurrence of the cancer [65]. Monitoring CA125 blood serum levels were found to be useful for determining how ovarian cancer is responding to treatment [66]. Thus, expression of CA125 in an elevated form may frequently occur in mammary carcinoma tissues. This tissue was strongly CA125 positive as compared to the surrounding normal tissues which had low but still higher than the reference range of serum CA125 levels.

In conclusion, CA125 is highly important as a disease marker and at several stages of disease prognosis. CA125 is conveniently predicted as a good determinant in cancer of certain stages and types. Not only as a disease marker, has CA125 played an important interactive role in cell-cell interaction, adhesion and disease manifestation.

DECLARATION OF CONFLICTING INTERESTS

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[1] Bischop, P.; Tseng, L.; Brioschi, P.A.; Herrmann, W.L. Cancer antigen 125 is produced by human endometrial stromal cells. *Human. Reproduction.*, **1986**, *1*, 423–426.

[2] Kenemans, P.; Yedema, C.A.; Bon, G.G.; Mensdorff-Pouilly, S.; CA 125 in gynecological pathology-a review. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **1993**, *49*, 115–124.

[3] Van Haaften-Day, C.; Shen, Y.; Xu, F.; OVX1, macrophage-colony stimulating factor, and CA125II as tumor markers for epithelial ovarian carcinoma: a critical appraisal. *Cancer*., **2001**, *92*, 2837–2844.

[4] Meyer, T.; Rustin, G.J.S. Role of tumor markers in monitoring epithelial ovarian cancer. *Br. J/. Cancer.*, **2000**, *82*, 1535-38.

[5] Berruti, A.; Tampellini, M.; Torta, M.; Buniva, T.; Gorzegno, G.; Dogliotti, L.; Prognostic value in predicting overall survival of two mucinous markers: CA 15-3 and CA 125 in breast cancer patients at first relapse of disease. *Eur. J. Cancer*., **1994**; *30A*: 2082–2084.

[6] Norum, LF.; Erikstein, B.; Nustad, K. Elevated CA125 in breast cancer-A sign of advanced diseas. *Tumor. Biol*., **2001**, *22*, 223–228.

[7] Meden, H.; Fattahi-Meibodi. A. CA 125 in benign gynecological conditions. *Int. J. Biol. Markers*, **1998**, *13*, 231–237.

[8] Ismail, M.A.; Rotmensch, J.; Mercer, L.J.; Block, B.S.; Salti, G.I.; Holt, J.A. CA125 in peritoneal fluid from patients with nonmalignant gynecologic disorders. *J. Reprod. Med*., **1994**, *39*, 510–512.

[9] Kitawaki, J.; Ishihara, H.; Koshiba, H.Usefulness and limits of CA125 in diagnosis of endometriosis without associated ovarian endometriomas. *Hum. Reprod*. **2005**, *20*, 1999–2003.

[10] Miller, K.A.; Deaton, J.L.; Pittaway, D.E. Evaluation of serum CA 125 concentrations as predictors of pregnancy with human in vitro fertilization. *Fertil. Steril.*, **1996**, *65*, 1184–1189.

[11] Kalambokis, G.; Kostoula, A.; Economou, M.; Tsianos, E.V. Tumor necrosis factor-alpha-related intraperitoneal release of CA 125 in cirrhotic patients with sterile ascites. *Clin. Chem.*, **2005**, *51*, 2207– 2208.

[12] Fernandez, J.; Quiros, B.; Telenti, M. CA125 Serum levels in tubercolosis patients. *Int. J. Biol. Markers*, **1995**, *10*, 180–181.

[13] Lloyd, K.O.; Yin, B.W.; Kudryashov, V.; Isolation and characterization of ovarian cancer antigen CA 125 using a new monoclonal antibody (VK-8): identification as a mucin-type molecule. *Int. J. Cancer*, **1997**, *71*, 842–850.

[14] Nouwen, E.J.; Dauwe, S.; De Broe, M.E.; Occurrence of the mucinous differentiation antigen CA125 in genital tract and conductive airway epithelia of diverse mammalian species (rabbit, dog, monkey). *Differentiation*., **1990**, *45*, 192–198.

[15] Duraisamy, S.; Ramasamy, S.; Kharbanda, S.; Kufe, D. Distinct evolution of the human carcinoma-associated transmembrane mucins, MUC1, MUC4 AND MUC16. *Gene*, **2006**, *373*, 28-34.

[16] Easton, R.L.; Panico, M. Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. *J. Biol. Chem.*, **2003**, *278*, 28619–28634.

[17] Patankar, M.S.; Jing, Y.; Morrison, J.C. Belisle, J.A.; Lattanzio, F.A.; Deng, Y.; Wong, N.K.; Morris, H.R.; Dell, A.; Clark, G.F. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecology Oncol.*, **2005**, *99*, 704–713.

[18] Scholler, N.; Fu, N.; Yang, Y. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. *Proc. Natl. Acad. Sci. USA.*, **1999**, *96*, 11531–11536.

[19] Hassan, R.; Laszik, Z.G.; Lerner, M.; Raffeld, M.; Postier, R.; Brackett, D. Mesothelin is overexpressed in pancreatic biliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. *Am. J. Clin. Pathol.*, **2005**, *124*, 838–845.

[20] Gubbels, J.A.; Belisle, J.; Onda, M. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. *Mol. Cancer*., **2006**, *5*, 50.

[21] Lindgren, P.R.; Backstrom, T.; Cajander, S.; Damber, M.G.; Mahlck, C.G.; Zhu, D.; Olofsson, J.I. The pattern of estradiol and progesterone differs in serum and tissue of benign and malignant ovarian tumors. *Int. J. Oncol.* **2002**, *21*, 583-589.

[22] Syed, V.; Ulinski, G.; Mok, S.C,.; Yiu, G.K.; Ho, S.M. Expression of gona- dotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. *Cancer. Res.*, **2001**, *61*, 6768-6776.

[23] Wimalasena, J.; Meehan, D.; Dostal, R.; Foster, J.S.; Cameron, M.; Smith, M. Growth factors interact with estradiol and gonadotropins in the regulation of ovarian cancer cell growth and growth factor receptors. *Oncol. Res.*, **1993**, *8*, 325-37.

[24] Yager, J.D. Endogenous estrogens as carcinogens through metabolic activation. *J. Natl. Cancer. Inst. Monogr.*, **2000**, *27*, 67-73.

[25] Grow, D.R. Metabolism of endogenous and exogenous reproductive hormones. *Obstet/ Gynecol. Clin. North Am.*, **2002**, *29*, 425-436.

[26] Cavalieri, E.; Frenkel, K.; Liehr, J.G.; Rogan, E.; Roy, D. Estrogens as endogenous genotoxic agents-DNA adducts and mutations. *J. Natl. Cancer. Inst. Monogr*., **2000**, *27*, 75-93.

[27] Yager, J.D.; Liehr, J.G.; Molecular mechanisms of estrogen carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.*, **1996**, *36*, 203-232.

[28] Wani. G.; Milo, G.E.; D'Ambrosio, S.M.; Enhanced expression of the 8-oxo-7,8-dihydrodeoxyguanosine triphosphatase gene in human breast tumor cells. *Cancer. Lett.*, **1998**, *125*, 123-130.

[29] Petrakova, K.; Palacova, M.; Schneiderova, M.; Standara, M. Hereditary Breast and Ovarian Cancer Syndrome. *Klin. Onkol*., **2015**, *29*, 14-21.

[30] Norum, L.F.; Erikstein, B.; Nustad, K. Elevated CA125 in breast cancer-A sign of advanced disease. *Tumor. Biol*., **2001**, *4*, 223-8.

[31] Jager, W.; Kissing, A.; Cilaci, A.; Melsheimer, R.; Lang, N. Is an increase in CA 125 in breast cancer patients an indicator of pleural metastases? *Br. J. Cancer*. **1994**, *70*, 493–495.

[32] Wang, G.; Qin, Y.; Zhang, J.; Zhao, J.; Liang,Y.; Zhang, Z.; Qin, M.; Sun, Y.; Nipple discharge of CA15-3, CA125, CEA and TSGF as a new biomarker panel for breast cancer. *Int. J. Mol. Sci*., **2014**, *15*, 9546-65.

[33] Zhang, S.J.; Hu,Y.; Qian, H.L.; Jiao, S.C.; Liu, Z.F.; Tao, H.T.; Han, L.; Expression and significance of ER, PR, VEGF, CA15-3, CA125 and CEA in judging the prognosis of breast cancer. *Asian. Pac. J. Cancer. Prev*., **2013**, *14*, 3937-40.

[34] Ogmundsdottir, H.M.; Gudlaugsdottir, S.; Bjornsson, J.; Jonasdottir, S. Altered expression of CA125 in breast carcinomas. *APMIS*., **1996**, *104*, 47-53.

[35] Acharyya, N.; Chattopadhyay, S.; Maiti, S. Chemoprevention against arsenic-induced mutagenic DNA breakage and apoptotic liver damage in rat *via* antioxidant and SOD1 upregulation by green tea (Camellia sinensis) which recovers broken DNA resulted from arsenic-H2O2 related in vitro oxidant stress. J. Environ. Sci. Health. C .*Environ. Carcinog. Ecotoxicol. Rev*., **2014**, *32*, 338-61.

[36] Jensen, L.J.; Kuhn, M.; Stark, M.; Chaffron. S,; Creevey, C.; Muller, J.; Doerks , T.; Julien, P.; Roth, A.; Simonovic, M.; Bork, P.; von Mering, C. STRING 8--a global view on proteins and their functional interactions in 630 organisms. *Nucleic. Acids. Res*., **2009**, *37*, 412-6.

[37] Taboada, B.; Verde, C.; Merino, E. High accuracy operon prediction method based on STRING database scores. *Nucleic. Acids. Res*., **2010**, *38*, 130.

[38] Von Mering, C.; Jensen, L.J.; Snel, B.; Hooper, SD.; Krupp, M.; Foglierini, M.; Jouffre, N.; Huynen, M.A.; Bork, P. STRING: known and predicted protein-protein associations, integrated and transferred across organisms. *Nucleic Acids Res.* **2005**, *33* (Suppl 1), D433-437.

[39] Zurawski, V.R.; Orjaseter, H.; Andersen, A.; Jellum, E. Elevated serum CA 125 levels prior to diagnosis of ovarian neoplasia: relevance for early detection of ovarian cancer. *Int. J. Cancer*. **1988**, *42*, 677– 680.

[40] Frierson, H.F.; Moskaluk, C.A.; Powell, S.M. Large-scale molecular and tissue microarray analysis of mesothelin expression in common human carcinomas. *Hum. Pathol.*, **2003**, *34*, 605–9.

[41] Wang, K.; Bodempudi, V.; Liu, Z.; Borrego-Diaz, E.; Yamoutpoor, F.; Meyer, A.; Woo, R.A.; Pan, W.; Dudek, A.Z.; Olyaee, M.S.; Esfandyari, T.; Farassati, F. Inhibition of mesothelin as a novel strategy for targeting cancer cells. *PloS. One*, **2012**, *7*(4), e33214.

[42] Rump, A.; Morikawa, Y.; Tanaka, M. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J. Biol. Chem*., **2004**, *279*, 9190–9198.

[43] Patankar, M.S.; Jing, Y.; Morrison, J.C.; Belisle, J.A.; Lattanzio, F.A.; Deng, Y.; Wong, N.K.; Morris, H.R.; Dell, A.; Clark, G.F. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecologic. Oncology*. **2005**, *99*, 704-13

[44] Belisle, J.A.; Gubbels, J.A.; Raphael, C.A. Peritoneal natural killer cells from epithelial ovarian cancer patients show an altered phenotype and bind to the tumor marker MUC16 (CA125). *Immunology*., **2007**, 122, 418-429

[45] Talbi, S.; Hamilton, A.E.; Vo, K.C. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology*, **2006**, *147*, 1097–1121.

[46] Saito, S.; Shiozaki, A.; Sasaki, Y.; Nakashima, A.; Shima, T.; Ito, M. Regulatory T cells and regulatory natural killer (NK) cells play important roles in feto-maternal tolerance. *Semin. Immunopathol.*, **2007**, *29*, 115–122.

[47] Seelenmeyer, C.; Wegehingel, S.; Lechner, J.; Nickel, W. The cancer antigen CA125 represents a novel counter receptor for galectin-1. *J. Cell. Sci*., **2003**, *116*, 1305-18.

[48] Sekiguchi, I.; Suzuki, M.; Tamada, T. Estrogen producing activity in epithelial ovarian tumors and dermoid cysts. *Asia. Oceania. J. Obstet. Gynaecol*., **1989**, *4*, 367-74.

[49] Song, E.S.; Lee, J.P.; Han, J.H.; Kim, H.Y.; Mun, S.H.; Ryu, H.S.; Chang, K.H. Dysgerminoma of the ovary with precocious puberty: a case report. *Gynecol. Endocrinol.* **2007**, *23*, 34-7.

[50] Hung, Y.C.; Chang, W.C.; Chen, L.M.; Chang, Y.Y.; Wu, L.Y.; Chung, W.M.; Lin, T.Y.; Chen, L.C.; Ma, W.L. Non-genomic estrogen/estrogen receptor α promotes cellular malignancy of immature ovarian teratoma in vitro. *J. Cell. Physiol*., **2014**, *229*, 752-61.

[51] Cecchi, E.; Lapi, F.; Vannacci, A.; Banchelli, G.; Mazzei, T.; Mugelli, A. Increased levels of CA 125 and CA 19.9 serum tumor markers following cyclic combined hormone replacement therapy. *J. Clin. Pharm. Ther*., **2009**, *34*, 129-32.

[52] Ponnusamy, M.P.; Batra, S.K. Ovarian cancer: emerging concept on cancer stem cells. *J. Ovarian. Res*., **2008**, *1*, 4.

[53] Blanpain, C.; Horsley, V.; Fuchs, E. Epithelial stem cells: turning over new leaves. *Cell*, **2007**, *128*, 445-58.

[54] Blanpain, C.; Fuchs, E.; Stem cell plasticity. Plasticity of epithelial stem cells in tissue regeneration. *Science*, **2014**, *13*, 344.

[55] Reya, T.; Morrison, S.J; Clarke, M.F; Weissman, I.L.; Stem cells, cancer, and cancer stem cells. *Nature*, **2001**, *414*, 105-111.

[56] Hu, W.Y.; Shi, G.B.; Lam, H.M.; Hu, D.P.; Ho, S.M.; Madueke, I.C.; Kajdacsy-Balla, A.; Prins, G.S. Estrogen-initiated transformation of prostate epithelium derived from normal human prostate stem-progenitor cells. *Endocrinology*., **2011**, *152*, 2150-63.

[57] Hogdall, E. Cancer antigen 125 and prognosis. *Curr. Opin. Obstet. Gynecol*. **2008**, *20*, 4–8.

[58] Norum, L.F.; Erikstein, B.; Nustad, K. Elevated CA125 in breast cancer-A sign of advanced disease. *Tumor. Biol*., **2001**, *22*, 223-8.

[59] Zhang, S.J.; Hu, Y.; Qian, H.L.; Jiao, S.C.; Liu, Z.F.; Tao, H.T.; Han, L. Expression and significance of ER, PR, VEGF, CA15-3, CA125 and CEA in judging the prognosis of breast cancer. *Asian. Pac. J. Cancer. Prev*., **2013**, *14*, 3937-40.

[60] Maiti, S.; Zhang, J.; Chen, G.; Redox regulation of human estrogen sulfotransferase (hSULT1E1). *Biochem. Pharmacol*., **2007**, *73*, 1474-81.

[61] Falany, C.N.; Wheeler, J.; Oh, T.S.; Falany, J.L. Steroid sulfation by expressed human cytosolic sulfotransferases. *J. Steroid. Biochem. Molec. Biol*., **1994**, *48*, 369-375.

[62] Falany, J. L.; Macrina, N.; Falany, C.N.; Regulation of MCF-7 breast cancer cell growth by beta-estradiol sulfation. *Breast. Cancer. Res. Treat.*, **2002**, *4*, 167-76

[63] Ren, X.; Wu, X.; Hillier, S.G.; Fegan, K.S.; Critchley, H.O.; Mason, J.I.; Sarvi, S.; Harlow, C.R. Local estrogen metabolism in epithelial ovarian cancer suggests novel targets for therapy. *J. Steroid. Biochem. Mol. Biol*., **2015**, *150*, 54-63.

[64] Gocze, P.; Vahrson, H. Ovarian carcinoma antigen (CA 125) and ovarian cancer (clinical follow-up and prognostic studies). *Orv. Hetil*., **1993**, *134*, 915–8.

[65] Santillan, A.; Gar, R.; Zahurak,M.L. Risk of epithelial ovarian cancer recurrence in patients with rising serum CA125 levels within the normal range. *J. Clin. Oncol*., **2005**, *23*, 9338–43.

[66] National Institute for Health and Clinical Excellence. Women should be offered a blood test for ovarian cancer. NICE. Guidance (2011) Retrieved, **2012**, 04-14.