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Aldehyde Dehydrogenase 1A1 Expression in Ovarian Epithelial Tumors

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Authors
Maha E. Muhammad[1] *MSc*,
Rasha A. Khairy[1] *MD*,
Sara E. Khalifa* *MD*,
Hala N. Hosni[1] *MD*,
Ahmed M. Abd E[l] *MD*,
Aziz[1] *MD*

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ABSTRACT

Aims Supporting evidences have been proposed that tumor initiating cells or cancer stem cells (CSCs) are principally sharing into tumor progress and relapse. Aldehyde dehydrogenase 1A1 (ALDH1A1), a stem cell marker, has currently been implicated in multiple human malignancies including ovarian carcinomas. The aims of this study were to assess immunohistochemical (IHC) expression of ALDH1A1 in ovarian epithelial tumors, tracking stem cells during ovarian cancer development and its relation with the clinicopathological features of such tumors.

Materials and Methods In this experimental study, 42 paraffin blocks of ovarian tumor cases were retrieved retrospectively from the department of pathology, faculty of medicine, Cairo University during January 2013 to January 2015. Ovarian tumor paraffin blocks included 14 benign cystadenomas 14 border line tumors and 14 carcinomas. IHC reactions were carried out by using ALDH1A1 monoclonal antibody. Cases were classified into two groups of low and high ALDH1A1 expression. The chi-squared test ($\chi 2$) was used and data analyzes by SPSS 22 software.

Findings High ALDH1A1 expression was reported in 50.0% of benign cystadenomas, 50.0% of borderline tumors and 85.7% of ovarian carcinomas with significant positive association with ovarian carcinomas (p=0.025). In ovarian carcinomas, positive relationship was detected between high ALDHA1 expression and advancing tumor grades but it didn't reach statistical significance (p=0.054), no any significant relations were detected between ALDH1A1 immunohistochemical expression and age of patients, the documented size, laterality, histologic types and FIGO stage in all tumors (p>0.05).

Conclusion ALDH1A1 is a potential biomarker for detecting CSCs in ovarian carcinomas and a prognostic marker. Also, it may act as a target for future therapy.

Keywords ALDH1A1; Ovarian Neoplasms; Carcinoma

CITATION LINKS

[1]Incidence,patternandmanagementofovariancerataatertiarymedicalcenterinEnugu,South East Nigeria [2] Ovarian cancer: Epidemiology, biology, and prognostic factors [3] Identification by cDNA microarray of genes involved in ovarian carcinogenesis [4] Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer [5] Aldehyde dehydrogenase 1 is amarker for normal and malignant human colonic Stem Cells (SC) and tracks SC overpopulation during colon tumorigenesis [6] ALDH1 expression correlates with favorable prognosis in ovarian cancers [7] ALDH1A1 expression correlates with clinicopathologic features and poor prognosis of breast cancer patients: A systematic review and meta-analysis [8] Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers [9] ALDH1-bright epithelial ovarian cancer cells are associated with CD44 expression, drug resistance, and poor clinical outcome [10] Pathology and genetics of tumours of the breast and female genital organs [11] Cancer stem cells--perspectives on current status and future directions: AACR workshop on cancer stem cells [12] Ovarian cancer: A clinical challenge that needs some basic answers [13] Biosensors for cancer markers diagnosis [14] Aldehyde dehydrogenase in combination with CD133 define angiogenic ovarian cancer stem cells that portend poor patient survival [15] Expression of stem cell markers in preinvasive tubal lesions of ovarian carcinoma [16] Differential expression of aldehyde dehydrogenase 1a1 (ALDH1) in normal ovary and serous ovarian tumors [17] Loss of ALDH1A1 expression is an early event in the pathogenesis of ovarian high-grade serous carcinoma [18] Cytotoxic effect of disulfiram/copper on human glioblastoma cell lines and ALDH-positive cancer-stem-like cells [19] Characterization of aldehyde dehydrogenase isozymes in ovarian cancer tissues and sphere cultures

*Pathology Department, Medicine Faculty, Cairo University, Cairo, Egypt
[1]Pathology Department, Medicine Faculty, Cairo University, Cairo, Egypt

Correspondence
Address: Kasr Al-Ainy Faculty of Medicine. El Manial, Cairo, Egypt. Postal Code: 11728
Phone: +22 (70) 11516
Fax: -
sarah.mekawy@kasralainy.edu.eg

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Introduction

Worldwide, ovarian malignancy ranks the fourth cause of cancer-related deaths. Increasing evidence has proposed that it may be more common to occur in developing countries [1].

Ovarian cancer patients usually present with insidious onset, and poor prognosis, and most of the patients (70%) presented with advanced disease, leading to increased mortality in spite of adequate surgery and aggressive chemotherapy [2]. Hence, it is crucial and urgent to discover ways for early diagnosis of ovarian cancer and to establish more effective and target therapies [3].

Aldehyde Dehydrogenase-1, family member A1 (ALDH1A1) is a cancer stem cells marker that has been found in many tumors and also non-neoplastic tissues [4]. It is a detoxifying enzyme that is implicated in oxidization of intracellular aldehydes along with resistance to alkylating agents. So it protects stem cells (SCs) against oxidative injury, leading to their durability of SC [5].

Positive cytoplasmic staining of ALDH1A1 was detected in many non-neoplastic, normal, tissues including the digestive system (e.g. esophageal, gastric, intestinal epithelium, liver and pancreas), the endocrine glands (adrenal, thyroid and salivary glands) and the gonads (both the ovary and testis) [5, 6].

In a large meta-analysis study performed in breast cancer, ALDH1A1was found as a poor prognostic marker and a detector of poor survival [7].

Regarding ALDH1A1 expression in ovarian malignancies, conflicting results were obtained as some being associated with poor prognosis mostly in serous carcinoma [8] while others showed the opposite [6]. The actual role of ALDH1A1 expression in ovarian cancer remains elusive with conflicting results [8].

The aims of the present study were to assess immunohistochemical (IHC) expression of ALDH1A1 in ovarian epithelial tumors, tracking stem cells during ovarian cancer development and its relation with the clinicopathological features of such tumors.

Material and Methods

In the present experimental study, 42 paraffin blocks of ovarian tumor cases were retrieved retrospectively from the department of pathology, faculty of medicine, Cairo University during January 2013 to January 2015. Ovarian tumor paraffin blocks included 14 benign cystadenomas (6 serous, and 8 mucinous), 14 border line tumors (9 serous, and 5 mucinous) and 14 carcinomas (6 serous, 5 mucinous, and 3 endometrioid). Patient's data were obtained from the patient's medical and pathological reports including age, laterality, tumor size, extra capsular extension, nodal status and distant metastases.

Five microns thick section was prepared from each paraffin block and stained by hematoxylin (H) and eosin (E) for routine histopathological examination. H and E stained slides were evaluated for the following: Histological type of the tumor, according to 2003 report of World Health Organization. For malignant tumors, the following criteria were assessed: Grade of the tumor, in keeping with the International Federation of Gynecology and Obstetrics (FIGO) grading system, Stage of the tumor, according to TNM Staging and FIGO Classification of Ovarian Carcinoma [9].

ALDH1A1 immunostaining: From each paraffin block, 3 to 5µm thick was cut then mounted on the positive charged slide. The slides were deparaffinized in xylene, followed by rehydration through a series of graded alcohols then were twice microwave-treated for 4 then 8minutes in 10mm sodium citrate buffer with pH 6.0. Endogenous peroxidase activity block was done using 3% H_2O_2 for 15min, followed by washing using tris-buffered saline (TBS). Subsequently, the sections were incubated with the ALDH1A1 monoclonal antibody (EP1933Y clone). 1.0ml concentrated, diluted at 1:200-400 for 1hour at the room temperature. Again tissue sections were washed in TBS then incubated with the avidin-biotin-peroxidase system (Dako; USA) for 30minutes. Finally, diaminobenzidine (DAB) was used as a chromogen and hematoxylin as a counterstain. A positive control was obtained from invasive duct carcinoma breast sections positive for ALDH1A1, as recommended by the manufacturer.

ALDH1A1 positively stained cells were characterized by the presence of brownish cytoplasmic coloration and was semiquantitatively scored as 0 (<5% positive tumor cells), 1 (5-20% positive cells), 2 (21 to 50% positive), 3 (>51% positive tumor cells). For statistical purposes; the cases were submitted into 2 groups, either low expression (score 0 and 1) or high expression (scores 2 and 3) [6].

The chi-squared test (χ^2) was used and data analyzes by SPSS 22 software.

Findings

ALDH1A1 immunostaining was observed in the ovarian tumors as cytoplasmic staining in tumor epithelial cells and was reported as low (Figures 1 and 2) and high expression (Figures 3 and 4).

Positive ALDH1A1 cytoplasmic immunostaining was also noted in stromal fibroblasts and served as internal control.

Immunohistochemical high expression of ALDH1A1 in tumor cells progressed from 50.0% (14/28) in benign and borderline tumors, to

85.7% (12 out of 14) in ovarian carcinoma, and that was statistically significant, while no significance was distinguished as relating with each histologic type (Table 1).

In ovarian carcinomas, positive relationship was detected between high ALDHA1 expression and advancing tumor grades but it didn't reach statistical significance where p value=0.054, while relating ALDH1A1 expression and patients age, tumor size, laterality, histologic type and FIGO stage revealed no statistical significance (p>0.05; Table 2).

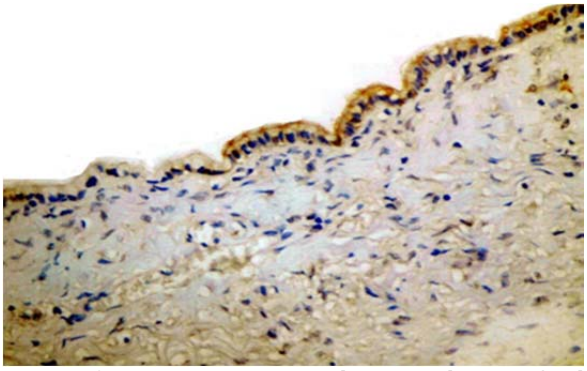

Figure 1) Benign serous cystadenomas showing focal cytoplasmic ALDH1A1 immunostaining (low ALDH1A1 expression; IHCx 400 original magnification)

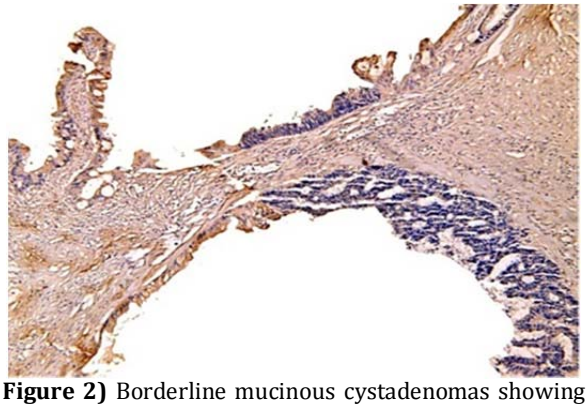

Figure 2) Borderline mucinous cystadenomas showing focal cytoplasmic ALDH1A1 immunostaining (low ALDH1A1 expression; IHCx 200 original magnification)

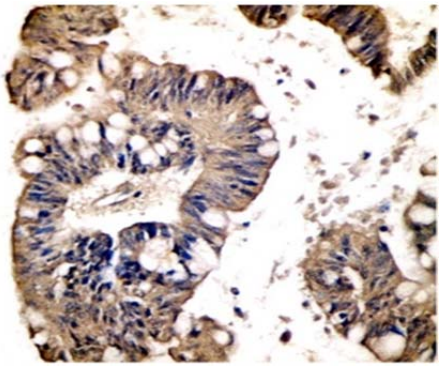

Figure 3) Mucinous adenocarcinoma, grade II showing diffuse cytoplasmic ALDH1A1 immunostaining (High ALDH expression; IHC x 400 original magnification)

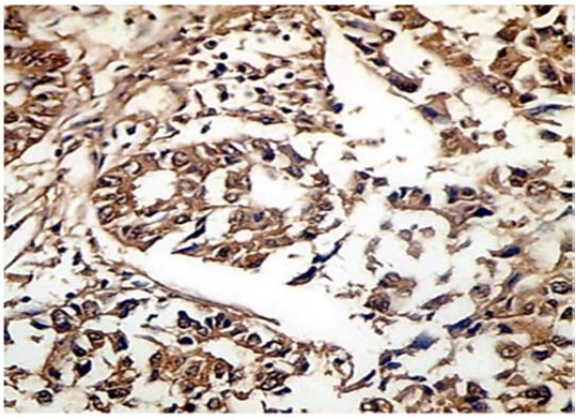

Figure 4) Endometrioid cystadenomas carcinoma, grade II showing diffuse cytoplasmic ALDH1A1 immunostaining (High ALDH1A1 expression; IHC x 400 original magnification)

Table 1) Relation of ALDH1A1 immunohistochemical expression with neoplastic types and histological subtypes in ovarian tumor cases

Variables	Total No.	ALDH1 expression (Low)	ALDH1 expression (High)	P value
Diagnosis				
Benign and borderline	28	14 (50.0%)	14 (50.0%)	0.025
Malignant	14	2 (14.3%)	12 (85.7%)	
Histologic type				
Serous	21	6 (28.6%)	15 (71.4%)	
Mucinous	18	10 (55.6%)	8 (44.4%)	0.083
Endometrioid	3	0	3 (100%)	

Table 2) Relation of ALDH1A1 immunohistochemical expression with the clinicopathologic characteristics of ovarian carcinoma

Clinicopathologic Characteristics	Total no.	ALDH1 expression Low	ALDH1 expression High	P value
Age				
<50 years	6	2 (33.3%)	4 (66.7%)	0.078
≥50 years	8	0	8 (100%)	
Tumor size				
<11cm	8	0	8 (100%)	0.078
≥11cm	6	2 (33.3%)	4 (66.7%)	
Laterality				
Unilateral	10	2 (20.0%)	8 (80.0%)	0.334
Bilateral	4	0	4 (100%)	
Histologic type				
Serous	6	0	6 (100%)	
Mucinous	5	2 (40.0%)	3 (60.0%)	0.122
Endometrioid	3	0	3 (100%)	
Histologic grade				
Grade I	4	2 (50.0%)	2 (50.0%)	P=0.054
Grade II	7	0	7 (100%)	
Grade III	3	0	3 (100%)	
FIGO stage				
Stage I	11	2 (18.2%)	9 (81.8%)	0.425
Stage II	3	0	3 (100%)	

Discussion

The aims of the present study were to assess immunohistochemical (IHC) expression of ALDH1A1 in ovarian epithelial tumors, tracking stem cells during ovarian cancer development and its relation with the clinicopathological features of such tumors.

In females, ovarian malignancy is considered as a principal source of gynecologic cancers related-deaths, with surface epithelial malignant tumors as the most prevalent ones [9]. Malignant cancer cell with a stem cell phenotypic character is a cancer stem cell, playing a central role within pathogenesis of epithelial ovarian carcinoma [10].

ALDH1A1 is detected in multiple malignant and normal tissues. It is as an intracellular enzyme that is involved in cellular detoxification, differentiation, drug resistance, and management of the differentiation pathways. It acts through intracellular aldehydes oxidation. Being a stem cell marker, it plays a central role in tumor-initiating cells' biology [11].

Also, it was reported that ALDH1A1 was associated with the resistance to chemotherapy in ovarian malignancies [12]. High ALDH1expression in breast, ovarian cancer cells and glioblastomas, was strongly related to poor prognostic outcomes [13]. Furthermore, Silva *et al*. noted that presence of stem cells which are positive for both ALDH and CD133 in ovarian cancers was correlated to diminished disease-free and overall survival [14].

In this study, the expression profiles of ALDH1A1 was immunohistochemically exmined in ovarian epithelial tumors to track ovarian CSCs (cancer stem cells) in tumor progression from benign to borderline to frank malignancy; however, to best of our knowledge, infrequent studies were done in this field. A total of 42 paraffin blocks of ovarian tumor cases was retrieved retrospectively, including 14 benign cystadenomas cases (6 serous and 8 mucinous), 14 borderline tumors (9 serous and 5 mucinous) and 14 ovarian carciomas (6 serous, 5 mucinous and 3 endometrioid).

Age of patients ranged from 18 to 72 years with a mean of 39.9±15.9 years. The ovarian carcinoma patients had mean age of 50.6 years.

Regarding histologic type, serous type was the most prevalent type as 21 out of 42 cases (50%), followed by mucinous type 18 out of 42 cases (43%) and lastly endometrioid type 3 out of 42 cases (7%).

For interpretation of ALDH1A1 positivity, tumor epithelail cells that showed distinct cytoplasmic staining were considered positive. Cytoplasmic immunostaining was also seen in stromal cells and represented an internal control [15]. ALDH1A1 expression was interpreted as low and high expression [6].

High ALDH1A1 expression was significantly increased in ovarian carcinoma 86% (12 out of 14 cases) than 50% in benign and borderline tumors (14 out of 28 cases) ($p<0.05$). This finding was contradictory to that detected in the study of Penumatsa *et al*. who found that ALDH1 expression in malignant tumors was significantly lower than what is expressed in normal ovaries and benign tumors with their percentages of positively stained cells (17.10±7.61%; n=5; 37.4±5.4%; $p<0.01$; n=5; 31.03±6.68%; $p<0.05$; n=5) respectively [16].

As regarding ALDH1A1 expression according to histologic type of ovarian tumors, higher ALDH1A1 expression was encountered in endometrioid tumors as 100% (3 out of 3 cases) and 71.4% (15 out of 21 cases) of serous tumors showed high ALDH1A1 expression compared to 44.4% (8 out of 18 cases) of mucinous tumors, although it didn't reach statistical significance ($p=0.083$).

A shift towards higher ALDH1A1 expression was noted with advanced histologic grades as 50% only of grade I ovarian carcinoma cases showed high ALDH1A1 expression compared to 100% of grade II and III cases ($P=0.054$). Contradictory results was obtained by Penumatsa *et al*. as well differentiated tumors in their study showed higher expression of ALDH1 compared to poorly differentiated malignancies and Chui *et al*. demonstrated that negativity of ALDH1A1 may act as a precursor of serous carcinoma [16, 17].

All cases (100%) with FIGO stage II showed high ALDH1A1 expression, compared to 82% of those with FIGO stage I although no statistical significance was reported ($p>0.05$). These results were compatible with findings noted by Wang *et al*. as high ALDH1 expression was more reported in advanced tumor stages III and IV (36.8%) compared to stage I and II (25.9%) although no statistical significance was reached ($p>0.05$) [9].

Silva *et al*. demonstrated that tumor cells co-expressing both ALDH1 and CD 133 are highly aggressive tumors, with rapid tumor growth, poor prognosis, and decreased free survival and overall survival [14]. In addition, Tothil reported that ALDH1A1 was associated with resistance to chemotherapeutic drugs in the ovarian CSC and Lui *et al*. stated that high ALDH1 activity detected in ovarian carcinomas being accompanied by poor clinical outcome [13, 18].

In the present work, 100% of ovarian serous carcinoma (3 out of 3 cases) and endometrioid carcinoma (6 out of 6 cases) showed high ALDH1A1 expression followed by 60% only of mucinous carcinoma (5 out of 5 cases). These findings was nearly consistent with the results of Wang *et al*. and Saw *et al*. as regards endometrioid tumors, where it was different as regards

mucinous and serous tumors as they demonstrated that ALDH1A1expression was more increased in mucinous and endometrioid tumors than in serous and clear cell ones [9, 19].

No significant relationship was found between ALDH1A1 expression in ovarian tumors and other clinicopathologic factors as age, tumor size and, tumor laterality status ($p>0.05$).

The differences in the percentages of ALDH1A1 expressions among studies may be due to using different antibodies, staining procedures, different sample sizes and different scoring systems for interpretation of ALDH1A1 expression.

In conclusion, ALDH1A1 expression in ovarian tumors revealed that CSCs play an important role in ovarian carcinogenesis, where malignant tumors showed the highest ALDH1A1 expression compared to benign and borderline tumors. Moreover, association of ALDH1A1 with advancing histologic grade supports being a prognostic marker and can be used as a target for further therapy. In practice, these findings are greatly appreciated because the immunoistochemical detection of ALDH1A1 is a simple technique and readily available. However, clinicopathological studies on a larger sample size to validate its prognostic value for improving therapeutic efficiencies of ovarian cancer are required.

The limitations of this research include unavailability of molecular testing for ALDH1A1 at the time of performing this research work that would add a lot to the sensitivity and accuracy of this work.

Further molecular and clinicopathologic follow-up studies would still be needed to delineate the role of ALDH1A1 being a new hope for therapeutic targeting and as a prognostic marker in ovarian cancer patients.

Conclusion

ALDH1A1 is a potential biomarker for detecting cancer stem cells in ovarian carcinomas and a prognostic marker. Also, it may act as a target for future therapy.

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Conflicts of interests: Authors declare no conflict of interests.

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References

1- Lyoke CA, Ugwu GO, Ezugwu EC, Onah N, Ugwu O, Okafor O. Incidence, pattern and management of ovarian cancer at a tertiary medical center in Enugu, South East Nigeria. Ann Med Health Sci Res. 2013;3(3):417-21.

2- Holschneider CH, Berek JS. Ovarian cancer: Epidemiology, biology, and prognostic factors. Semin Surg Onco. 2000;19(1):3-10.

3- Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, et al. Identification by cDNA microarray of genes involved in ovarian carcinogenesis. Cancer Res. 2000;60(18):5007-11.

4- Landen CN Jr, Goodman B, Katre AA, Steg AD, NICK AM, Stone RL, et al. Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. Mol Cancer Ther. 2010;9(12):3186-99.

5- Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, et al. Aldehyde dehydrogenase 1 is amarker for normal and malignant human colonic Stem Cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res. 2009;69(8):3382-9.

6- Chang B, Liu G, Xue F, Rosen DG, Xiao L, Wang X, et al. ALDH1 expression correlates with favorable prognosis in ovarian cancers. Mod Pathol. 2009;22(6):817-23.

7- Liu Y, Lv DL, Duan JJ, Xu SL, Zhang JF, Yang XJ, et al. ALDH1A1 expression correlates with clinicopathologic features and poor prognosis of breast cancer patients: A systematic review and meta-analysis. BMC Cancer. 2014;14:444.

8- Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, et al. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PLoS One. 2010;5(4):e10277.

9- Wang YC, Yo YT, Lee HY et al. ALDH1-bright epithelial ovarian cancer cells are associated with CD44 expression, drug resistance, and poor clinical outcome. Am J Pathol. 2012;180(3):1159-69.

10- International Agency for Research on Cancer. Pathology and genetics of tumours of the breast and female genital organs. Eble GN, Tavassoli FA, Devilee P, editors. Lyon: IARC Press; 2003. pp. 114-29.

11- Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells--perspectives on current status and future directions: AACR workshop on cancer stem cells. Cancer Res. 2006;66(19):9339-44.

12- Lawrenson K, Gayther SA. Ovarian cancer: A clinical challenge that needs some basic answers. PLoS Med. 2009;6(2):e25.

13- Tothill IE. Biosensors for cancer markers diagnosis. Semin Cell Dev Biol. 2009;20(1):55-62.

14- Silva IA, Bai S, McLean K, Yang K, Griffith K, Thomas D, et al. Aldehyde dehydrogenase in combination with CD133 define angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res. 2011;71(11):3991-4001.

15- Chene G, Ouellet V, Rahimi K, Barres V, Meunier L, De Ladurantaye M, et al. Expression of stem cell markers in preinvasive tubal lesions of ovarian carcinoma. BioMed Research Int. 2015;808531.

16- Penumatsa K, Edassery SL, Barua A, Bradaric MJ, Luborsky JL. Differential expression of aldehyde dehydrogenase 1a1 (ALDH1) in normal ovary and serous ovarian tumors. J Ovarian Res. 2010;3:28.

17- Chui MH, Wang Y, Wu RC, Seidman J, Kurman RJ, Wang TL, et al. Loss of ALDH1A1 expression is an early event in the pathogenesis of ovarian high-grade serous carcinoma. Mod Pathol. 2015;28(3):437-45.

18- Liu P, Brown S, Goktug T, Channathodiyil P, Kannappan V, Hugnot JP, et al. Cytotoxic effect of disulfiram/copper on human glioblastoma cell lines and

ALDH-positive cancer-stem-like cells. Br J of Cancer. 2012;107(9):1488-97.
19- Saw YT, Yang J, Ng SK, Liu S, Singh S, Singh M, et al. Characterization of aldehyde dehydrogenase isozymes in ovarian cancer tissues and sphere cultures. BMC Cancer. 2012;12:329.