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Research Article

Association between Expression of CD133 and Aldehyde Dehydrogenase 1A1 (ALDH1A1) with FIGO Stage in Serous Ovarian Cancer

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Abstract:
Ovarian cancer (OC) is the fifth most common cancer in women and has become the main cause of gynecologic malignancy death. The incidence rate of OC increase and overall survival (OS) is relatively low because most of patients are diagnosed at advanced stages. Serous ovarian cancer (SOC) is the most frequent histopathological type and often occurs at advance stage. Stage and optimal treatment are independently associated with chemo-response in SOC. FIGO staging system in SOC can provide prognostic information and guidance on personalized management of SOC. Cancer stem cells (CSC) are pivotal players in SOC progression and prognosis. CD133 and Aldehyde Dehydrogenase 1A1 (ALDH1A1) are related CSC markers in SOC.This study aimed to investigate the expression of CD133 and ALDH1A1 in SOC and their association with FIGO stage.
This research was carried out as analytic-observational with cross-sectional design using paraffin block of patients diagnosed with SOC in the Department of Anatomic Pathology Hasan Sadikin Hospital Bandung. Samples were divided in two groups: 20 cases of early FIGO stage (FIGO stage I and II) and 20 cases of advanced FIGO stage (FIGO stage III and IV). All samples were stained by immunohistochemistry CD133 and ALDH1A1. All data were analysed using Chi-Square test with significant level 5%.
The results of this study showed that 16 patients (80%) showed high expression of CD133 at advanced FIGO stage and high expression of ALDH1A1 in 10 patients (50%) at advanced FIGO stage. There was a association between expression of CD133 with FIGO stage in SOC (p value 0.004) and there was no association between expression of ALDH1A1 with FIGO stage in SOC (p value 0.197). It can be concluded that higher CD133 expression showed higher tumour cells ability to do invasion and metastasis and had higher influenced on FIGO stage.

Keywords: Serous ovarian cancer, CD133, ALDH1A1, Cancer Stem Cells, FIGO stage

Introduction

Ovarian cancer (OC) is an epithelial malignancy of ovary, the seventh most common cancer and the eighth cause of cancer-related death among women worldwide.[1,2] GLOBOCAN 2012 showed that incidence of OC were 239,000 cases with 152,000 deaths.[2] Incidence of OC is getting higher by year. According to GLOBOCAN 2018, incidence of OC became 295.414 cases with 184.799 deaths.[3] The incidence of OC in Asia were higher in China, India and Indonesia. In Indonesia, the incidence of OC were 10.238 cases with 7.075 deaths.[4] Serous ovarian cancer (SOC) is the most common type of OC (70%) and it is classified as Low grade SOC (LGSOC) and High grade SOC (HGSOC).[5]

The prognosis of SOC tends to poor in majority of patients, it is five years survival rate from 9-56% and influenced by FIGO stage and chemo-response.[6] The Cancer Genomic Atlas (TCGA) concludes that FIGO stage and optimal treatment (including optimal surgery and 6 cycles of platinum-based chemotherapy) are independently associated with chemo-response in SOC.[7]

FIGO staging system in ovarian cancer can provide prognostic information and guidance on personalized management of ovarian cancer. FIGO stage is determined based on the location of involvement, invasion, dissemination and metastasis of tumor mass seen by radiological examination (CT scan / MRI) before surgical staging and pathological examination (histopathology / cytology) obtained from surgical staging. CT scan is a screening modality recommended for staging on SOC with a sensitivity of 85-93% and specificity 91-96%. However, the sensitivity may decrease to 25-50% in detecting tumors with a diameter of <1 cm (micro-metastasis). Thus, histopathology examination becomes the gold standard to determine metastasis.[8]

In the last few years, the existence of Cancer Stem Cells (CSC) has been considered as a main role of poor prognosis in many kinds of cancer. CSC are subpopulation of tumor cells called cell progenitors that can increase proliferation and mobilization of tumor cells which then affect the aggressiveness of tumor cells. CSC are considered to play an important role in the occurrence of resistance, proliferation, progression and initiation of tumor cell metastasis.[1,9]

Identification of CSC in SOC has an important potential in providing optimal management and become predictors in determining aggressiveness and can subsequently become a reference in the development of anti-CSC therapy.[10] There are many CSC markers in SOC: CD24, CD44, CD117, CD133 and aldehyde dehydrogenase isoform 1 (ALDH1). CD24

affects the ability of differentiation, self-renewal and resistance of tumor cells to chemotherapy. CD44 plays a role in chemoresistance. CD177 or c-Kit is involved in cell signal transduction, increasing cell differentiation and proliferation capabilities.[11]

CD133 and ALDH1A1 are CSC marker that can be identified in OC and correlate with stage in OC.[12] CD133 promote cell proliferation, tumorigenesis, tumor invasion and metastasis through induction of NF-κB and upregulation of MMP9.[13] CD133 is also involved in the activation of the PI3K / Akt and Wnt / β Catenin pathways which induce self renewal potential, tumorigenesis and metastasis.[14]

ALDH1A1 is one of ALDH1 isoform with the highest affinity in catalysed retinal oxidation into retinoic acid (RA). RA will bind to nuclei receptors, regulate gene expression in promoting cell proliferation and regulate antiapoptotic through upregulation of c-MYC and cyclyn D1.[15] The expression ALDH1A1 is related to the Wnt / β Catenin signaling pathway.[9] ALDH1A1 also involved in the formation of multicellular spheroids / MCS that facilitate the dissemination of tumor cells to the peritoneum and surrounding organs through Epithelial Mesenchymal Transition (EMT) process.[16]

This study aimed to investigate the expression of CD133 and ALDH1A1 on SOC and to analyse its role in determining SOC aggressiveness based on FIGO stage so that it can be used in predicting patient aggressiveness. This can then be considered to determine appropriate treatment options for SOC patients, especially for those whose previous incomplete surgery has been carried out and who have been referred to undergo optimal cytoreduction surgery so that it can be a reference for clinical and operators to gain optimal surgery results without residue.

Appropriate management is expected to increase life expectancy and decrease the mortality rate of SOC patients. In addition, this research is expected to be the basis for the development of anti-CSC therapy on SOC.

Materials and Methods

This study uses analytic observational method with cross-sectional study design and retrospective data retrieval /collection. Ethical clearance has been approved / assessed by Health Research Ethic Commission, Padjadjaran University, assessment number 1154/UN6.KEP/EC/2018.

Sample preparation: the samples were obtained from registered patients of Hasan Sadikin Hospital who were diagnosed with SOC from January 2013 to March 2018. Samples were divided in two groups: 20 cases of early FIGO stage (FIGO stage IA, IB, IC, IIA, IIB) and 20 cases of advanced FIGO stage (FIGO stage IIIA, IIIB, IIIC, IVA, IVB).

Analysis of expression: samples from paraffin blocks were prepared for IHC analysis; IHC analysis was performed based on the protocol provided by anatomical pathology laboratory.

Under the microscope, the slides were visualized by three reviewers who had not had knowledge about clinicopathologic data.

Histoscore calculation: Positive result was shown/visualized as brown staining on the tumour cell. Analysis on CD133 was evaluated by brown staining in cytoplasmic membrane of tumour cells and ALDH1A1 expression was evaluated by brown staining assessed in the cytoplasm. The stain intensity and distribution measured under the microscope were then converted into histoscore and categorized as high and low expression. This study used antibody CD133 (Polyclonal antibody, Elabscience) with dilution 1:200 and antibodyALDH1A1 (Polyclonal antibody, Santacruz) with dilution 1:8000. The intensity of tumor-cell staining was scored as having no expression (0), or weak (1), moderate (2), or strong (3). The distribution of tumor-cell staining was scored as having no expression (0), or <10% (1), 10-50% (2), or > 50% (3). Histoscores obtained from the samples were represented on a scale of 0-9. CD133 histoscores were classified to weak (histoscore ≤6) and strong (histoscore > 6). ALDH1A1 histoscores were classified to weak (histoscore ≤2) and strong (histoscore > 2).

This research was performed as analytic-observational with cross-sectional design. Statistical analysis was performed with SPSS using Chi-Square test with significant level 5%.

Result

From January 2013 to March 2018 there were 77 cases of SOC registered in Anatomic Pathology Department but only 40 samples matched to inclusive criteria. The age ranged from 26 to 63 years old. The most common histopathology grade was Low grade SOC in 22 patients (55%) (Table1.)

Table 1. Characteristic of research subject

Variable	N=40
Age (years)	
Mean±Std	49.22±9.616
Median	51.00
Range (min-max)	26.00-63.00
Parity	
P0	7(17.5%)
P≥1	33(82.5%)
Histopathology grade	
(Low Grade serous carcinoma)	22(55.0%)
(High Grade serous carcinoma)	18(45.0%)

Table 1. Characteristic of this research subject based on age, parity and histopathology grade

This study found 20 patients with early FIGO stage and 20 patients with advance FIGO stage. Thirteen patients (65%) showed low expression of CD133 at early FIGO stage and 16 patients (80%) showed high expression of CD133 at advanced FIGO stage (Figure 1A). Statistical analysis showed significant association between CD133 ($p<0.05$) expression with FIGO stage in SOC (Table 2). The expression of

ALDH1A1 at early FIGO stage showed low expression in 14 patients (70%) and high expression in 10 patients (50%) (Figure 1B). Statistical analysis showed there was no significant association between ALDH1A1 ($p>0.05$) expression with FIGO stage in SOC (Table 2).

Table 2. CD133 and ALDH1A1 data on FIGO stage of SOC group

Variable	FIGO stage of SOC		p Value
	Early (n=20)	Advance (n=20)	
Histoscore CD133			0.004**
Low	13 (65.0%)	4(20.0%)	
High	7 (35.0%)	16(80.0%)	
Histoscore ALDH1A1			0.197
Low	14(70.0%)	10(50.0%)	
High	6 (30.0%)	10(50.0%)	

Table2. The significant association between CD133expression ($p<0,05$) with FIGO stage in SOC and no significant association between ALDH1A1 expression ($p>0,05$) with FIGO stage in SOC in this study

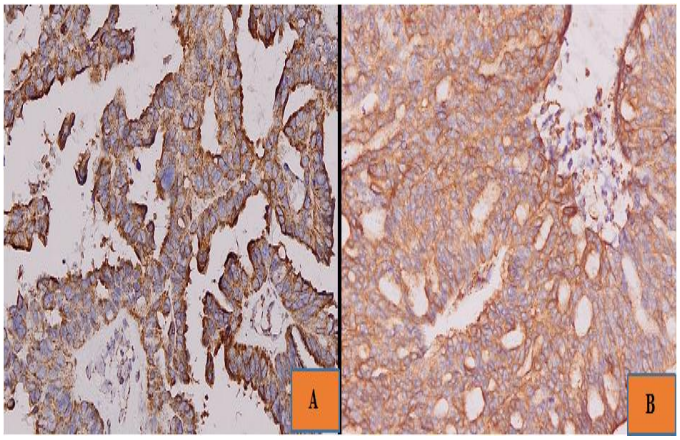

Figure 1: CD133 and ALDH1A1 expression of SOC in this study.

(A) CD133 strong expression, tumour cell stained with CD133 antibody in the cytoplasmic membrane (200x magnification)

(B) ALDH1A1 strong expression, tumour cell stained with ALDH1A1 antibody in the cytoplasm (200x magnification)

Discussion

Cancer stem cells (CSC) are small proportion of tumor cells that are proposed to be able to proliferate, self-renew and do invasion extensively.[17] This study analyzed the association between CSC markers expression (CD133 and ALDH1A1) with FIGO stage in SOC. The theory of CSC in OC was first examined by Bapat by culturing clone OC-cells in mice peritoneum. This clone of the tumor cell continued to differentiate and was capable of generating new tumors in the peritoneal cavity of mice[1, 18]

CD133 is a transmembrane glycoprotein, a product from a single-copy gene of human chromosome 4 (4p.15.33). Expression of CD133 is also regulated by some epigenic factors, demethylation in the CD133 gene can increase CD133 expression. Demethylation on CD133 promoter-1 is induced by TGFβ1. Demethylation in CD133 promoter-1 is found in colorectal carcinoma, gastric carcinoma, glioma, glioblastoma, hepatocellular carcinoma and OC.[19] CD133 increases the expression of Chemokine (C-C motif) ligand5 (CCL5) and its receptors (CCR1, CCR3, CCR5). CCL5 is a chemokine that can induce tumour cell metastasis. CCL5 can be produced by MSC (Mesenchymal Stem Cells), CAF (Cancer Associated Fibroblasts) and CSC. CCL5 expression is increased and binds to its receptors (CCR1, CCR3, CCR5) which further decreases E-cadherin levels and increases snail levels.[20] Snail will also reduce E-cadherin levels and trigger EMT (Epithelial-Mesenchymal Transition).[21] High CCL5 expression will mediate the activation of the NF-κB pathway so that tumour cells have a high capability in invasion and metastasis. NF-κB will induce the EMT process with increasing levels of Twist, MMP2 and MMP9.[22] NF-κB is a protein in nucleus which transcribes extracellular signals into a genetic code that promotes tumour growth, as an oncogen in OC. NF-κB triggers anti-apoptosis by regulation of CyclinD1, Cyclin E and c-Myc.[23] CD133 can also activate the PI3K / Akt and Wnt / β-Catenin pathways which induce self renewal and tumorigenesis.[9]

This study showed statistically significant association between CD133 expression and FIGO stage in SOC ($p = 0.004$). The higher expression of CD133 associated with higher FIGO stage on SOC. This study is in line with Zhao et al. who obtained a high CD133 expression in OC associated with advance FIGO stage and large tumour residual size. CD133 expression was also related to metastasis in lymph nodes in OC.[24] Cioffi et al. also obtained the same results which stated that high CD133 expression was significantly associated with advanced FIGO stage on SOC.[25] Wang et al. concluded that high expression of CD133 OC was significantly associated with advanced FIGO stage and gave a low survival rate.[26]

ALDH1A1 is an enzyme with high affinity in catalysing oxidation retinal (retinaldehyde) to retinoic acid (RA). RA will bind to RA receptors (RAR) and retinoid X receptors (RXR) in nucleus.[15] RAR is an RA receptor in the nucleus which is a factor affecting prognosis in OC.[27] The binding of RA with RAR will induce c-MYC and cyclinD1 expression so tumour cells will continue to proliferate.[15] ALDH1A1 also plays a role in inhibiting the effect of Reactive Oxygen Species (ROS) so that DNA damage can be avoided and tumour cells will continue to proliferate.[28] In this study ALDH1A1 was strongly expressed in 16 cases of SOC. Based on statistical analysis there was no significant association between ALDH1A1 expression with FIGO stage in SOC ($p = 0.197$). This result is in line with Landen et al. who stated that the results of ALDH1A1 expression were not related to FIGO stage and not related to survival rates on OC. High expression of ALDH1A1 was more significantly associated with resistance to chemotherapy. Landen et al. also obtained high results of ALDH1A1 expression found in low-grade group OC and in

tumors with low histopathological degrees.[29]

Opdenaker et al. also found that ALDH1A1 was not significantly associated with stage in triple negative invasive breast carcinoma. The role of ALDH1A1 in the determination of prognosis is still very controversial, some mention that ALDH1A1 is associated with a poor prognosis, but some said that ALDH1A1 is not associated with a poor prognosis. Opdenaker found that other ALDH1 isoforms, namely ALDH1A3, were more significantly associated with poor prognosis and were associated with stages of triple negative invasive breast carcinoma. ALDH1A3 is an ALDH enzyme isoform that is strongly associated with ALDH activity in CSC. While ALDH1A1 is more associated with resistance to chemotherapy.[30]

In contrast to the results of this study, Tao et al. mentioned that high expression of ALDH1 associated with FIGO stage and invasion of lympho-vascular vessels in OC.[1] Kim et al. also found that ALDH1 expression related with tumour size and thus affected stage in invasive breast carcinoma. However, ALDH1 was not associate with lymph node metastasis in invasive breast carcinoma.[31] This may relate with the nature of ALDH1 which plays a role in tumour cell proliferation, so invasive breast carcinoma tumour cells with high ALDH1 will have high proliferation. Consequently, the tumour size will be larger. Tumour size (T) is one component of TNM staging in invasive breast carcinoma. This is different from FIGO stage in SOC which does not make tumour size (T) as a component in staging.

Condello et al. mentioned that high expression of ALDH1A1 will facilitate tumor cells to spread to the peritoneal cavity. The role of ALDH1A1 in the formation of MCS is because it works as an enzyme with a detoxification function so that it can prevent tumor cells from damage and regulate tumor cell proliferation. Condello et al. also mentioned that ALDH1A1 expression increased in MCS OC compared to tumor cells that formed a group of monolayers. MCS also found high expression of β-catenin. β-catenin inhibition decreases ALDH1A1 expression. Condello concludes that β-catenin strongly influences the expression of ALDH1A1. This results differ from the results of this study, that ALDH1A1 expression was not significantly associated with FIGO stage in SOC. There was a sample difference between this study and Condello et al .'s study conducted with cell culture which analyzed the expression of β-catenin and ALDH1A1 on the OC xenograft model. We suggest that there are differences in the overall expression of ALDH1A1 in OC with ALDH1A1 expression in SOC. Until now, research on the expression of ALDH1A1 in SOC is still rare.

ALDH1 is an enzyme with many isoforms. ALDH1A1 and ALDH1A3 are high affinity isoforms that have been identified in CSC in various organs including the ovary. The difference of these isoforms will result in differences of regulation as CSC on OC. Saw et al. found that high expression of ALDH1A1 more related to certain histopathological subtypes in OC. High expression of ALDH1A1 was more likely to be found in endometrioid OC and mucinous OC, whereas in SOC and clear cell OC expression of ALDH1A1 tended to be low. The role of ALDH1A3 as CSC in breast carcinoma has been shown to be related to staging and poor prognosis, but the role of ALDH1A3 in OC in general still needs further research.[32]

Based on FIGO staging system theory, FIGO stage describes the extent of tumour cell invasion and its metastases to other organs. The early FIGO stage means the tumour mass confines to the ovary with or without extension to the tube, uterus with or without extension to intraperitoneal pelvic. If there is metastasis to the surrounding organs, to lymph nodes or distant metastasis beyond peritoneal metastasis it is referred to as advanced FIGO stage.[33] Tumour invasion and metastasis are the important factors influencing the stage. Tumour invasion and metastasis are strongly influenced by EMT (Epithelial-Mesenchymal Transition).

EMT is a process of epithelial tumour cells capable of assuming the characteristics of mesenchymal cells so that mesenchymal cells facilitate the migration of tumour cells through the extracellular matrix. EMT occurs due to down-regulation of E-cadherin and up-regulation of Snail1 and Twist1. EMT is also influenced by Wnt / β-catenin signaling pathway. During the period of tumour cell progression, EMT causes dissemination of group tumour cells, facilitates invasion by regulation of matrix metalloprotease (MMP) production and alters the cytoskeletal structure of tumour cells.[11] Hosono et al. found that Twist1 expression is higher in OC especially Clear cell carcinoma and associated with advance FIGO stage. High expression of Twist1 associates with the incidence of OC metastasis. This is due to the high expression of Twist1 which decreases E-cadherin levels.[34] The importance of the role of EMT in the mechanism of OC metastasis makes CD133 is able to become a marker in determining FIGO stage on SOC. Whereas ALDH1A1 plays a role in the proliferation and tumorigenesis processes. The role of ALDH1A1 in SOC and its correlation with β Catenin in the formation of MCS in SOC still needs further investigation. There may be other pathways between ALDH1A1 expression and MCS formation in SOC.

Conclusion

There was a significant association between CD133 and FIGO stage in SOC. But there was no significant association between ALDH1A1 expression and FIGO stage in SOC. Patient SOC with high expression of CD133 (histoscore >6) will associate with advance FIGO stage. It can be concluded that higher CD133 expression showed higher tumour cells ability to do invasion and metastasis and had higher influenced on FIGO stage, so the clinician has to do an appropriate management to increase survival rate and reduce the mortality rate of SOC patients.

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