Expression Of Rad6, Ddb2 And Cancer Stem Cell / Csc Protein ($Cd44^{+}/Cd24^{-}$) On Therapy Response To The Administration Of Chemotherapy In Ovarian Cancer: A Prospective Flow Cytometry Study

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Abstract

Objective: Ovarian cancer is the 8th deadliest women's cancer in the world. In 2018 there were 295.414 new cases with 184.799 deaths. In Indonesia, there are 13.310 (7,1%) ovarian cancer of 188.231 female cancer with an incidence of 9,7/100,000. Almost all patients experienced chemoresistance, recurrence, and poor prognosis after cytoreductive surgery followed by platinum-based chemotherapy. Chemo-resistant cancer cells have characteristic expressions of RAD6, DDB2, and cancer stem cell proteins (CSCs, $CD44^+/CD24^-$). RAD6 is a UBE2 enzyme required for DNA repair, mutagenesis, and proliferation. DNA damage-binding protein 2 (DDB2) protein is an amino acid that repairs the excision of nucleotides in DNA. $CD44^+/CD24^-$ protein is a transmembrane glycoprotein. Increased expression of $CD44^+/CD24^-$, RAD6, and decreased DDB2 are believed to be associated with chemoresistance, recurrence, and poor prognosis of the disease.

Methods: This study is a prospective cohort of 32 people in each group at the Obstetrics-gynecology and pathology Department of Cipto Mangunkusumo, Tarakan, Dharmais, and Fatmawati Hospital. All suspected ovarian cancer patients will undergo cytoreductive debulking and histopathological examination. Chemotherapy will be given for six series followed by six months of observation. We determine therapy response with the RECIST Criteria (Response Criteria in Solid Tumors) then classify it into chemo-resistant or chemo-sensitive groups. The patient will perform Flow cytometry blood tests to examine the expression of RAD6 and DDB2 and $CD44^+/CD24^-$. We used univariate, bivariate, and multivariate (logistic regression) analysis in this research.

Results: There was a significant relationship between increased levels of $CD44^+/CD24^-$, RAD6, and reduced DDB2 protein ($p<0,05$) with chemoresistance of ovarian cancer. Logistic regression test showed that the results of $CD44^+/CD24^-$ also had significant results compared to other variables.

Conclusion: These results indicate that $CD44^+/CD24^-$, RAD6, and DDB2 are significantly associated with ovarian cancer chemoresistance, and $CD44^+/CD24^-$ is the primary marker to predict ovarian cancer chemoresistance.

Introduction

Ovarian cancer is the 8th deadliest of women's cancer worldwide. In 2018, there were 295.414 new cases with 184.799 deaths. In Indonesia, there were 13.310 (7,1%) ovarian cancer cases of 188.231 cancer with an incidence of 9,7 per 100.000 (1). Usually, 70% of patients present with an advanced stage (III-IV) (2).

The standard treatment for ovarian cancer is cytoreductive surgery followed by platinum-based chemotherapy. Almost all patients experienced disease recurrence. Recent studies said that standard therapy had a chemosensitivity and chemoresistance rate of 77,4% and 18,1%, respectively. Recent studies said that ovarian cancer patients had a 12-month progression-free survival (PFS) with overall

survival (OS) was about 30 months (3, 4). The low survival rate of ovarian cancer patients is due to chemotherapy resistance caused by cancer stem cells (CSCs) protein.

CSCs are a new term that is believed to play an essential role in the initiation, tumor growths, metastasize, recurrence, and the presence of resistance. Meng *et al*. said that ovarian stem cell cancer with $CD44^{+}/CD24^{-}$ was 71–93% resistant to all chemotherapy agents, a recurrence rate of 83% (p = 0,003), and median PFS of 6 months (5). Hu *et al.* found that $CD44^{+}$ was positively expressed in the chemotherapy-resistant ovarian cancer significantly 91,17% with p < 0,05.(6)

Attention to CSCs mechanism has focused on the DNA damage response (DDR) in the tumorigenic process. Increased DDR can prevent the formation of CSCs and chemo-resistant cells. The DDR pathway consists of post replication repair (PRR), nucleotide excision repair (NER), etc. The process of NER occurs through several DNA binding proteins, such as DNA damage binding protein 2 (DDB2 or XPE) while in the PRR process, there is an expression of E2 ubiquitin-conjugating enzymes (UBE2) protein, such as RAD6 (7).

RAD6 is a UBE2 protein required for DNA regulation, associated with mitotic abnormalities and cell transformation. Increased expression of RAD6 enhances stemness function, chemoresistance, progression, and metastasize. The RAD6 on DNA is associated with chemoresistance and poor clinical prognosis in ovarian cancer. Somasagara et al. reported that RAD6 expression < 5 and > 5 was associated with 37,5% and 70% recurrence, respectively (8).

DDB2 is an amino acid in nucleotide excision DNA repair. Low expression of DDB2 causes increased chemotherapy resistance and leads to a poor prognosis (7). Han C *et al.* found that DDB2 mRNA expression levels > 0,5 had a better prognosis than values <0,5 (9). This decrease in protein expression causes a reduction in life expectancy (10).

Most cancer cells are sensitive to chemotherapy, but some CSCs are not detected and develop into disease recurrence. The CSC resistance will make the cells continue to move into the mitotic and interphase (G1, S, G2), while the G0/rest phase is inactive, continuously replicating (11). The aim of this study was to find relationships between $CD44^{+}/CD24^{-}$, RAD6 and DDB2 with chemotherapy response in ovarian cancer and the ability of the three markers to predict ovarian cancer chemotherapy response

Materials And Methods

Study Design

This study is a prospective cohort study at the obstetrics-gynecology and anatomical pathology department of Cipto Mangunkusumo Hospital, Tarakan Hospital, Dharmais Hospital, and Fatmawati Hospital from February 2018 until February 2022.

Participants

The research subjects were patients with ovarian carcinoma inclusion, stage II-IV ovarian cancer patients, and were willing to participate in the study. The sample exclusion criteria were pregnant patients and patients diagnosed with other types of cancer. The number of samples in this study was 32 people in each group with consecutive sampling methods.

Data Collection

Every patient suspected of ovarian cancer will undergo cytoreductive debulking and histopathological examination. If the pathology result is malignant, chemotherapy will be given for six series followed by six months observation. We determine therapy response with the RECIST Criteria (Response Criteria in Solid Tumors) then classify it into chemo-resistant or chemo-sensitive groups. The patient will perform Flow cytometry blood tests to examine the expression of RAD6 and DDB2 and CSC ($CD44^+/CD24^-$). In addition, demographic data, cancer stage, operation type, chemotherapy response, tumor cell differentiation (cancer stage), cancer histopathology, cancer size, cancer residue, ascites, lymph node metastasize, and serum Ca-125 levels were also taken. The staging of the disease was carried out using the FIGO criteria.

Flow cytometry

Blood is taken from peripheral blood at five cc and then centrifugated. The supernatant was discarded, and 50 µL was left, after which the cell mixture was resuspended. Their markers identified expression $CD44^+/CD24^-$, RAD6, and DDB2. Samples were reacted with fluorescent-labeled antibody against $CD44^+/CD24^-$ (monoclonal anti-human) $CD44^+$ labeled as PerCP, $CD24^-$ labeled as APC, RAD6 labeled as PE, and DDB2 labeled FITC. The four reagents were removed for leukocytes with CD45 labeled pacific blue. The samples in the Falcon tube were added with 2,5 µL of CD44 marker, 2,5 µL of CD24 marker, and 2,5 µL of RAD6 and DDB2, then incubated for 15 minutes in the dark at room temperature. After incubation, cells were lysed using 300 µL of lysing solution, then set again for 15 minutes in a dark room and at room temperature. Next, 1 mL of facs flow solution was added and centrifuged at 500 g for 5 minutes. The supernatant formed was discarded, then added with 500 µL perm wash buffer and centrifuged at 500 g for 5 minutes; the supernatant created was discarded. To be more optimal, 1mL perm wash buffer was added again and centrifuged at 500 g for 5 minutes. The last step was to add 200 µL of 1% paraformaldehyde in phosphate-buffered saline (PBS). After that, the analysis was carried out using a flow cytometer using four fluorochrome colors.

Cell identification was carried out using an automated flow cytometer (*BD Facs Calibur*). CSCs were identified through the positive expression of $CD44^+/CD24^-$ markers while the RAD6 and DDB2 through a positive expression of RAD6 and DDB2 markers with four different colors. Protein percentage is the percentage of expression of protein markers CSCs ($CD44^+/CD24^-$), RAD6, and DDB2 in the blood.

Statistical Analysis

We analyze univariate (descriptive data), followed by bivariate and multivariate analysis. Each categorical variable was tested with the chi-square or Fisher test while the numerical variables were

tested with an unpaired t-test or Mann-Whitney test if the data were not normally distributed. Multivariate analysis was also performed using logistic regression. Research ethics approval was obtained from the Health Research Ethics Committee of the Universitas Indonesia, Cipto Mangunkusumo Hospital.

Results

Basic Participants Characteristics

The total sample in this study was 32 samples in each group. All samples had undergone chemotherapy with 32 (50%) chemoresistance and 32 (50%) chemosensitive. The distribution of profiles and clinical characteristics of ovarian cancer patients can be seen in Table 1.

Table 1
Essential Clinical Characteristics of Ovarian Cancer Patient

Variable	Number (%)
• Chemoresistant	32 (50)
• Chemosensitive	32 (50)
Age (years old)	
• < 40	4 (6,3)
• 40–50	19 (29,7)
• > 50	41 (64,1)
Ca-125	
• ≤35	30 (46,9)
• > 35	34 (53,1)
Ovarian cancer stage	
• Early stage: II	5 (7,8)
• Advance stage: III - IV	59 (92,2)
Operation type:	
• Optimal Debulking	56 (87,5)
• Suboptimal Debulking	8 (12,5)
Differentiation/cancer grade	
• Good	13 (20,3)
• Intermediate	16 (25,0)
• Poor	35 (53,1)
Tumor histology type	
• Serous	24 (37,5)
• High-grade serous	14 (21,9)
• Mucinous	3 (4,7)
• Endometrioid	12 (18,8)
• *Clear cell*	10 (15,6)
• Others	1 (1,6)

Variable	Number (%)
Lymph nodes metastasize	
• Positive	32 (50)
• Negative	32 (50)
Ascites	
• Positive	36 (56,3)
• Negative	28 (43,7)
Tumor size	
• 5 cm	17 (26,6)
• 5–10 cm	15 (23,4)
• > 10 cm	32 (50)
Tumor residue	
• < 1cm	56 (87,5)
• > 1cm	8 (12,5)

Flow cytometry of Ovarian cancer

Figure 2 and 3. shows the results of flow cytometry. The proportion of $CD44^{+}/CD24^{-}$, RAD6, and DDB2 values were calculated based on the percentage of the total cells.

Bivariate Analysis

Table 2 shows that $CD44^{+}/CD24^{-}$, RAD6, DDB2, Ca-125, type of surgery, lymph node metastasize, tumor size and tumor residue have significant difference results ($p < 0,05$) with each odds ratio (OR) and relative risk (RR) values

Table 2
Bivariate Analysis of The Variables in Ovarian Cancer Patients.

Variable	Therapy Response		P value	OR	RR
	Chemoresistant (%)	Chemosensitive (%)			
$CD44^{+}/CD24^{-}$ expression			0,001*	10,7	18,0
• High (≥32.692)	25 (78,1)	8 (25)			
• Low (< 32.692)	7 (21,9)	24 (75)			
RAD6 expression			0,007*	4,76	7,27
• High (≥5.846.136)	15 (46,9)	5 (15,6)			
• Low (< 5.846.136)	17 (53,1)	27 (84,4)			
DDB2 Expression			0,046*	2,78	4,0
• High (≥7.370.316)	20 (62,5)	12 (37,5)			
• Low (< 7.370.316)	12 (37,5)	20 (62,5)			
Ca-125 Level	2 (6,25)	28 (87.5)	0,001*	105	42,4
• ≤35	30 (93,75)	4 (12,5)			
• > 35					
Ovarian cancer stage			0,162	4,42	1,95
• Early stage: II	1 (3,13)	4 (12,5)			
• Advance stage: III - IV	31 (96,87)	28 (87,5)			
Surgery type			0,023*	8,68	5,14
• Optimal Debulking	25 (84,4)	31 (96,87)			
• Suboptimal Debulking	7 (15,6)	1 (3,13)			
Differentiation/cancer grade	6 (18,75)	7 (21,88)	0,760	0,97	1,2
• Good	26 (81,25)	25 (78,12)			
• Intermediate - Poor					
Lymph nodes metastasize			0,012*	3,65	6,25
• Positive	21 (65,63)	11 (34,37)			
• Negative	11 (34,37)	21 (65,63)			

Note: *: p < 0,05, Significant results.

Variable	Therapy Response		P value	OR	RR
	Chemoresistant (%)	Chemosensitive (%)			
Ascites			1,000	1	0,0
• Positive	18 (56,25)	14 (43,75)			
• Negative	14 (43,75)	18 (56,25)			
Tumor size			0,001*	3,1	23,1
• 5 cm	0 (0)	17 (53,13)			
• 5–10 cm	32 (100)	15 (46,87)			
• > 10 cm					
Tumor residue			0,023*	8,6	5,1
• < 1cm	25 (84,4)	31 (96,87)			
• > 1cm	7 (15,6)	1 (3,13)			
Note: *: p < 0,05, Significant results.					

ROC and AUC Curves

ROC curve in Fig. 1 and Table 3 data showed that the $CD44^+/CD24^-$ protein has the best ROC curve and AUC value. The AUC value of the $CD44^+/CD24^-$ is 0,783, which means it has a moderate level of accuracy, but the value is significant (p < 0,05). The sensitivity of this protein is 78%, and its specificity is 75% for detecting chemoresistance. RAD6 protein had an AUC of 0,586 (very weak accuracy), not significant (p > 0,05), with a sensitivity of 84% and specificity of 46%. DDB2 had an AUC value of 0,61 (weak accuracy), not significant (p > 0,05), sensitivity 37,5%, and specificity of 37,5%.

Table 3

AUC analysis of *cancer stem cells* ($CD44^+/CD24^-$), RAD6, and DDB2 variables

Variable	AUC	SD	95% *CI*	Sensitivity (%)	Specificity (%)	Cutoff value	P value
$CD44^+/CD24^-$	0,783	0,60	0,66 – 0,89	78	75	32.692	0,001*
RAD6	0,586	0,74	0,44 – 0,73	84	46	5.846.136	0,237
DDB2	0,611	0,74	0,46 – 0,75	37.5	37.5	7.370.316	0,126
Note : *: p < 0,05, significant							

Multivariate Analysis

In logistic regression, Table 4 found the Hosmer & Lemeshow test showed a p-value of 0,936 ($p > 0.05$), which means the regression model is fit or appropriate. We found the variables used as regression models are the $CD44^+/CD24^-$ and Ca-125. The following are the regression model:

Table 4
Logistic Regression Result

Variable	*p value*	Omnibus test *p value*	Hosmer & Lemeshow test *p value*	Result
$CD44^+/CD24^-$	0,001	0,00	0,936	Modeled
RAD6	0,007			Eliminated
DDB2	0,046			Eliminated
Ca-125	0,001			Modeled
Operation type	0,023			Eliminated
Lymph nodes metastasize	0,012			Eliminated
Cancer size	0,001			Eliminated
Cancer residue	0,023			Eliminated

$$\log\frac{p}{1-p} = \beta 0 + \beta 1.x1 + \beta 2.x2...$$

$$\log\frac{p}{1-p} = -5,991 + ((-1,958).(CD44CD24)) + ((4,418).(Ca125))$$

The results of Table 5 and Table 6 show that $CD44^+/CD24^-$ and Ca-125 has p value < 0,05, which means that they both have a significant effect on ovarian chemoresistancs. The value of Cox & Snell R Square is 0,571 and Nagelkerke's R Square is 0,762. This study's maximum − 2 Log-Likelihood Estimator (MLE) value is 54,183.

Table 5
Logistic regression of $CD44^+/CD24^-$ and Ca-125

No	Variables	Beta value (β)	Standard deviation	Wald	p value	*95% CI*
X1	$CD44^+/ CD24^-$	-1,958	0,928	4,449	0,035	0,023 − 0,871
X2	Ca-125	4,418	0,969	20,780	0,000	12,4-553,9
Constant		**-5,991 ($\beta 0$)**	**1,618**	**13,712**	**0,000**	**-**

Table 6

Logistic Regression Model Characteristics of $CD44^+/CD24^-$ and Ca-125

No	Variable	-2 log Likelihood (initial)	-2 log Likelihood (final)	MLE	Cox & Snell R Square	Nagelkerke R square
X1	$CD44^+/CD24^-$	88,723	34,540	54,183	0,571	0,762
X2	Ca-125					
Note: MLE = *Maximum – 2 Log Likelihood Estimator*						

Discussion

This study showed that there is overexpression of $CD44^+/CD24^-$ and RAD6 while there is lower expression of DDB2 in chemoresistance ovarian cancer patients. $CD44^+$ (cluster of differentiation 44) and $CD24^-$ expression is associated with increased ovarian cancer oncogenesis and progression (5, 12). $CD44^+$ overexpression has also been found in pancreatic cancer (13), breast cancer (14), gastric cancer (15), urothelial bladder cancer(16) and colorectal cancer (17). It is associated with metastasize, recurrence, chemoresistance, and poor survival rates in the ovarian cancer (18). $CD24^-$ is a cell surface adhesion molecule frequently detected in invasive ovarian carcinoma. High $CD24^-$ expression in invasive ovarian cancer predicts shorter overall survival than low $CD24^-$ markers (19).

$CD44^+/CD24^-$ is a good predictor of ovarian cancer chemoresistance. We found that higher $CD44^+/CD24^-$ has a significant result ($p < 0,05$) with moderate accuracy (AUC 0,7 – 0,8) with a sensitivity of 78% and specificity of 75%. Meng *et al.*, (2012) found that ovarian cancer cells with high $CD44^+/CD24^-$ expression have stem cells characteristics: higher aggressive, invasive, progressive, and multiplicative properties in each tumor histology type. This ovarian cancer cell also has higher chemoresistance properties, recurrence rate, and aggravating prognosis (5).

Li *et al.*, (2017) found that high $CD44^+/CD24^-$ protein levels indicated breast tumor malignancy higher rates of cell proliferation, tumorigenesis, and metastasize. Breast cancer with CSC has resistance to chemotherapy and radiotherapy (20). Yan *et al.*, (2013) also found that cells with high $CD44^+/CD24^-$ expression showed higher migration and invasion properties and were the cause of chemoresistance (21).

There was higher $CD44^+$ expression in paclitaxel-resistant cell lines than paclitaxel-sensitive cell lines in a mouse model using ovarian cancer xenografts while patients with ovarian cancer showed that $CD24^-$ cells were more resistant to cisplatin and increased tumorigenesis ability (22). A meta-analysis study showed that $CD44^+$ protein was associated with poorer cancer-specific survival rates in patients undergoing chemoradiotherapy [(23)]. The study by Zhang *et al.*, (2019) found that high expression of

markers CD105, $CD44^{+}$, and CD106 related to chemoresistance, poorly differentiated, and advanced-stage ovarian cancer (18).

RAD6 has a significant role in activating several DNA repair pathways and is substantial in chemoresistance in ovarian cancer (24). RAD6 overexpression is associated with mitotic abnormalities and tumor progression (25). We found that there was a significant increase in RAD6 levels ($p < 0,05$) in chemoresistance patients. However, ROC and AUC results were not significant ($p > 0,05$), and the accuracy was very weak (AUC 0,5 – 0,6), sensitivity 84%, specificity 46%.

Clark *et al.*, (2018) investigated the role of RAD6 in chemoresistant ovarian cancer by inhibiting RAD6A and RAD6B in several ovarian cancer. These cells showed decreased expression of CSC markers, activation of DDR protein, and concomitant sensitivity to carboplatin responses suggesting that RAD6 expression increases after chemotherapy and causes chemoresistance in cancer cells through stimulating CSC protein expression and increasing DNA repair activity (25). The study by Somasagara et al., (2016) found association between chemoresistance and increased RAD6 in ovarian cancer cells through RAD6-mediated ubiquitin signaling, which led to increased DDR and CSC protein expression. In addition, a higher RAD6 ($\geqslant$5,1) was also associated with a disease recurrence rate of 70%. (26) Another study concluded that RAD6 related to the severity of ovarian cancer, breast cancer, and melanoma. Rad6 levels were significantly increased in severe ovarian cancer with platinum chemoresistance (27).

RAD6 overexpression can increase stem cell characteristics, making them more aggressive, metastasize and relapse. The epigenetic influence of RAD6 causes the ubiquitination of some histone variants which then regulates genes related to DNA repair, cell resistance, and chemoresistance (27). RAD6 is also closely related to RAD18, a protein E3 ubiquitin ligase that regulates the DNA repair pathway in Fanconi anemia and the BRCA gene in breast cancer (26) R.AD6 was involved in breast cancer chemoresistance in which researchers inhibited RAD6 with a small molecule inhibitor and found an increased sensitivity to cisplatin (28). In bladder cancer, it was also found that overexpression of enzymes from the UBE2 group, one of which was RAD6, could affect the growth of bladder cancer cells. An experiment was carried out by stopping the expression of UBE2, then the cells would stop growing in the G2/M phase and increase the apoptosis of these cancer cells (29).

DDB2 is a protein localized in the cell nucleus that contribute to gene transcription, cell cycle progression, and protein degradation (30). The decrease in DDB2 also affects ovarian cancer's aggression, metastasize, and severity (8). This study found that DDB2 protein expression was found significantly lower in chemotherapy-resistant patients ($p < 0,05$). ROC and AUC analysis results are $p > 0,05$, weak accuracy (AUC 0.6–0.7), sensitivity 37,5%, specificity 37,5%.

DDB2 levels are high in the chemo-sensitive cancer patient group because DDB2 participates in the tumor suppression process in at least three ways, namely: promoting the nucleotide excision repair (NER) process, supporting apoptosis, and inducing cell aging after DNA damage has occurred.(31) The loss of DDB2 function in normal cells can lead to susceptibility to tumor growth. DDB2 gene mutation causes

loss of function and gives rise to the phenotypic characteristics of Xeroderma Pigmentosum group E, which is characterized by malignant skin tumors. Mice with low DDB2 levels are not only hypersensitive to UV-related carcinogenesis processes but also have a high incidence of broad-spectrum spontaneous malignant tumors from internal organs. Thus, DDB2 acts as a mediator in the suppression of the p53 and BRCA1 pathways, thereby being a tumor cell suppressor, protecting against cancer by regulating the cell cycle and increasing the occurrence of apoptosis as opposed to being directly involved in the repair of DNA damage.(32)

Barakat *et al.* investigated the expression of DDB2 in several cisplatin-resistant ovarian cancer cell lines, and the results obtained were lower DDB2 expression than cisplatin-sensitive cells (33). The study also further explained that low DDB2 expression is associated with chemoresistance poor patient prognosis (9). DDB2 can reduce excess CSC protein ($CD44^+/CD24^-$) in large ovarian cancer. Overexpression of DDB2 in human ovarian cancer cells decreased the ability of these tumor cells to replicate (9). Han *et al.*, (2014) stated that low DDB2 protein expression was associated with poor prognosis ovarian cancer patients. DDB2 deficiency causes ovarian tumors to relapse due to the expansion of the CSCs population (34).

Yang *et al.*, (2018) found that DDB2 protein expression was associated with the onset, progression, and prognosis of colorectal cancer. Increased DDB2 expression was significantly associated with a better colorectal cancer prognosis (35). DDB2 was found to be able to inhibit colon cancer metastasizing through the mechanism of decreasing gene expression, which is an activator of Epithelial-to-Mesenchymal Transition (EMT) in colon cancer, and NF-κB found in breast cancer (9). Decreased DDB2 also affects EMT activation, triggering squamous cell carcinomas in the head and neck region (10). A Study on non-small cell lung cancer (NSCLC) found that DDB2 can facilitate the process of breaking the cancer cell growth. Conversely, if DDB2 decreases, the G2 cycle will continue, tumor growth and therapy resistance will occur (36).

This study applies multivariate analysis. Multivariate analysis with logistic regression found that two variables with almost the same effect and can be used as logistic regression models are the $CD44^+/CD24^-$ and Ca-125 variables. There have been no internationally published studies describing the $CD44^+/CD24^-$ and Ca-125 regression models. Furthermore, it may be necessary to carry out further research to confirm this regression model.

In this study, some shortcomings made us unable to prove that RAD6 and DDB2 can predict ovarian cancer chemoresistance. Although the results showed a significant difference ($p < 0,05$), the accuracy was still weak. It cannot yet be used as a good predictor of ovarian cancer chemoresistance and need further research.

Conclusion

We conclude that there is a significant relationship between increased levels of $CD44^+/CD24^-$, RAD6, and reduced DDB2 protein ($p < 0,05$) with chemoresistance of ovarian cancer. Logistic regression results indicate that $CD44^+/CD24^-$ is significantly associated with ovarian cancer chemoresistance and can be used as a good predictor of ovarian cancer chemoresistance whereas RAD6 and DDB2 did not yet have similar results.

Declarations

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Figures

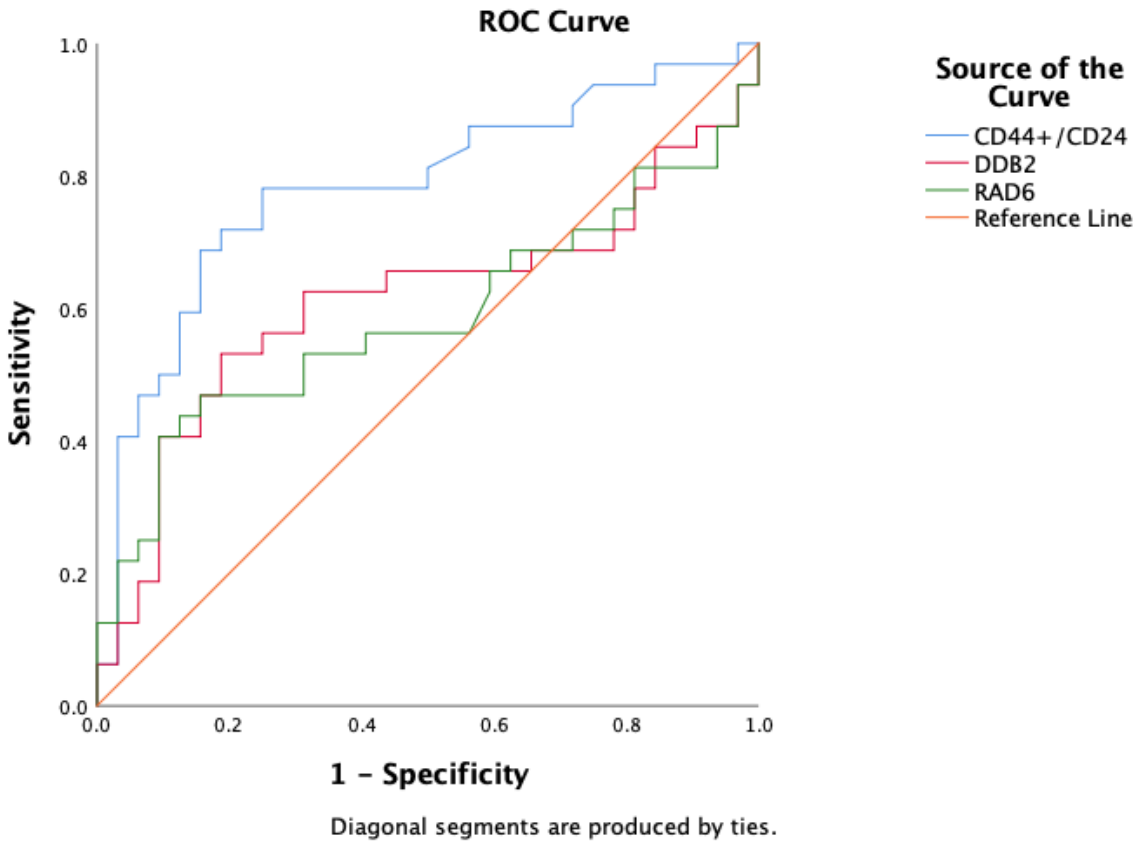


Figure 1

ROC Curve of $CD44^{+}/CD24^{-}$, DD82, and RAD6 with therapy response

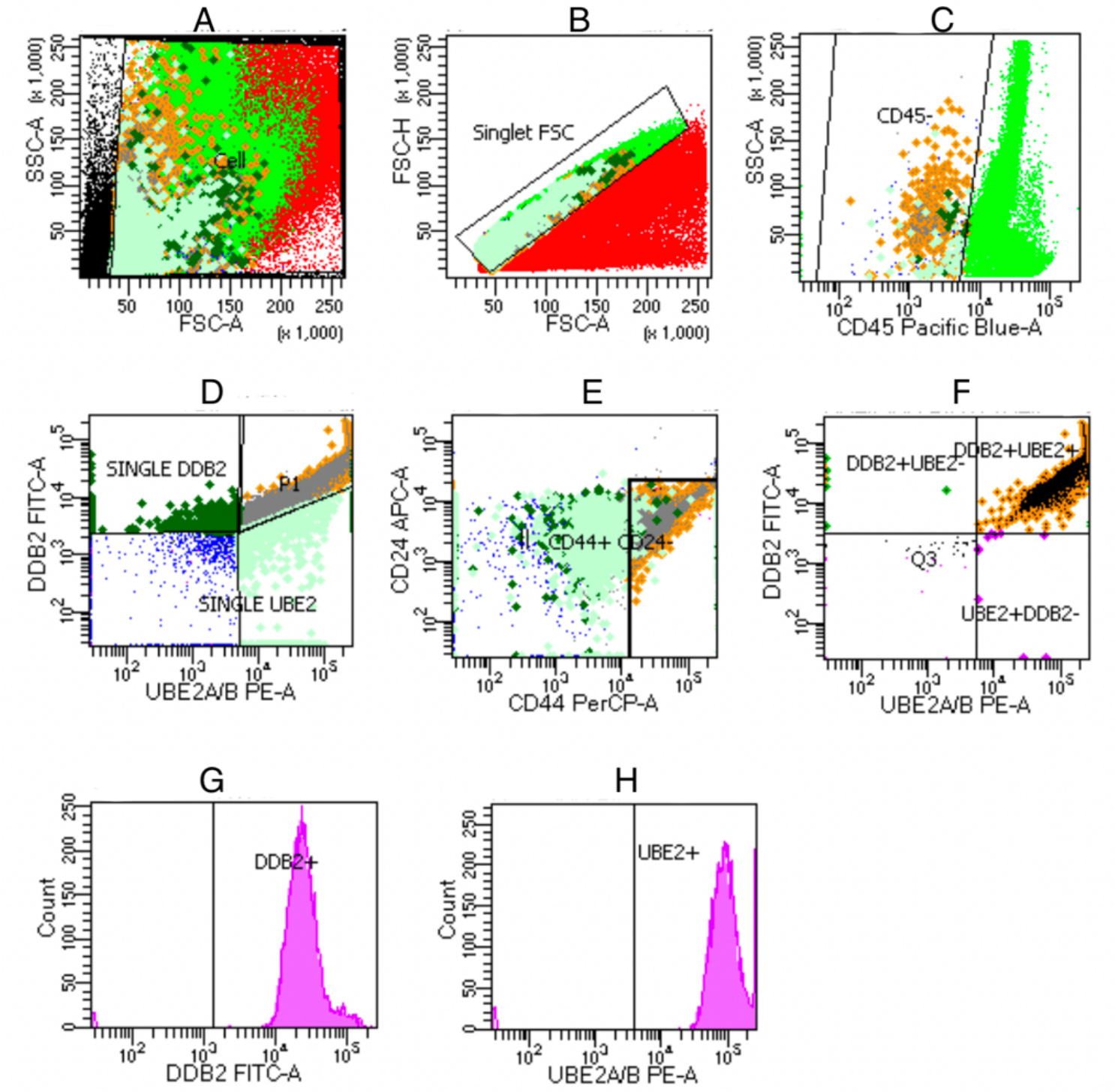
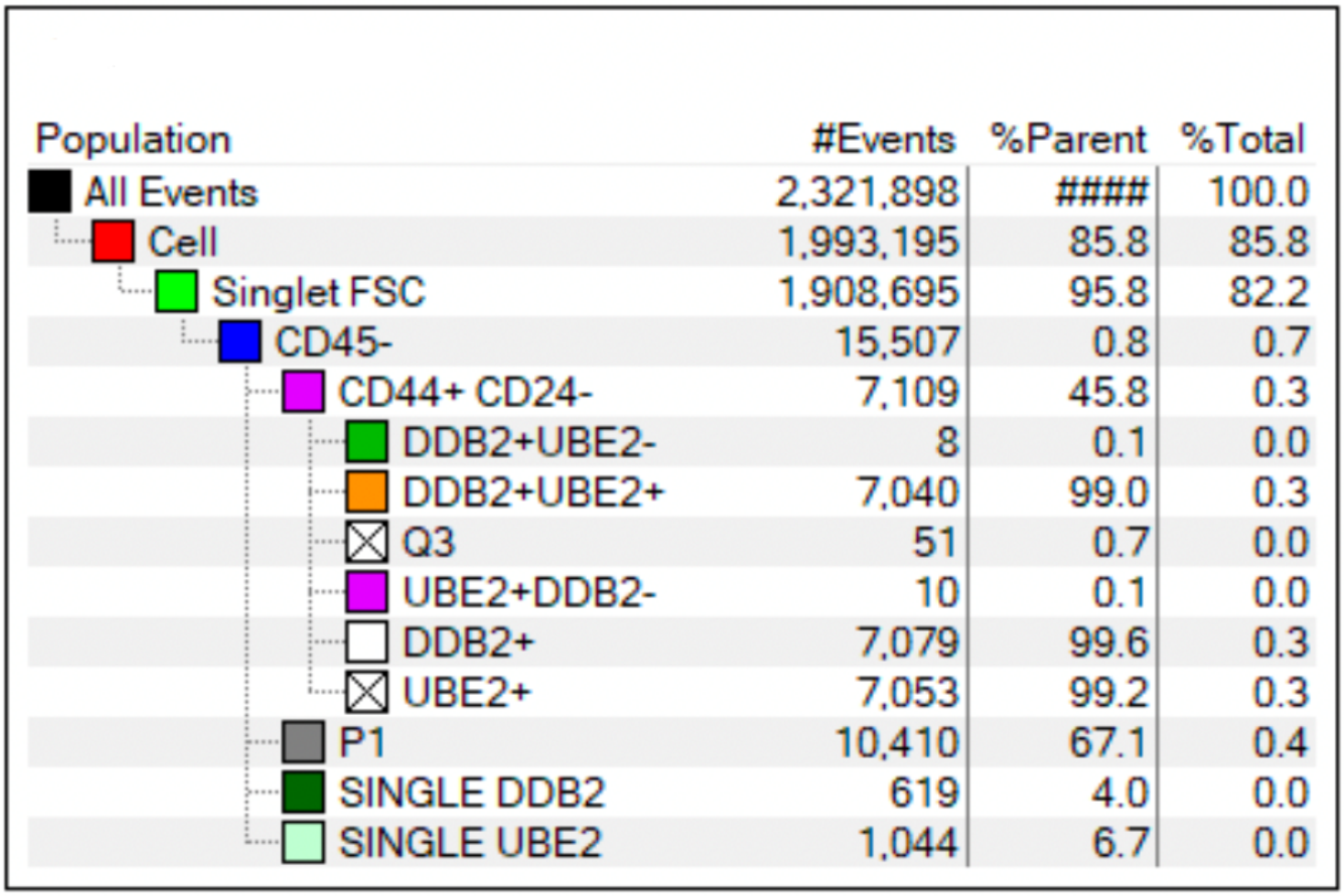


Figure 2

Overview of Flow cytometry Results. (A): total cells, (B): Singlet FSC, (C): CD45 labeled pacific blue, (D): UBE2A/B labeled PE-A, (E): CD44 labeled PerCP, (F): UBE2A/B labeled PE-A(G): graphic DDB2 cell count labeled FITC-A, (H): graph of UBE2A/B cell count labeled PE-A



Population	#Events	%Parent	%Total
All Events	2,321,898	####	100.0
Cell	1,993,195	85.8	85.8
Singlet FSC	1,908,695	95.8	82.2
CD45-	15,507	0.8	0.7
CD44+ CD24-	7,109	45.8	0.3
DDB2+UBE2-	8	0.1	0.0
DDB2+UBE2+	7,040	99.0	0.3
Q3	51	0.7	0.0
UBE2+DDB2-	10	0.1	0.0
DDB2+	7,079	99.6	0.3
UBE2+	7,053	99.2	0.3
P1	10,410	67.1	0.4
SINGLE DDB2	619	4.0	0.0
SINGLE UBE2	1,044	6.7	0.0

Figure 3

Details of Flow cytometry Cell Calculation Results. $CD44^{+}/CD24^{-}$ was calculated based on the proportion of purple CD44+/CD24- cells. RAD6 (UBE2) is calculated based on the ratio of orange, purple and single UBE2 cells; DDB2 is calculated based on the proportion of green, orange, and white colors.