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DDX4 (DEAD box polypeptide 4) colocalizes with cancer stem cell marker CD133 in ovarian cancers

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ABSTRACT

DDX4 (DEAD box polypeptide 4), characterized by the conserved motif Asp-Glu-Ala-Asp (DEAD), is an RNA helicase which is implicated in various cellular processes involving the alteration of RNA secondary structure, such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. DDX4 is known to be a germ cell-specific protein and is used as a sorting marker of germline stem cells for the production of oocytes. A recent report about DDX4 in ovarian cancer showed that DDX4 is overexpressed in epithelial ovarian cancer and disrupts a DNA damage-induced G2 checkpoint.

We investigated the relationship between DDX4 and ovarian cancer stem cells by analyzing the expression patterns of DDX4 and the cancer stem cell marker CD133 in ovarian cancers via tissue microarray. Both DDX4 and CD133 were significantly increased in ovarian cancer compared to benign tumors, and showed similar patterns of expression. In addition, DDX4 and CD133 were mostly colocalized in various types of ovarian cancer tissues. Furthermore, almost all CD133 positive ovarian cancer cells also express DDX4 whereas CD133-negative cells did not possess DDX4, suggesting a strong possibility that DDX4 plays an important role in cancer stem cells, and/or can be used as an ovarian cancer stem cell marker.

1. Introduction

The germ cell-specific marker DDX4, which is the human ortholog of the *Drosophila* gene vasa, was first mapped and cloned by radiation hybrid mapping [1]. Vasa has been reported to play a central role in several aspects of germ cell development. It encodes a member of the DEAD (Asp-Glu-Ala-Asp) box family of ATP-dependent RNA helicases [2,3]. A null mutation removing the entire vasa coding region results in female sterility with severe defects in oogenesis, including abnormal germ-line differentiation and oocyte determination [4]. The expression of the DDX4 gene is also restricted exclusively to ovary and testis and is not detected in any somatic tissues. DDX4 protein is cytoplasmic and is most abundant in spermatocytes and mature oocytes, suggesting that it is a highly specific marker of germ cells [1].

In a recent report, Tilly and colleagues described improvements in the technology of isolating primitive germ cells, which can serve as stem cells to produce oocytes. They confirmed that the carboxy-terminal portion of DDX4 is exposed on the outer surface of primitive germ cells, and they sorted these cells by fluorescence activated cell sorting (FACS) using a DDX4 antibody. The sorted germ cells can be expanded for months and can spontaneously generate oocytes, as determined by morphology, gene expression, and haploid status [5].

Recent studies have revealed the existence of cancer stem cells with the exclusive ability to regenerate tumors. These cancer stem cells share many characteristics with normal stem cells, including

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self-renewal and differentiation. Cancer stem cells also have been identified in ovarian cancer and most other carcinomas as a small population of cells that can self-renew. CD133 has emerged as one of the most promising markers of ovarian cancer stem cells [6]. DDX4 is a specific marker of pluripotent germ cells in the ovary and may be involved in stem-like characteristics in cancer stem cells, especially ovarian cancer stem cells (OCSC). Only one report has shown expression of DDX4 in epithelial ovarian cancers [7]. The authors analyzed DDX4 expression in 75 epithelial ovarian cancers and found DDX4 expression in 21 of those 75 cases. The expression pattern of DDX4 positively correlated with high age and serous histology. Based on those results, they suggested that DDX4 may play a direct role in the progression of EOC or serve as a valuable marker of tumorigenesis [7].

Here, we report the first analysis and comparison of the expression patterns and colocalization of DDX4 and CD133 in 59 ovarian cancer patients via tissue microarray and immunofluorescence analysis to identify the expression patterns and relationship of those two markers in ovarian cancer cells.

2. Materials and methods

2.1. Tissue microarrays

Tissue microarray slides of cervical cancer were purchased from Super Bio Chips (SuperBioChips Laboratories, Seoul, Korea). No clinical information, except the age and gender of each patient, and stage of cancer, was available for the tissue on these arrays. The detailed information of the patients is presented in Table 1.

2.2. Immunofluorescence

Paraffin-embedded sections were deparaffinized and hydrated. For antigen retrieval, slides were immersed in citrate buffer (0.01 M, pH 6.0) and heated twice in a microwave oven (700 W or high) for 5 min each. Immunofluorescence was analyzed as described previously [8]. In short, slides were permeabilized by incubation in PBS containing 0.1% Triton X-100 for 5 min and incubated with 10% normal serum in PBS for 1 h to block nonspecific antibody binding. Slides were then incubated with a mixture of mouse anti-DDX4 monoclonal (1:100 dilution; Abcam Inc., Cambridge, MA, USA) and rabbit anti-CD133 polyclonal antibody (1:100 dilution; Abcam Inc.) at 4 °C overnight. After primary antibody incubation, slides were washed three times in PBS for 5 min each and incubated with secondary antibodies (Alexa Fluor 546 anti-mouse antibody and Alexa Fluor 488 anti-rabbit antibody; Invitrogen, Carlsbad, CA, USA) for 1 h. The slides were observed for epifluorescence with a confocal laser-scanning microscope.

Ovarian cancer cells (OVCAR-3 and SKOV-3) were grown on coverslips and visualized by confocal microscopy, as previously described [10]. Briefly, cells on glass coverslips were rinsed three times with PBS, fixed for 10 min at room temperature by incubation in 4% paraformaldehyde, and then permeabilized by incubation in PBS containing 0.1% Triton X-100 for 5 min. The cells were incubated with 1% bovine serum albumin in PBS for 1 h to block nonspecific antibody binding and then incubated for 1 h at room temperature with anti-DDX4 monoclonal (1:100 dilution; Abcam Inc.) and rabbit anti-CD133 polyclonal antibodies (1:100 dilution; Abcam Inc.) at 4 °C overnight. After primary antibody incubation, slides were washed three times in PBS for 5 min each and incubated with secondary antibodies (Alexa Fluor 546 anti-mouse and Alexa Fluor 488 anti-rabbit antibodies; Invitrogen, Carlsbad, CA, USA) for 1 h. The slides were observed for epifluorescence with a confocal laser-scanning microscope. The number of DDX4 and CD133 double positive ($DDX4^+/CD133^+$) and single positive ($DDX4^+/CD133^-$ or $DDX4^-/CD133^+$) cells of the OVCAR-3 and SKOV-3 cell lines were counted under 400× magnification. Twelve fields were counted and the mean value was calculated for each cell line.

2.3. Semiquantitative assessment

For protein expression assessment, staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), 3 (strong), or 4 (very strong). Focality of expression was evaluated and factored into the overall score ((0.5) focally weak, (1.5) focally moderate, (2.5) focally strong, (3.5) focally very strong). Three well-trained and blinded observers read the slides and scored the expression of DDX4 and CD133. The detailed expression scores are presented in Table 1.

2.4. Statistical analysis

The protein expression levels and number of blood vessels per area were measured for calculation of mean values and 95% confidence intervals. Statistical significance of differences among the groups was determined using a two-tailed Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

2.5. Western blot analysis

Western blot analysis was conducted as previously described [10]. Briefly, 100 μg of cell lysate was separated by electrophoresis on a Novex 4–20% Tris–glycine gel (Invitrogen, Carlsbad, CA, USA). The protein concentrations of the lysates were determined by the bicinchoninic acid protein assay system (Pierce, Rockford, IL, USA). Equal protein loading was confirmed by Coomassie blue staining of duplicate gels after electrophoresis. The gels were incubated in a blotting buffer containing 1× Novex Tris–glycine transfer buffer (Invitrogen) and 20% methanol for 30 min at room temperature. Proteins were transferred to a nitrocellulose membrane (Invitrogen) by electrotransfer. The membrane was preincubated for 2 h in Tris-Buffered Saline (TBS) containing 5% skim milk and 0.05% Tween 20 (TBS-T). The membranes were incubated overnight at 4 °C in TBS-T plus mouse anti-DDX4 monoclonal antibody (1:1000 dilution; Abcam Inc.), rabbit anti-CD133 polyclonal antibody (1:1000 dilution; Abcam Inc.), and mouse anti-GAPDH monoclonal antibody (1:1000 dilution; Abcam Inc.). The membranes were washed five times with TBS-T and then incubated with species-appropriate horseradish peroxidase-conjugated secondary antibodies (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for 1 h at room temperature. The membranes were washed 5 times with TBS-T, and bound antibody was detected with an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech).

3. Results

3.1. Expression of DDX4 and CD133 in ovarian cancers

The expression patterns of DDX4 and CD133 in various kinds and stages of ovarian cancers were analyzed (Table 1). First, the expressions of DDX4 and CD133 in benign tumors, tumors of borderline malignancy (TBM) or uncertain malignant potential (UMP), and cancer were compared. As shown in Fig. 1A, the expression levels of both DDX4 and CD133 were significantly increased in cancer, compared with benign tumors ($P = 0.03109$ and $P = 0.00064$, respectively). Comparing the expression patterns in various tumor stages, both DDX4 and CD133 were increased in stage IV compared with stage I, but only CD133 showed significant induction (Fig. 1B). Neither DDX4 nor CD133 expression changed significantly

Table 1
Patients information.

No.	Age	Diagnosis	pTNM	Stage	Tissue types	Expression	
						DDX4	CD133
1	65	Serous papillary cystadenoma of borderline malignancy	T1aN0M0	I A	5	1.333	1.667
2	26	Mucinous cystadenoma of borderline malignancy	T1aN0M0	IA	5	1.833	1.848
3	35	Serous papillary cystadenoma of borderline malignancy	T1aN0M0	IA	5	1.333	1.121
4	65	Serous cystadenocarcinoma	T2bN0M0	IIB	6	1.500	1.727
5	48	Endometrioid carcinoma	T2bN0M0	IIB	6	2.333	1.394
6	44	Granulosa cell tumor	T1aN0M0	IA	5	0.500	0.818
7	49	Endometrioid carcinoma	T2bN0M0	IIB	6	2.333	2.303
8	67	Mucinous cystadenoma of borderline malignancy	T2bN0M0	IIB	5	0.500	0.818
9	75	Serous cystadenofibroma	T1aN0M0	IA	4	0.500	0.818
10	50	Papillary serous cystadenocarcinoma , poorly differentiated	T2cN0M0	IIC	6	1.333	1.576
11	44	Fibroma	T1aN0M0	IA	4	1.167	0.788
12	50	Papillary serous cystadenocarcinoma	T3cN0M1	IV	6	4.000	4.091
13	47	Endometrioid carcinoma	T3cN0M1	IV	6	3.000	3.455
14	38	Mixed Brenner tumor and mucinous cystadenoma	T1aN0M0	IA	4	1.000	1.273
15	58	Papillary serous cystadenocarcinoma	T2bN0M0	IIB	6	2.333	2.303
16	70	Papillary serous cystadenocarcinoma	T3bN0M0	IIIB	6	2.833	3.030
17	65	Struma ovarii	T1aN0M0	IA	4	0.667	0.970
18	79	Endometrioid adenoacanthofibroma	T1aN0M0	IA	4	0.667	0.788
19	57	Serous surface papillary carcinoma	T2bN0M0	IIB	6	1.167	1.606
20	43	Papillary serous cystadenocarcinoma	T3bN0M0	IIIB	6	0.833	1.030
21	68	Undifferentiated carcinoma	T1bN0M0	IB	6	2.333	1.758
22	50	Clear cell carcinoma	T1aN0M0	IA	6	1.500	1.364
23	23	Dysgerminoma	T2bN0M0	IIB	6	0.167	0.970
24	51	Common epithelial carcinoma , poorly differentiated	T2bN0M0	IIB	6	3.167	3.333
25	70	Malignant muellerian mixed tumor	T1aN0M0	IA	6	3.167	2.606
26	21	Dysgerminoma	T1aN0M0	IA	6	1.500	1.727
27	34	Fibrothecoma	T1aN0M0	IA	4	1.333	1.212
28	61	Papillary serous cystadenocarcinoma	T3bN0M0	IIIB	6	2.667	2.970
29	54	Sertoli-leydig cell tumor	T1bN0M0	IB	4	2.500	2.636
30	53	Fibrothecoma	T1aN0M0	IA	4	0.500	0.727
31	54	Fibrothecoma	T1aN0M0	IA	4	2.333	1.303
32	34	Papillary serous cystadenocarcinoma	T2bN0M0	IIB	6	2.667	2.970
33	31	Dysgerminoma	T2cN0M0	IIC	6	2.500	2.727
34	51	Papillary serous cystadenocarcinoma	T2bN1M0	IIIC	6	2.667	1.697
35	61	Granulosa-theca cell tumor	T1aN0M0	IA	5	1.167	1.515
36	24	Sertoli-leydig cell tumor	T1aN0M0	IA	4	2.167	1.242
37	27	sclerosing stromal tumor	T1aN0M0	IA	5	2.667	1.515
38	41	Clear cell carcinoma	T2bN0M0	IIB	6	0.667	0.879
39	56	Malignant muellerian mixed tumor	T1aN0M0	IA	6	3.667	3.879
40	41	Clear cell carcinoma	T1aN0M0	IA	6	0.500	0.727
41	27	Embryonal carcinoma	T2aN0M0	IIA	6	0.500	0.727
42	55	Granulosa cell tumor	T1aN0M0	IA	5	0.667	0.970
43	23	Dysgerminoma	T1aN0M0	IA	6	1.333	2.212
44	64	Papillary serous cystadenocarcinoma	T2cN1M0	IIIC	5	2.667	3.061
45	43	Metastatic adenocarcinoma (most likely from breast)	M1	IV	6	0.167	0.879
46	48	Malignant lymphoma		II	6	2.833	3.121
47	50	Adenocarcinoma from salpinx	T2bN0M0	IIB	6	2.500	2.818
48	25	Fibrothecoma	T1aN0M0	IA	4	2.000	1.909
49	46	Metastatic undifferentiated carcinoma	M1	IV	6	2.667	3.061
50	37	Metastatic signet ring carcinoma from stomach	M1	IV	6	0.667	0.879
51	55	Serous cystadenocarcinoma, moderately differentiated	T3bN0M0	IIIB	6	0.167	0.606
52	55	Fibrothecoma	T1aN0M0	IA	4	0.333	0.485
53	45	Serous cystadenocarcinoma, moderately differentiated	T2cN0M0	IIC	6	3.333	3.121
54	37	Endometrioid carcinoma	T2bN0M0	IIB	6	2.333	2.758
55	58	Fibrothecoma	T1aN0M0	IA	4	1.333	0.848
56	65	Adenocarcinoma, poorly differentiated	T3cN0M0	IIIC	6	2.667	2.333
57	39	Clear cell carcinoma	T2cN1M0	IIIC	6	1.667	2.333
58	70	Fibroma	T1aN0M0	IA	4	1.500	0.909
59	58	Clear cell carcinoma	T1aN0M0	IA	6	1.333	2.121

Tissue type: (1) normal tissue from a non-cancer patient; (2) normal tissue from a cancer patient, but the cancer involves an unrelated organ; (3) normal tissue adjacent to the cancer; (4) benign tumor; (5) tumor with borderline malignancy or uncertain malignant potential; (6) cancer.
Expression: (0) no expression; (0.5) weak focal expression; (1) weak expression; (1.5) moderate focal expression; (2) moderate expression; (2.5) strong focal expression; (3) strong expression; (3.5) very strong focal expression; (4) very strong expression.

according to ovarian cancer type (Fig. 1C and D). The expression levels of both DDX4 and CD133 were analyzed by age group. As shown in Fig. 2A, the expression of neither DDX4 nor CD133 varied by age among the pooled samples (which include benign tumors, TBM or UMP, and cancer), but in the cancer samples, they were significantly increased in the oldest age group (60–70) (Fig. 2B). This result of increased levels of DDX4 in cancers in the oldest age group is in agreement with a previous study by Hashimoto et al. [7]. To determine whether the induction of both DDX4 and CD133 in cancers in the oldest age group was related to the tumor stage of ovarian cancers, we analyzed tumor stages in all ovarian cancer samples. However, we found that tumor stages were significantly higher in the middle age groups (30–39 and 40–49) than in the youngest and oldest age groups, suggesting that the elevated expression of both DDX4 and CD133 is not related to tumor stage, but rather to age (Fig. 2C). Analyzing the overall patterns of DDX4

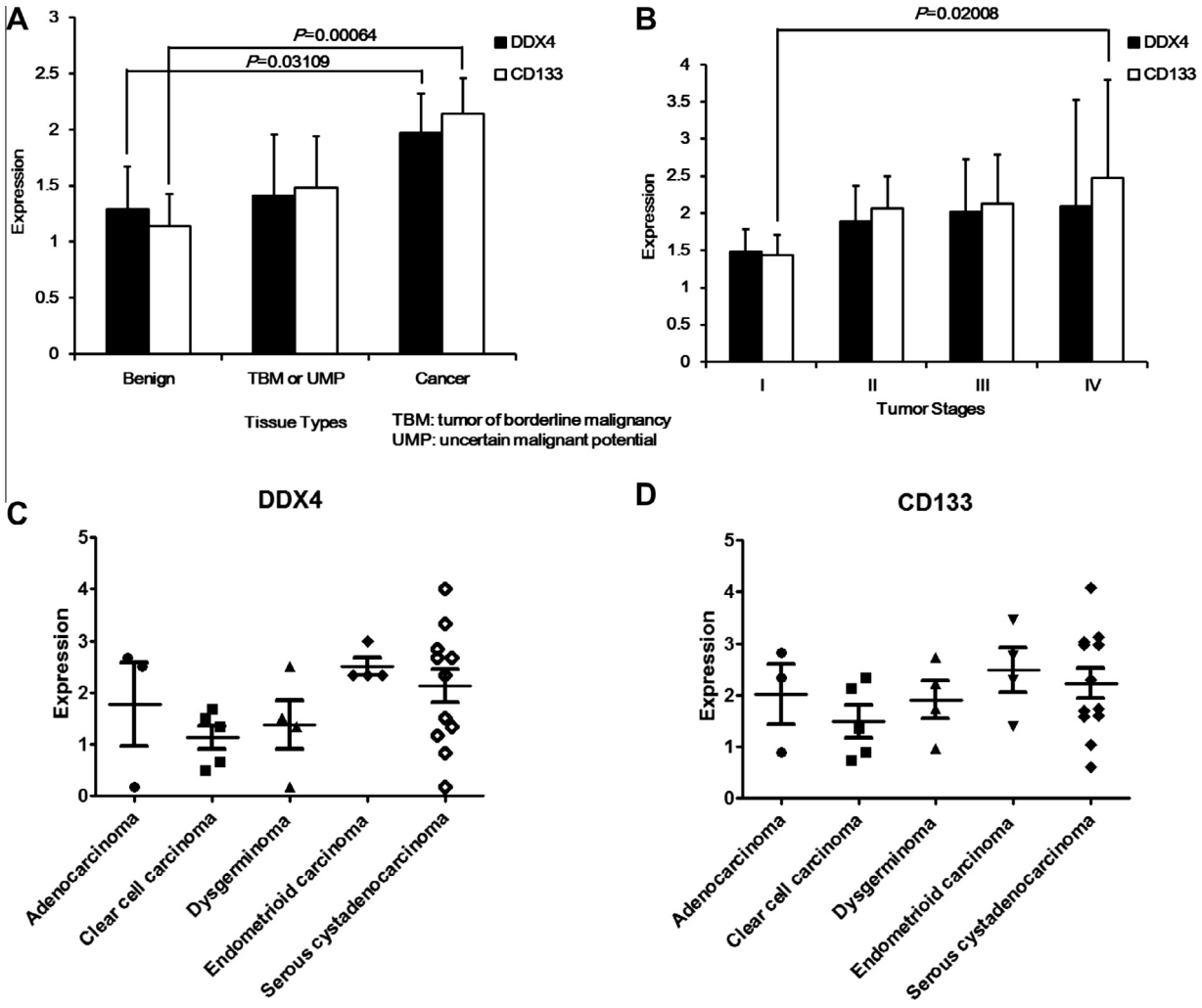

Fig. 1. Expression patterns of DDX4 and CD133 in ovarian tumors. (A) Expression of DDX4 and CD133 in various types of ovarian tumors, including benign, tumor of borderline malignancy (TBM) or uncertain malignant potential (UMP), and cancer. (B) Expression of DDX4 and CD133 by tumor stage of ovarian cancers. (C) Expression of DDX4 in various types of ovarian cancers. (D) Expression of CD133 in various types of ovarian cancers. Tissue microarray slides were immunostained with mouse anti-DDX4 monoclonal (1:100 dilution; Abcam Inc., Cambridge, MA, USA) and rabbit anti-CD133 polyclonal antibody (1:100 dilution; Abcam Inc., Cambridge, MA, USA) and incubated with secondary antibodies (Alexa Fluor 546 anti-mouse antibody and Alexa Fluor 488 anti-rabbit antibody; Invitrogen, Carlsbad, CA, USA). The slides were observed for epifluorescence with a confocal laser-scanning microscope and expression intensity was scored as 0 (negative), 1 (weak), 2 (moderate), 3 (strong), or 4 (very strong). Focality of expression was evaluated and the score was factored into the general score (0.5: focally weak, 1.5: focally moderate, 2.5: focally strong, 3.5: focally very strong).

and CD133 expression in various ovarian cancers, we found that the expression patterns of both markers are significantly similar, which suggests that these markers are highly related or colocalized within cancer cells.

3.2. Colocalization of DDX4 and CD133 in ovarian cancer tissues

In order to analyze the relationship and colocalization of DDX4 and CD133, we conducted simultaneous immunofluorescence with primary antibodies against DDX4 and CD133 in tissue microarrays of ovarian cancers. As shown in Fig. 3, DDX4 and CD133 expression was colocalized in almost all samples. These data suggest that DDX4 expression is strongly related to CD133 expression and is a characteristic of ovarian cancer stem cells.

3.3. Colocalization of DDX4 and CD133 in ovarian cancer cells

Colocalization of DDX4 and CD-133 was also confirmed in ovarian cancer cell lines. We chose two ovarian cancer cell lines, SKOV-3 and OVCAR-3, expressing different levels of CD133. As shown in Fig. 4A, OVCAR-3 expressed much more CD133 than SKOV-3 did. Comparing the DDX4 expression levels in the two cell lines, OVCAR-3 also shows much higher expression of DDX4 than SKOV-3 does (Fig. 4A). These results suggest that the expression level of CD133 is related to the expression of DDX4 in both ovarian cancer cell lines. To confirm the relationship of CD133 and DDX4 expression in these cell lines, we analyzed the colocalization of CD133 and DDX4 in both cell lines. As shown in Fig. 4B, almost all DDX4 and CD133 expression colocalized in both cell lines. The proportion of cells expressing CD133 and DDX4 differed according to cell type, but almost all of the cells expressing CD133 also expressed DDX4. Conversely, CD133-negative cells did not possess DDX4 (Fig. 4B).

4. Discussion

Germ cells are the precursor cells of gametes, the cells of sexual reproduction. In many animals, the germ cells undergo cell division of two types, mitosis and meiosis, followed by cellular differentiation into mature gametes, either eggs or sperm. Under special conditions *in vitro*, germ cells can acquire properties similar to those of

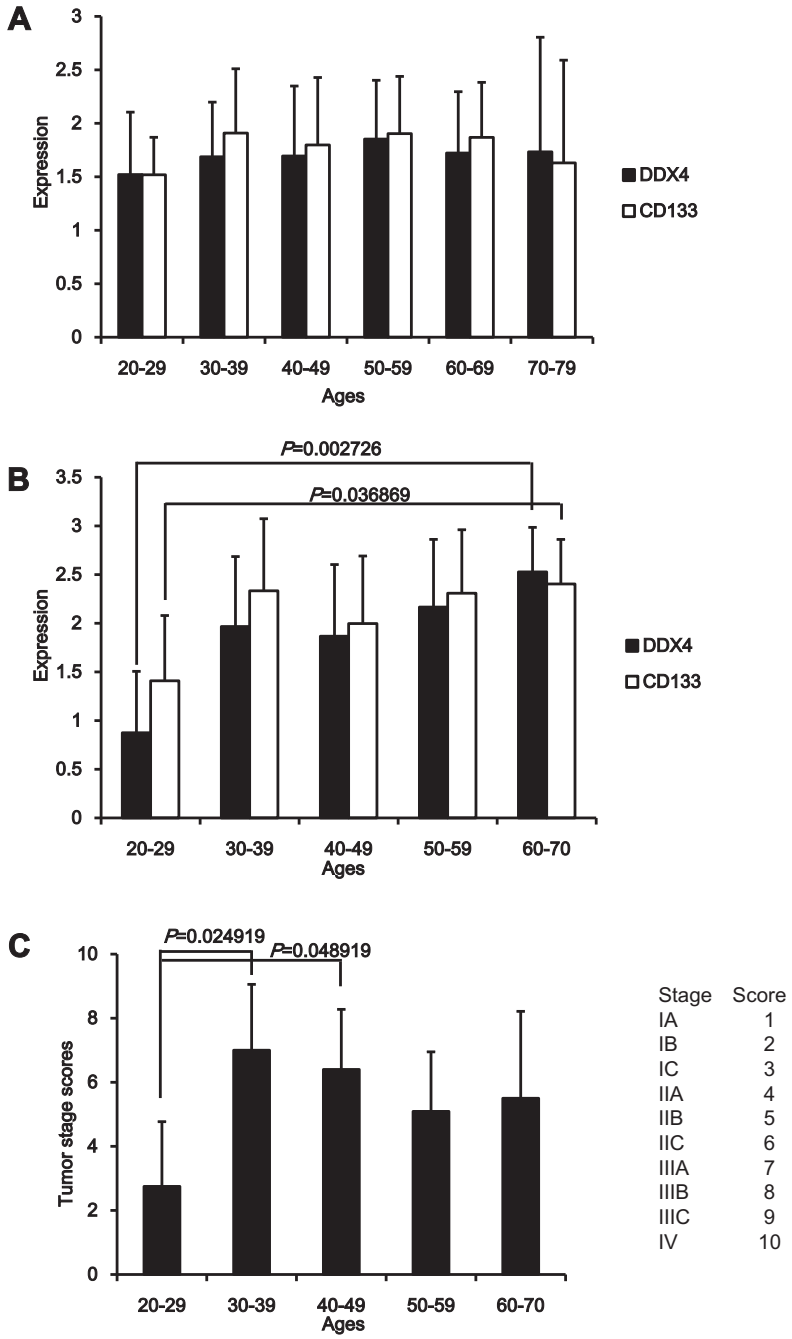

Fig. 2. Expression patterns of DDX4 and CD133 according to age. (A) Expression patterns of DDX4 and CD133 according to age in pooled tumor types (benign, TBM or UMP, and cancer). (B) Expression patterns of DDX4 and CD133 according to age in ovarian cancers. (C) Tumor stage scores according to age. Tissue microarray slides were immunostained with mouse anti-DDX4 monoclonal and rabbit anti-CD133 polyclonal antibodies and observed with a confocal laser-scanning microscope as described previously.

embryonic stem cells (ES). The underlying mechanism of that change is still unknown. These changed cells are then called embryonic germ cells (EG). Both EG and ES are pluripotent *in vitro*, but only ES have proven pluripotency *in vivo*. Several studies have demonstrated that it is possible for ES to give rise to primordial germ cells [11]. Recently, Zou et al. [9] established proliferative germ cells referred to as female germline stem cells (FGSCs) from both newborn and adult ovaries by isolating mouse VASA homolog (MVH)-positive cells. MVH-positive FGSCs proliferate long-term in culture and accept and maintain expression of the transgenic marker, green fluorescent protein (GFP). When FGSCs were transplanted into the ovaries of conditioned mice, those cells underwent oogenesis and the mice produced offspring that had the GFP transgene. Furthermore, White et al. successfully isolated human FGSCs from ovarian cortical tissue using a fluorescence-activated cell sorting-based protocol with a human VASA homolog (DDX4) antibody [5]. These cells are also rare mitotically active cells and have a gene expression profile that is consistent with primitive germ cells. Human FGSCs can be expanded for months and can spontaneously generate oocytes that can be implanted into the ovaries of immunodeficient female mice [5].

Cancer-initiating cells, or cancer stem cells (CSCs), are thought to constitute a small subset of cells within a tumor that both initiate the primary cancer and its recurrence because of their capacity

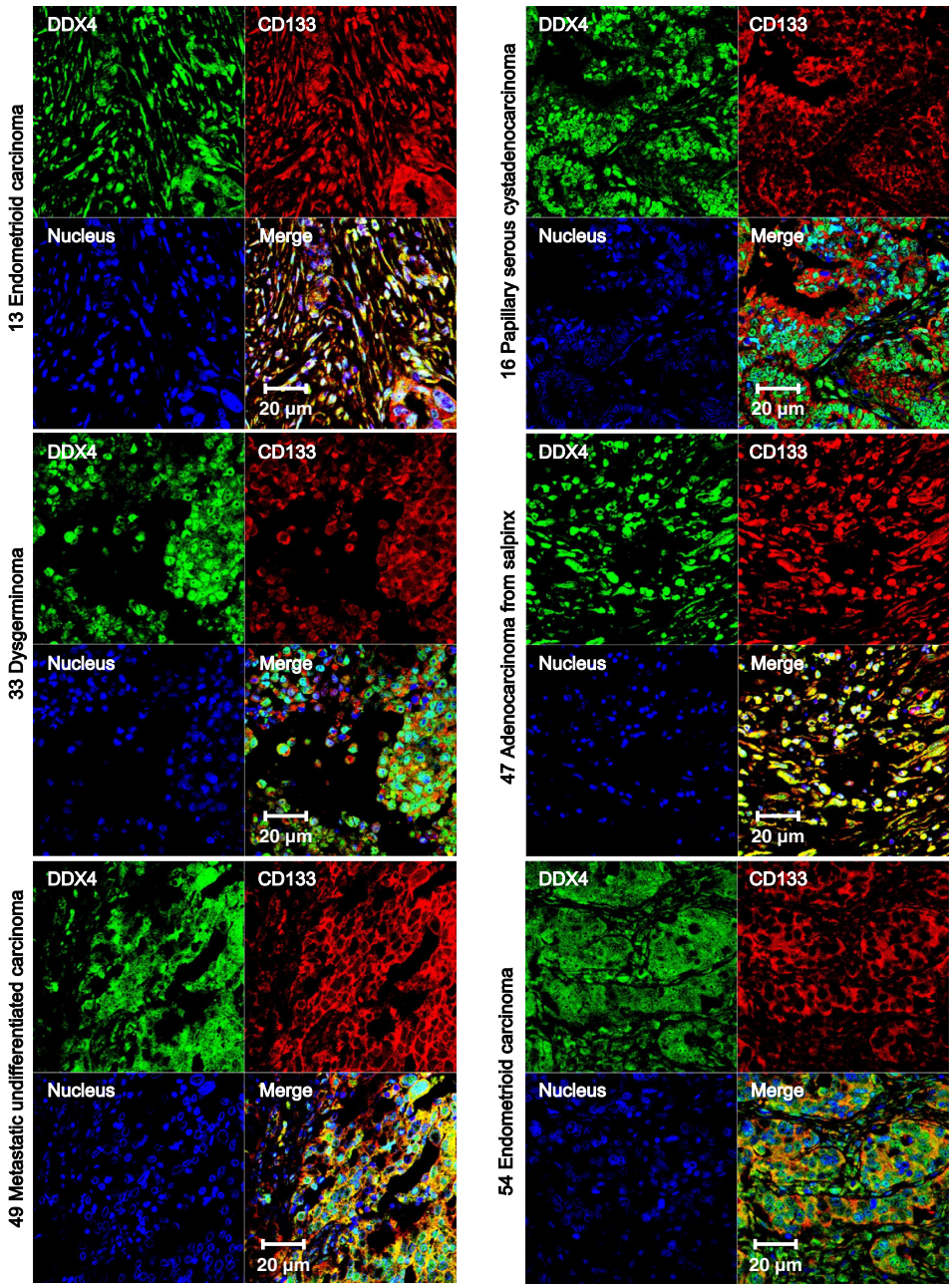

Fig. 3. Colocalization of DDX4 and CD133 in various ovarian cancers. Tissue microarray slides were immunostained with mouse anti-DDX4 monoclonal and rabbit anti-CD133 polyclonal antibodies and observed with a confocal laser-scanning microscope as described previously.

for self-renewal and inherent chemoresistance. CSCs differ considerably from the majority of cells of the tumor mass. It is assumed that the unlimited growth capacity of the tumor as well as the capability to develop metastases depend on the CSC population. CSCs are very similar to normal stem cells in many aspects. They apparently express the same markers as normal stem cells. In addition, several stem cell markers are upregulated in cancer, e.g., ABCG or Bmi-1 [12]. In other instances, isotypes of stem cell markers such as CD44v or ALDH1A3 are preferentially expressed on tumor cells [13,14], although this finding is not wholly accepted [15]. DDX4, a typical marker of FGSCs, has also been reported to be overexpressed in epithelial ovarian cancers [7] and these reports strongly suggest that DDX4 may be an ovarian cancer stem cell marker.

Several surface markers have been reported to be associated with cancer stem-like or progenitor cells. In ovarian cancer, early progenitor cells are associated with some specific surface markers like CD44, CD133, and CD117 [16–20]. However, $CD44^+$ and $CD133^+$ subpopulations in ovarian cancer are believed to be heterogeneous and consist of differentiated cells as well as progenitor cells [18,21]. The pentaspan transmembrane glycoprotein CD133, also known as Prominin-1, was originally described as a hematopoietic stem cell marker [22] and was subsequently shown to be expressed by a number of progenitor cells, including those of the epithelium, where it is expressed on the apical surface [23]. CD133 expression distinguishes a number of cancer-initiating cells, including those associated with brain [24,25], pancreatic [26], liver [27], skin [28], prostate [29,30], and colon cancers [31,32]. Recent studies have substantiated that ovarian cancer may arise from aberrant stem cells and that CD133 can be a CSC marker in ovarian cancers. Ferrandina et al. [33] isolated $CD133^+$ cells from ovarian cancers by flow cytometry and found that these cells were more

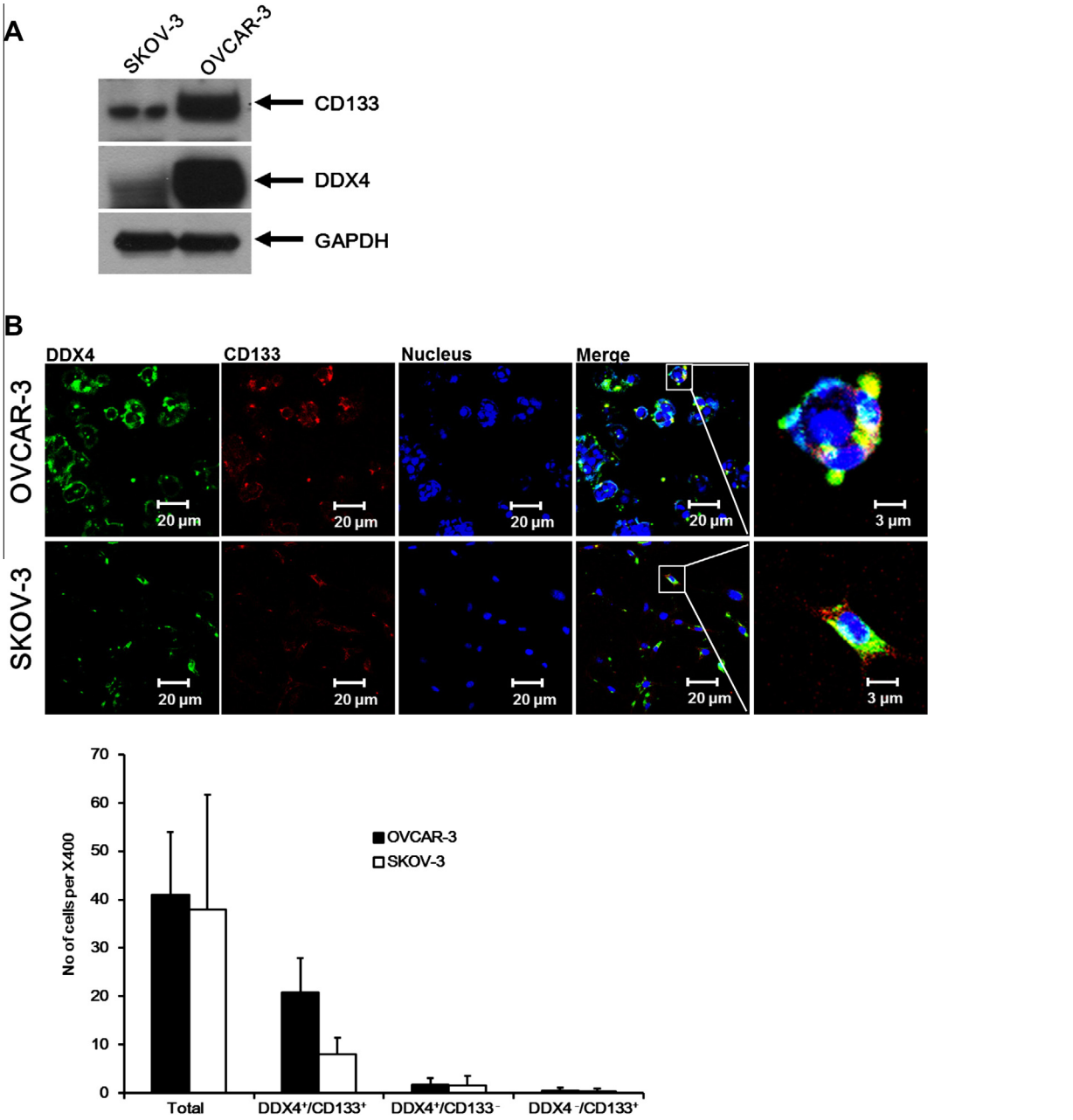

Fig. 4. Expression and colocalization of CD133 and DDX4 in SKOV-3 and OVCAR-3 ovarian cancer cell lines. (A) Expression of CD133 and DDX4 in SKOV-3 and OVCAR-3 ovarian cancer cell lines. CD133 and DDX4 were detected by immunoblotting; GAPDH levels are shown to provide an internal loading control. (B) Colocalization of CD133 and DDX4 in SKOV-3 and OVCAR-3 ovarian cancer cell lines. Cells were cultured on coverslips and stained with mouse anti-DDX4 monoclonal and rabbit anti-CD133 polyclonal antibodies and observed with a confocal laser-scanning microscope. The number of DDX4 and CD133 double positive ($DDX4^+/CD133^+$) and single positive ($DDX4^+/CD133^-$ or $DDX4^-/CD133^+$) cells were counted under 400× magnification.

frequently detected in primary ovarian cancers than in their metastatic lesions or in normal ovaries. Another report showed that sorted $CD133^+$ ovarian cancer cells exhibit enhanced resistance to platinum-based chemotherapy and form more aggressive tumor xenografts at a lower inoculum than their $CD133^-$ progeny do, suggesting that CD133 demarcates an ovarian cancer-initiating cell population [34]. Our previous report showed that thymosin β4, which was overexpressed in a side population of cancer stem cells and CD133-positive colorectal cancer stem cells, also colocalized with CD133 expression in primary ovarian cancers [35]. These data suggest that CD133 is a significant CSC marker in ovarian cancer.

In this study we analyzed the potential of DDX4 as a CSC marker by analyzing the expression patterns of both DDX4 and CD133 in tissue arrays of ovarian cancers. The expression patterns of DDX4 and CD133 are approximately quite similar. If the expression of CD133 is high in a certain tissue sample, the expression of DDX4 is also high, whereas if CD133 is low, the expression of DDX4 is also low in that same tissue. The relationship between CD133 and DDX4 was also confirmed in the ovarian cancer cell lines, SKOV-3 and OVCAR-3. OVCAR-3, which expresses more CD133 than SKOV-3 does, also expresses more DDX4. Moreover, almost all CD133-positive cells were also DDX4-positive in both cell lines. These results strongly indicate the colocalization of both DDX4 and CD133 in ovarian cancer, and suggest the possibility that DDX4 is another CSC marker of ovarian cancer cells.

Acknowledgments

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