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Research Article

Stem cells in ovarian cancer

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Abstract

Background: Identification of ovarian cancer stem cell will give a hope in radical cure of this disease which is highly chemo resistant and recur after chemotherapy if the researches reach to target the cancer stem cells to eradicate it. **Objective:** to evaluate expression of stem cells in cases of ovarian cancer in comparison with borderline ovarian tumors, benign ovarian tumors, and normal ovarian tissues**. Patients and methods:** 80 patients were divided to 4 groups: normal ovarian tissues group, benign ovarian tumors group, borderline ovarian tumors, and ovarian cancer group. Each group included 20 patients. 2 slides from each specimen had been obtained :the first slide was stained by H&E for histopathological diagnosis, and the second slide was stained by oct-4 for stem cell scoring using Edessy stem cell score (EISS). **Results:** stem cell score in ovarian cancer group was in range from 7/10 to 10/10 while in the rest of groups the score was in range from 5/10 to 7/10 which was statistically highly significant. **Recommendations:** targeting cancer stem cells by treatment and extension of stem cell researches.

Keywords: ovarian cancer – stem cells – Oct-4

Introduction

Ovarian cancer is the fifth most common cause of cancer-related death in women and is the most lethal gynecological malignancy with 30% to 40% overall survival (OS) at 5 years **(Agarwal & Kaye, 2005)**.

Due to the non-specific symptoms and inadequate screening methods at the early stages, more than 60% of ovarian cancer patients are diagnosed at advanced stage. Clinically, ovarian cancer is characterized by an initial response to combined cytoreductive surgery and chemotherapy. Subsequently, recurrence and disease progression ensues contributing to the poor patient outcome **(Di J, Duiveman et al., 2013).**

The terms cancer stem cells (CSCs) or cancer initiating cells (CICs) are a very small subgroup of tumor cells with the ability to self-renew, differentiate, and form secondary/tertiary tumors after serial xenotransplantation into immune-compromised animal models. Actually, the reason for 90% of tumors arising from ovary surface epithelium is that stem cells reside in the area. In early stage of ovarian cancer, the number of EOC stem cells can be used to predict progression of the disease **(Gupta et al., 2009).**

The elimination of ovarian CSCs has been challenging in part due to heterogeneity. Thus the efficacy of any single drug was limited for cancer patients. Combined treatments that target CSCs will be a new direction in the future. Nevertheless, drug treatment for CSCs may increase the risk of toxicity since CSCs share common features with normal stem cells **(Yin et al., 2013).**

Current methods to eliminate CSCs cannot be successfully applied in all clinical situations. One way to eradicate CSCs is to induce their differentiation, resulting in loss of their stemness property. Thus, the understanding of regulation of differentiation processes is necessary for designing new agents to

eliminate CSCs. In 2012, Yin and his colleagues observed that TWIST-1 (a basic helix-loop-helix transcription factor) played a key role in triggering differentiation of epithelial ovarian cancer (EOC). Jain et al. recently reported that p53 capable for regulating molecular networks can activate two miRNAs (miR-34a and miR-145). These miRNAs were then shown to prompt differentiation of human embryonic stem cells. Indeed, emerging evidence indicated that miRNAs were involved in self-renewal and differentiation of normal and cancer stem cells. It is suggested that such miRNAs should be a new therapeutic target for cancer treatment **(Yin et al., 2013).**

Oct-4 (octamer-binding transcription factor 4) also known as POU5F1 (POU domain, class 5, transcription factor 1) is a protein that in humans is encoded by the *POU5F1* gene. Oct-4 is a homeodomain transcription factor of the POU family. This protein is critically involved in the self-renewal of undifferentiated embryonic stem cells. As such, it is frequently used as a marker for undifferentiated cells. Oct-4 expression must be closely regulated; too much or too little will cause differentiation of the cells. The octamer (made of eight units) in this family of transcription factors is the DNA nucleotide sequence "ATTTGCAT", the etymology for the naming of the octamer transcription factor **(Niwa et al., 2000).**

Several studies suggest a role for Oct-4 in sustaining self-renewal capacity of adult somatic stem cells (i.e. stem cells from epithelium, bone marrow, liver, etc.) **(Kim et al., 2009)**. Other scientists have produced evidence to the contrary, and dismiss those studies as artifacts of in vitro culture, or interpreting background noise as signal and warn about Oct-4 pseudogenes giving false detection of Oct-4 expression. Oct-4 has also been implicated as a marker of cancer stem cells **(Kim et al., 2012).**

Subjects and Methods

This is a prospective controlled study which was conducted at Al Azhar university hospital in Assiut and Cairo and Cancer institute in Assiut and Cairo - Egypt in the interval from March 2013 to August 2014. 80 subjects were divided into four groups:

- The first group: normal ovarian tissues group and included 20 patients.
- The second group: Benign ovarian tumour group and included 20 patients.
- The third group: borderline ovarian tumour and included 20 patients.
- The fourth group: malignant ovarian tumours and include 20 patients.

Two specimens from each case were obtained the first slide was stained by haematoxline and eosin to assure the histopathological diagnosis and The second slide was stained by Oct- 4 (octamer-binding transcription factor 4) for stem cell expression.

Inclusion criteria

- Ovarian stem cell candidate.

Exclusion criteria:

- Another malignanacy
- Preoperative chemotherapy or radiotherapy.

A written consent was obtained from all patients woh were then subjected to:

- Complete history taking,
- Careful general, abdominal and vaginal examination,
- Ultrasound examination,
- Computed tomography or MRI evaluation,
- Tumour markers: especially CEA and CA-125,
- Routine investigations, IVU (intravenous urograghy),
- Immunohistochemistry evaluation for stem cell scoring using Oct-4 and
- Pathological evaluation of the specimen:

Samples were obtained from the ovarian tissues; 20 samples from malignant ovarian neoplasms whatever the type of malignancy, 20 samples from borderline ovarian neoplasms, 20 samples from benign ovarian tissues, and 20 samples from normal ovarian tissues. These samples were obtained via laparotomy or laparoscopy procedures. Biopsy samples were fixed in 10% neutral-buffered formalin at 4 C overnight and were subsequently paraffin embedded.

Before performing immunohistochemistry, sections of the tissues were stained with Hematoxyline- Eosin (H&E) to select tissues with ectopic cells.

Serial sections of the same selected samples, 5-mm thick, were used for immunohistochemistry.

Commercially available monoclonal antibodies (m Ab) were used for the detection of Oct- 4.

Tissue sections were dewaxed and rehydrated conventionally and the quenching of the endogenous peroxidase was achieved by incubation with 0.3% hydrogen peroxidase in menthanol for 30 minutes at room temperature.

All tissue sections were exposed to anon immune block with normal horse serum for 30 minutes at room temperature.
Incubations with the first antibody were carried at 4 C overnight with a dilution of 1:100 for the monoclonal mouse anti human Fas-L and with the dilution of 1:50 for the monoclonal mouse anti human Fas antigen.

Thereafter tissue sections were labeled with avidin-biotin-peroxidase detection system Vectastain (Vector Laboratories, Burlington, VT).

Each step was followed by meticulous washing with phosphate-buffered saline (PBS). Finally 3, 30-diaminobenzidine was used as a chromogen.

Conterstaining was performed with Meyer hematoxyline. The positive controls were ovarian tissue that showed expression of Oct- 4.

A semi quantitative analysis of specific stainig was performed using the histochemical score (HSCORE) system according to **(McCarthy et al., 1985)** to score the immunohistochemistry slides and perform statistical analysis.
The HSCORE was calculated using the following eqution: HSCORE 1/4 SPi (ib1), where is the intensity of the staining with the value of 1, 2, 3 (weak, strong, or very strong) and pi is the percentage of stained cells for each intensity varying from 0% to 100%. For scoring, the Edessy stem cell score was applied by giving a score for each finding 0, 1, 2 as shown in table (1).

Table (1) Edessy stem cell score (Edessy et al., 2014)

Score factor	0	1	2
Intensity Of SC Marker	Negative to mild	moderate	strong
Percentage Of Stained Cells	0	0-50%	> 50-100%
Focality	None	focal	diffuse
Distribution	None	Epithelial or mesenchmal	both
Localization Of The Stain	None	Cytoplasmic or nuclear	both

Statistical Analysis:

The collected data was organized, categorized, tabulated, and analyzed by using the computer software (Statistical Package for Social Science {SPSS} version 12). Suitable statistics was used for quantitative data. Yates corrected chi-square (χ^2) and Fisher exact (FE) were used as tests of significance. The significance level for them was accepted if P-value <0.05

Results

Evaluation of immunohistochemical parameters revealed that the most important factors for evaluation of stem cells were intensity of stem cell marker, percentage of stained cells, focality of positive stained cells, distribution of positive stained cells either epithelial, mesenchmal, or both, localization of this stain either cytoplasmic, nuclear, or both. Each specimen was examined histopathologically and immunohistochemistry for stem cell score as shown in the following example:

Table (2): Demographic characteristics of the study groups

	Group I (n= 20)		Group II (n= 20)		Group III (n= 20)		Group IV (n= 20)		P_1	P_2	P_3	P4	P5	P6
	No.	%	No.	%	No.	%	No.	%						
Age: (years)														
• < 30	7	35	4	20	11	55	1	5	0.001	0.004	0.000	0.031	0.12	0.00
• 30 – < 40	13	65	4	20	3	15	2	10						
• 40 – < 50	0	0	6	30	3	15	5	25						
• ≥ 50	0	0	6	30	3	15	12	60						
BMI														
• Normal	14	70	4	20	13	65	5	25	0.333	0.677	0.156	0.353	0.49	0.09
• Overweight	5	25	14	70	3	15	6	30						
• Obese	1	5	2	10	4	20	9	45						
Occupation:														
• House wife	20	100	17	85	13	65	16	80	0.072	0.004	0.035	0.144	0.67	0.28
• Worker	0	0	3	15	7	35	4	20						
Marital status:														
• Single	4	20	2	10	4	20	0	0	0.376	1.000	0.035	0.658	0.46	0.11
• Married	16	80	18	90	16	80	20	100						
Level of education:														
• Illiterate	2	10	0	0	0	0	5	25	0.013	0.325	0.435	0.003	0.005	0.04
• Secondary	11	55	19	95	11	55	10	50						
• High education	7	35	1	5	9	45	5	25						
Social class:														
• Low	10	50	0	0	0	0	2	10	0.001	0.000	0.005	0.001	0.055	0.13
• Middle	10	50	19	95	10	50	13	65						
• High	0	0	1	5	10	50	5	25						

1: Comparison between Group I and Group II

2: Comparison between Group I and Group III

3: Comparison between Group I and Group IV

4: Comparison between Group II and Group III

5: Comparison between Group II and Group IV

6: Comparison between Group III and Group IV

Table (3): Age of menarche for the study groups

	Group I (n= 20)		Group II (n= 20)		Group III (n= 20)		Group IV (n= 20)		P_1	P_2	P_3	P4	P5	P6
	NO.	%	NO.	%	NO.	%	NO.	%						
Age at menarche:														
< 12 years	0	0.0	16	80	11	55	15	75	0.000	0.000	0.000	0.024	0.859	0.037
≥ 12 years	20	100	4	20	9	45	5	25						

1: Comparison between Group I and Group II
2: Comparison between Group I and Group III
3: Comparison between Group I and Group IV
4: Comparison between Group II and Group III
5: Comparison between Group II and Group IV
6: Comparison between Group III and Group IV

Table (4): Premenopause And Postmenopause Of The Study Groups

	Group I (n= 20)		Group II (n= 20)		Group III (n= 20)		Group IV (n= 20)		P_1	P_2	P_3	P4	P5	P6
	NO.	%	NO.	%	NO.	%	NO.	%						
menopause														
premenopause	20	100	17	85	14	70	8	40	0.000	0.000	0.002	0.88	0.24	0.23
postmenopause	0	0.0	3	15	6	30	12	60						

1: Comparison between Group I and Group II
2: Comparison between Group I and Group III
3: Comparison between Group I and Group IV
4: Comparison between Group II and Group III
5: Comparison between Group II and Group IV
6: Comparison between Group III and Group IV

Table (5): Tumor markers

	Group III (n= 20)		Group IV (n= 20)		P-value
	NO.	%	NO.	%	
CEA: (ng/ml)					0.021
< 50	12	60	7	35	
≥ 50	8	40	13	65	
CA- 125:(U/ml)					0.014
< 100	10	50	4	20	
≥ 100	10	50	16	80	

- Normal range of CEA is 0-4 ng/ml.
- Normal range of CA-125 is 0-35 U/ml.

Table (6): Histopathological diagnosis

Diagnosis	Group I (n= 20)		Group II (n= 20)		Group III (n= 20)		Group III (n= 20)	
	No	%	No	%	No	%	No	%
Normal ovarian tissue	20	100	0	0	0	0	0	0
Benign mucinous cystadenoma	0	0	3	15	0	0	0	0
Benign papillary mucinous cystadenoma	0	0	1	5	0	0	0	0
Benign serous cystadenoma	0	0	14	70	0	0	0	0
Borderline serous cystadenoma	0	0	0	0	20	100	0	0
Mucinous cystadenocarcinoma	0	0	0	0	0	0	2	10
Papillary Serous cystadenocarcinoma	0	0	0	0	0	0	3	15
Serous cystadenocarcinoma	0	0	0	0	0	0	15	75

Table (7) Distribution of stem cell score in the studied groups

score	Normal		Benign		Borderline		Malignant		P1	P2	P3	P4	P5	P6
	NO.	%	NO.	%	NO.	%	NO.	%						
5/10	18	90	5	27.78	4	20			001	001	0.99	-		
6/10	1	5	11	61.11	12	60			001	002	0.99	-		
7/10	1	5	2	11.11	4	20	4	20	0.69	0.65	0.33	0.65	0.33	0.99
8/10	-	-	-		-		8	40	--	--	--	-	-	-
9/10	-	-	-		-		3	15	--	--	--	-	-	-
10/10	-	-	-		-		5	25	--	--	--	-	-	-

1: Comparison between Group I and Group II
2: Comparison between Group I and Group III
3: Comparison between Group I and Group IV
4: Comparison between Group II and Group III
5: Comparison between Group II and Group IV
6: Comparison between Group III and Group IV

Table (8) Distribution of stem cell score in the malignant group according to histopathological diagnosis

score	Serous cystadenocarcinoma		Papillary Serous cystadenocarcinoma		Mucinous cystadenocarcinoma		P1	P2	P3
	NO.	%	NO.	%	NO.	%			
7/10	2	13.33	1	33.33	1	50	**0.396**	**0.201**	0.709
8/10	7	46.67	-	-	1	50	-	0.929	-
9/10	3	20	-	-	-	-	-	-	-
10/10	3	20	2	66.67	-	-	099	-	-

Table (9) Distribution of stem cell score in the benign group according to histopathological diagnosis

EISS	Benign serous cystadenoma		Benign mucinous cystadenoma		Benign papillary mucinous cystadenoma		P1	P2	P3
	NO.	%	NO.	%	NO.	%			
5/10	4	28.57	1	33.33	-	-	0.869	-	-
6/10	8	57.14	2	66.67	1	100	0.761	0.398	0.505
7/10	2	14.29	-	-	-	-	-	-	-

Table (10): Stem cell score

Stem cell score	Group I (n= 20)	Group II (n= 20)	Group III (n= 20)	Group IV (n= 20)	P_1	P_2	P_3	P4	P5	P6
Mean ± SD	5.15 ± 0.49	5.84 ± 0.60	5.94 ± 0.64	8.45 ± 1.10	0.005	0.001	0.000	0.622	0.000	0.000
Range	5 – 7	5 – 7	5 – 7	7 – 10						

(11): Relation between Stem cell score and age

Stem cell score	Age (years)								P-value
	< 30		30 – < 40		40 – < 50		≥ 50		
	NO.	%	NO.	%	NO.	%	NO.	%	
	23	28.8	22	27.5	14	17.5	21	26.3	0.004
Range of score	5 – 8		5 – 8		5 – 10		5 – 10		

Table (12): Relation between Stem cell score and parity

Stem cell score	Parity								P-
	0		1		2-4		5+		
	NO.	%	NO.	%	NO.	%	NO.	%	
	11	15.7	9	12.9	38	54.3	12	17.1	0.487
Range of score	5 – 9		5 – 8		5- 10		5-10		

Table (13): Relation between Stem cell score and BMI

Stem cell score	BMI						P-value
	Normal		Overweight		Obese		
	NO.	%	NO.	%	NO.	%	
	36	45	28	35	16	31.3	0.531
Range of score	5 – 10		5 – 9		5 – 10		

Table (14): Relation between Stem cell score and age of menarche

Stem cell score	Age of menarche				P-value
	< 12 years (n= 42)		≥ 12 years (n= 18)		
	42	52.5	38	47.5	<001
Range of score	5 – 10		5 – 9		

Table (15): Relation between Stem cell score and menopause

Stem cell score	Menopause				P-value
	Pre-menopause		Post-menopause		
	NO.	%	NO.	%	
	59	73.8	21	26.2	0.001
Range of score	5 – 10		5 – 10		

Table (16): Relation between Stem cell score and histopathological diagnosis in (group IV)

Stem cell score	Diagnosis						P-value
	Mucinous cystadeno-carcinoma		Papillary serous cystadeno-carcinoma		Serous cystadeno-carcinoma		
	NO.	%	NO.	%	NO.	%	
	2	10	3	15	15	75	0.343
Range of score	7 – 8		7 – 10		7 – 10		

Table (17): Relation between Stem cell score and histopathological diagnosis in (Group II)

Stem cell score	Diagnosis				P-value
	Mucinous cystadenoma		Serous cystadenoma		
	NO.	%	NO.	%	
	4	20	15	75	0.741
Range of score	5 – 6		5 – 7		

-The stem cell score was statistically significant in the malignant group in comparison to the other groups.
-The histopathological difference does not change the results.
-The borderline ovarian tumor group was all the same in histopathological diagnosis (serous borderline ovarian tumor).
-The score increases with the age regardless the diagnosis.
-The score increases in those who had earlier menarche regardless the diagnosis.
- The score increases in the postmenopause group regardless the diagnosis.
-The score is ranging from 5/10 to 7/10 in the first three groups (normal ovarian tissues, benign ovarian tumors and borderline ovarian tumors).
- The score is ranging from 7/10 to 10/10 in the fourth group (the malignant group).
-The parity, body mass index, clinical presentation, and values of tumor markers did not influence the score.

Discussion

The existence of ovarian cancer-initiating cells was previously supported by others in studies whereby cancer cells isolated from ascitic fluid of ovarian cancer patients exhibited characteristics consistent with those expected of a cancer stem cell **(Bapat et al., 2005; Szotek et al., 2006).** More recently, CD44+ ovarian cancer cells have been shown to possess cancer-initiating capabilities **(Zhang et al., 2008)**. However, CD133 expression was not examined

in these studies, so the potential overlap (as reported for prostate cancer; **Collins et al., 2005; Maitland and Collins, 2008)**, or independence (as reported for breast cancer; **Wright et al., 2008)**, of CD44+ and CD133+ cells in ovarian malignancies remains unclear. Our results extend more recent findings that Oct-4 cells are present in epithelial ovarian cancers.

Zhang et al successfully identified and separated human ovarian CSCs from human ovarian cancer tissue the first time. This finding makes it possible to thoroughly prevent the occurrence and development of ovarian cancer. With the deepening of the study of the theory of ovarian CSCs, the importance of ovarian CSCs in the nature of ovarian cancer etiology has gradually been recognized. Studies have showed that CSCs have the following biological characteristics:

- self-renewal capacity;
- differentiation potential;
- the expression of stem cell marker genes;
- chemotherapy drug resistance;
- tumorigenicity in immunodeficient mice **(Suzuki et al.,2011).**

These results suggest that these ovarian CSCs have a strong differentiation potential and confirm the point that ovarian cancers are originated from ovarian CSCs. One of the characteristics of the tumor stem cells different from the mature and differentiated cells is that stem cells are resistant to chemotherapy **(Stevenson et al., 2009)**.

Studies have also demonstrated that a two-fold increase in Oct4 expression results in the conversion of ESCs towards a primitive endoderm and mesoderm state. Conversely, a 50% decrease in Oct4 expression can induce differentiation of ESC into trophectoderm. This suggests that the precise level of Oct4 protein expression in ESCs is crucial to maintain lineage-specific ESC differentiation and different developmental fates **(Kellner & Kikyo, 2010).**

These results suggest that isolated Oct4 positive VSELs may serve as a good source of pluripotent stem cells in adult tissues and have a potential application in regenerative medicine **(Shin et al., 2010).**

Recently, Oct4 expression has been described in immature teratoma of the ovary, in Fallopian tube epithelium and serous and mucinous epithelial ovarian tumors of different histological grades using immunohistochemical analysis **(Zhang et al., 2010**).

In this study, Oct4 expression was shown to be significantly increased from benign/borderline tumors to serous and mucinous carcinomas, suggesting that the expression of Oct4 is associated with the initiation and progression of serous ovarian cancer. However, this study found no significant difference among benign, and malignant tumors in Oct4 expression regarding the histological type whether serous or mucinous

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