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Alteration of *BRCA-1* tumor suppressor gene expression in serous and mucinous ovarian neoplasms in the benign-borderline-malignant pathway

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ABSTRACT

Alteration of expression of the tumor suppressor gene *BRCA-1* has been widely studied in breast and ovarian carcinoma. However, pattern of this alteration in the benign-borderline-carcinoma sequence in serous and mucinous ovarian neoplasms have not yet fully described. Tissue sections from 214 formalin-fixed paraffin-embedded ovarian specimens were stained immunohistochemically with *BRCA-1* antibody. Specimens were 10 normal ovarian surface epithelium, 10 fallopian tube epithelium, 70 benign adenoma (50 serous and 20 mucinous), 28 borderline (13 serous and 15 mucinous), 78 carcinoma (58 serous and 20 mucinous), and 18 metastatic deposit (13 serous and 5 mucinous). Expression was evaluated into 0, +1, +2, and +3. Score +3 staining similar to normal tissues was considered normal and other scores were considered altered expression. Strong expression was seen in all normal epithelium specimens. Altered expression was seen in 34 serous neoplasms; 17 of 50 (34%) of benign cystadenomas, 6 of 13 (46%) of borderline tumors, 43 of 58 (74%) of primary carcinoma, and in 8 of 13 (62%) of metastatic carcinoma. This alteration was significantly associated with higher histopathologic grade ($P = 0.049$), presence of necrosis ($P = 0.0001$), and higher proliferation rate ($P = 0.001$). In mucinous neoplasms; altered *BRCA-1* was detected in 25 specimens; 7 of 20 (41%) of benign cystadenomas, 5 of 15 (33%) of borderline neoplasms, 9 of 20 (45%) of primary carcinoma, and 4 of 5 (80%) of the metastatic deposits. This alteration was not associated with any of the clinicopathologic tumor characteristics. In conclusion, alteration of *BRCA-1* expression is more

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frequent in serous than in mucinous carcinomas and is associated with tumors of higher grades and high proliferation rate.

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Introduction

Ovarian cancer is the eighth cancer affecting women and ranks the fifth in cancer deaths among women. According to the recent figures released by the American cancer society, about 22,440 women will be diagnosed with ovarian cancer 14,080 of whom will die from the disease in 2017.[1]

In spite of the significant development of our understanding of the mechanisms of carcinogenesis and tumor progression of ovarian carcinoma, the outcome of the disease is still grim. The conventional chemotherapeutic agents are not efficiently successful in treating the disease. After surgery and chemotherapy, the 5-year survival rate is still very poor (30%). The recent interest in ovarian oncology research is directed toward the development of targeted therapy that can selectively "kill and eradicate" the tumor cells.[2]

Our understanding of the molecular basics of ovarian carcinogenesis has been reshaped during the last decade. A complex network of molecular changes participate both in malignant transformation and in tumor progression. A central player in which is alteration of *BRCA-1* gene function. *BRCA-1* is a tumor suppressor gene located on chromosome 17. It plays an essential role in maintaining stability of the genome by repairing DNA damages. *BRCA-1* is involved in 2 main forms of DNA repair; mismatch repair and repairs of double-strand breaks in the DNA.[3]

Impairment of *BRCA-1* gene function has been implicated in development of breast, ovarian, pancreatic,[4] and prostatic carcinoma.[5] Impairment of *BRCA* may result from germ line mutation, sporadic mutation,[6] or by epigenetic changes through hypermethylation of the gene.[7]

The recent understanding of the important role of *BRCA-1* in ovarian carcinoma and its molecular function lead to the development of a targeted therapeutic agent "Olaparib" in *BRCA-1* mutated breast and ovarian carcinoma. Olaparib was developed by AstraZeneca as an oral inhibitor of poly (ADP-ribose) polymerase (PARP) proteins. The drug works by blocking the DNA repair mechanism that is mediated by PARP causing "synthetic lethality" and selective tumor cell death. Clinical trial studies proved the successful use of Olaparib in treating advanced ovarian carcinomas with *BRCA-1* mutation.[8]

Genetic mutation of *BRCA-1* has been extensively studied in malignant ovarian neoplasms but the alteration in *BRCA-1* protein expression has not yet been fully studied in the benign-borderline-malignant sequence in both serous and mucinous neoplasms. Aims of the following study were: (1) to assess pattern of *BRCA-1* expression in a spectrum of ovarian mucinous and serous neoplasms starting from benign cystadenomas to borderline tumors ending by malignant and metastatic neoplasm, (2) to assess association between alteration in *BRCA-1* expression with clinicopathologic criteria of serous and mucinous carcinoma.

Material and methods

Specimens

Two hundred and fourteen formalin-fixed paraffin-embedded blocks of a spectrum of benign, borderline, malignant primary ovarian tumors, and metastatic deposits were obtained from the archive of the Gynecological and the Surgical Pathology Laboratories, Assiut University Hospital

and Woman Health Hospital, Faculty of Medicine, Assiut University. Specimens were as follows: (1) 10 specimen of normal ovarian surface epithelium; (2) 10 normal tubal epithelium; (3) 50 benign serous cystadenoma; (4) 20 benign mucinous cystadenoma; (5) 13 borderline serous neoplasm; (6) 15 borderline mucinous neoplasm; (7) 58 primary serous carcinoma; (8) 20 primary mucinous adenocarcinoma; (9) 13 extraovarian metastatic deposit of serous carcinoma; and (10) 5 extraovarian metastatic deposit of mucinous carcinoma.

Representative sections from each specimen were reviewed for detailed histopathologic examination. Tumors were classified according to the recent World Health Organization (WHO) classification (2003). Serous and mucinous neoplasms were diagnosed in benign, borderline, and malignant forms according to the amount of epithelial cell proliferation, the degree of nuclear atypia (mild, moderate, and severe), number of mitosis, and the presence or absence of stromal invasion (WHO, 2003). Malignant serous tumors were graded according to the MD Anderson (2 tiered) grading system that depends on the degree of nuclear atypia and mitotic index (MI) into low-grade serous carcinoma with moderate nuclear atypia and MI $<$ 12/10 high power field (HPF) and high-grade serous carcinoma (HGSC) with severe nuclear atypia and MI $>$ 12/10 HPF.[9] The growth pattern of malignant mucinous tumors was reported as expansile or infiltrative. The expansile type had back to back glands or cysts lined by malignant mucinous epithelium and is associated with good prognosis. The infiltrative type had irregular penetration of stroma by misshapen glands, clusters, cords, or nests of cells with stromal response and associated with poor prognosis.[10] Tumor staging was done according to American Joint Committee on Cancer/Tumour size, Node and Metastasis (AJCC/TNM) staging system. After that manual tissue microarray (TMA) was done for construction of the TMA blocks.

TMA construction

In the current study, manual TMA was used for construction of the TMA blocks using Arraymold kit B 150 core TMA, 1.5-mm paraffin/Cryo Arrays, Catalog No.IW-111, IHC WORLD (www.ihcworld.com) according to the manufacturer instructions. Details were described previously.[11]

Immunohistochemical staining

Tissue sections from each of the TMA and of the whole specimens were stained using the Rabbit anti-*BRCA-1* polyclonal antibody. Staining was performed using an immunoperoxidase technique with the autostainer Bench-Mark. In summary, paraffin sections were deparaffinized, rehydrated then treated in 3% H_2O_2 for 5 minutes and washed with phosphate buffer solution for 15 minutes. After blocking nonspecific reaction, tissues were incubated for 45 minutes at room temperature with the primary antibody (*BRCA-1*, Origene; TA310042, polyclonal; 1:100). After rinsing in phosphate buffer solution, the reaction products were visualized by immersing into diaminobenzidine, then counterstained with hematoxylin, dehydrated, and mounted.

Assessment of *BRCA* expression was done using a semiquantitation approach. The intensity of nuclear staining was scored in a scale of 0-3, 0: negative, 1: weak, 2: moderate and 3: strong. The same approach was used in several studies.[12]

Results

Pattern of BRCA-1 expression in normal and in neoplastic ovarian tissues

Positive expression was seen in all specimens in the normal surface ovarian epithelium and in the fallopian tube epithelium.

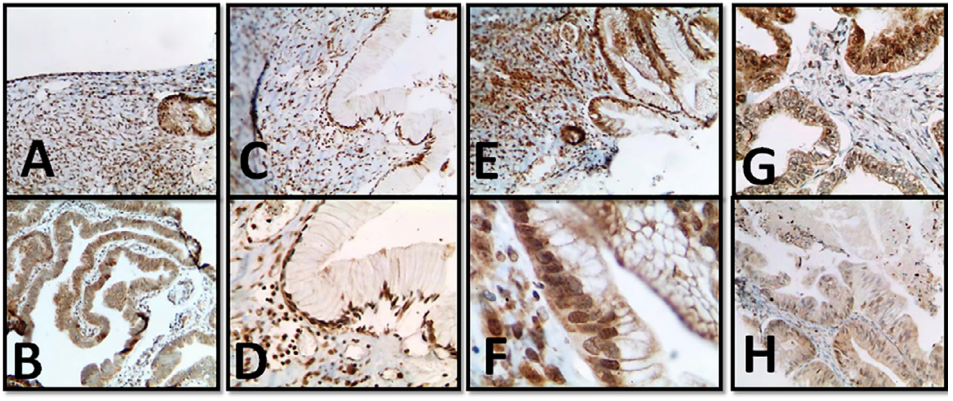

Fig. 1. (A-D) Are ovarian tissue sections stained with *BRCA-1* antibodies? (A) A benign serous cystadenoma showing strong positive nuclear staining ×100. (B) Section from a borderline serous neoplasm showing strong staining ×100. (C) Section from a malignant serous carcinoma showing strong positive nuclear staining ×200. (D) Section from another serous carcinoma showing lost expression for *BRCA-1* × 200.

Table 1
Expression of *BRCA-1* in the spectrum of serous and mucinous tumors examined.

		Benign (%)	Borderline (%)	Malignant (%)	Deposits (%)	*P* value*
Serous	Altered (0, +, ++)	17 (34)	6 (46)	43 (74)	8 (62)	**<0.001**
	Strong positive (+++)	33 (66)	7 (54)	15 (26)	5 (39)	
	Total	50	13	58	13	
Mucinous	Altered (0, +, ++)	7(41)	5 (33)	9 (45)	4 (80)	**0.001**
	Strong positive (+++)	10 (59)	10 (66)	11 (55)	1 (20)	
	Total	20[a]	15	20	5	

Bold numbers indicates statistical significance.
* *P* value was assessed using chi-square test.
[a] Three specimens were lost from the TMA blocks and were not assessed.

In whole specimens, complete negative expression was detected in 37 specimens; 3 (4%) of the benign adenomas, 3 (11%) of the borderline neoplasms, 19 (24%) of the primary malignant tumors, and in 3 (16%) of the metastatic extraovarian deposits, $P = 0.001$.

In serous neoplasms, alteration of *BRCA-1* expression (negative, score +1 or score +2) was significantly increased from benign toward malignant tumors. Altered expression was seen in 34 specimens; 17 of 50 (34%) of the benign serous cystadenomas, 6 of 13 (46%) of borderline tumors, 43 of 58 (74%) of primary serous carcinoma, and in 8 of 13 (62%) of metastatic serous carcinoma (Fig 1).

In mucinous tumors, alteration of *BRCA-1* expression was detected in 25 specimens; 7 of 20 (41%) of benign cystadenomas, 5 of 15 (33%) of borderline neoplasms, 9 of 20 (45%) of primary malignant mucinous carcinoma, and 4 of 5 (80%) of the metastatic deposits of mucinous carcinomas (Fig 2). Further details are mentioned in Table 1.

Association between BRCA-1 expression and clinicopathologic characteristics in serous and mucinous carcinoma

Clinicopathologic characteristics of tumors with normal *BRCA-1* expression were compared with those of tumors with altered expression. In serous neoplasms, significant association was

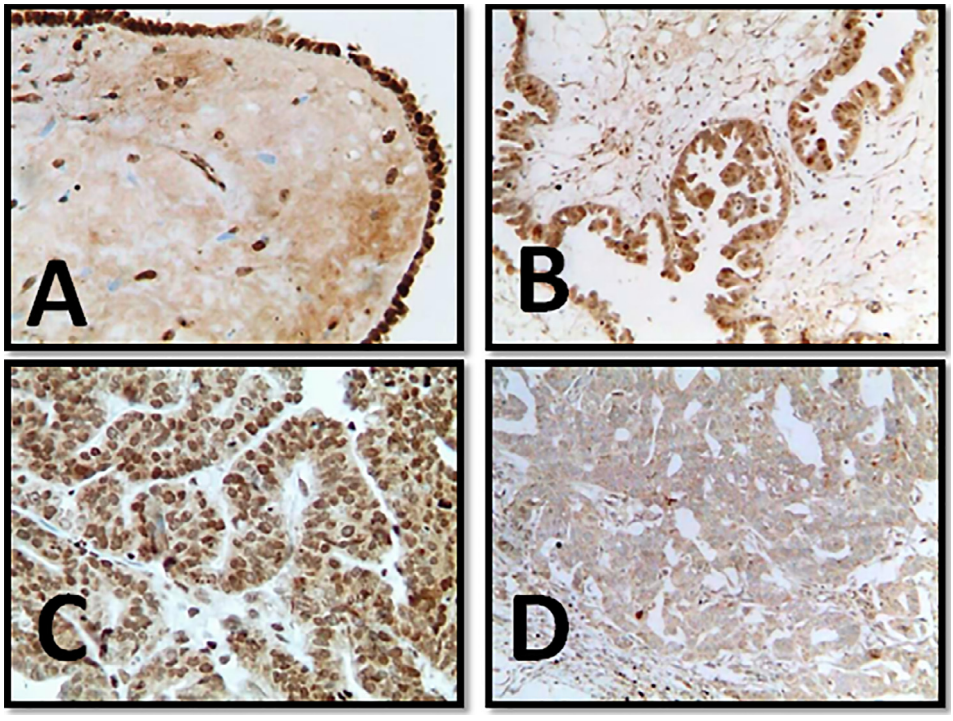

Fig. 2. (A-H) Are ovarian tissue sections stained with *BRCA-1* antibody. (A) Normal surface ovarian epithelium showing strong positive expression in surface cells and also in the ovarian stromal cells and the epithelial cells of primordial follicle (right) ×100. (B) Normal fallopian tube epithelium showing strong positive expression ×100. (C) A benign mucinous cystadenoma showing strong positive expression ×100. (D) The same section in (C) with higher magnification ×200. (E) Section from a borderline mucinous neoplasm showing strong expression ×100. (F) The same section in C with higher magnification ×400. (G) Section of a mucinous carcinoma showing heterogeneous expression of *BRCA-1* with strong expression above and weak and lost expression in the lower part ×200. (H) Section of another example of mucinous carcinoma showing altered expression (negative and very weak staining) ×200.

detected between alteration of *BRCA-1* expression and tumors of higher histopathologic grade ($P = 0.049$), with the presence of tumor necrosis ($P = 0.0001$) and with tumors of higher proliferation rate ($P = 0.001$). No association was seen with the patient age ($P = 0.723$), tumor size ($P = 0.775$), histopathologic growth pattern ($P = 0.157$), or with the presence of associated benign neoplasms ($P = 0.598$), further details are summarized in Table 2.

In mucinous neoplasms, no significant association was seen with any of the clinicopathologic tumor characteristics. Details are summarized in Table 3.

Discussion

The following study assessed alteration of *BRCA-1* protein expression in a spectrum of serous and mucinous ovarian specimens including normal-benign-borderline-malignant and metastatic specimens.

We found that all the normal epithelial tissues examined (ovarian surface epithelium and fallopian tube mucosa) showed strong positive expression. *BRCA-1* is essential for DNA repair and therefore it functions as a tumor suppressor gene, strong positive reactivity is an expected observation in normal epithelial ovarian tissues. As described in the Human Protein Atlas, *BRCA* protein is expressed ubiquitously in majority of human adult tissues including, skin, muscle, testis, brain, lymph node, prostate, breast, and ovary.[13]

Table 2
Clinicopathologic features of serous carcinoma in association with altered *BRCA-1* expression.

Histopathological criteria		Altered staining (0, 1, 2)	Strong positive staining (3)	*P* value
Patient age	<50	15 (71)	6 (29)	
	>50	28 (76)	9 (24)	0.723
Tumor size	<20	32 (74)	11 (26)	
	>20	7 (70)	3 (30)	0.775
Associated benign or borderline lesions	Absent	25 (71)	10 (29)	
	Benign	3 (60)	2 (40)	
	Borderline	13 (81)	3 (19)	0.598
Growth pattern of serous tumors	Papillary		7 (44)	
	Papillary and glandular	9 (70)	4(31)	
	Solid (pure or mixed)	21	3	0.157
Grading of serous tumors	Low	5 (50)	5 (50)	
	High	38 (79)	10 (21)	**0.049**
Deposits outside ovary	Absent	9 (60)	6 (40)	
	Present	26 (79)	7 (21)	
	Unknown	2 (67)	1 (33)	0.390
Presence of necrosis	Absent	13 (52)	12 (48)	
	Present	30 (91)	3 (9)	**<0.0001**
No. of mitosis	<5/10 HPF	5 (39)	8 (62)	
	≥5/10 HPF	38 (84)	7 (16)	**0.001**

Bold numbers indicates statistical significance.

Table 3
Clinicopathologic features of mucinous carcinoma in association with altered *BRCA-1* expression.

Histopathological criteria		Altered staining (0, 1, 2)	Strong positive staining (3)	*P* value
Patient age	<50	4 (44)	5 (56)	
	>50	5 (45)	6 (54)	0.964
Tumor size	<20	6 (46)	7 (54)	
	>20	3 (43)	4 (57)	0.888
Associated benign or borderline lesions	Absent	3 (50)	3(50)	
	Benign	1(33)	2(67)	
	Borderline	5(55)	4(44)	
	Both	0	2(100)	0.519
Growth pattern of mucinous tumors	Expansile	1 (17)	5 (83)	
	Infiltrative	8 (57)	6 (43)	0.095
Deposits outside ovary	Absent	4(50)	4 (50)	
	Present	2 (33)	4 (67)	
	Unknown	3 (60)	2 (40)	0.665
Presence of necrosis	Absent	2 (33)	4 (66)	
	Present	7 (50)	7 (50)	0.492
No. of mitosis	<5/10 HPF	1 (20)	4 (80)	
	≥5/10 HPF	8 (53)	7 (46)	0.194

In this study, a gradual increase in *BRCA-1* alteration (lost or weak expression) was seen in the direction of the benign-borderline-malignant sequence. It has been found that alteration of *BRCA-1* protein expression can take several forms from complete loss to weak expression of the protein. This alteration can be caused by germ line or somatic mutation.[14] In our specimens,

complete negative expression was detected in 4% of the benign adenomas, 11% of the borderline neoplasms, 24% of the primary malignant tumors, and in 16% of the metastatic extraovarian deposits.

The reported rate of *BRCA-1* mutation among ovarian cancer patients is variable among studies and among different ethnic groups.[15] For example, prevalence of *BRCA-1* mutation is high in countries such as Greece where it accounts for 10% of all ovarian carcinomas and 60% of serous carcinomas. On the other hand, it was low in countries such as Denmark and Sweden where it accounts for 6% of overall carcinoma and 5%-8% of serous carcinoma.[16]

Interestingly, in our specimens, *BRCA-1* alteration was more frequent in serous carcinoma (74%) comparing with mucinous carcinoma (45%). From overall studies, nearly 30% of ovarian serous carcinoma may have either defects or altered expression of *BRCA-1/2*. However, based on several studies of *BRCA-1* protein expression analyses (by immunohistochemistry [IHC]), the absence of or low *BRCA-1* expression is reported in about 41%-65% of ovarian cancer.[12] Our rate is higher than reported in the fore mentioned studies. It should be noted that in this study, the alteration in *BRCA-1* protein expression was assessed and not the genetic mutation, our figures are therefore higher because they assess loss in the *BRCA-1* protein either due to mutation (germ line or somatic) or epigenetic changes.

The great advances in studying molecular profiles of neoplasms has reshaped our understanding for the molecular basics in ovarian carcinogenesis. The original view was that all surface ovarian tumors develop from metaplastic changes in the ovarian surface epithelium, this paradigm however, has been challenged after the gene expression profiling studies. The new model for ovarian carcinogenesis now is that surface ovarian neoplasms are heterogenous group of neoplasms that can be divided into 2 main categories based upon their molecular changes during carcinogenesis.[17] Serous carcinoma, especially high-grade type, was found to originate from epithelial precursors in the fallopian tube fimbria. These precursor lesions were found in a high frequency in women with *BRCA-1* germ line mutation,[18] while mucinous neoplasm mostly originate in the benign-borderline-malignant pathway. The molecular changes in both types of neoplasm are then different.

We further compared carcinomas (serous and mucinous) with aberrant expression of *BRCA-1* with those with normal *BRCA-1* expression. In serous—not in mucinous—carcinoma we found that alteration of *BRCA-1* was associated with higher grade tumors where 79% of HGSC showed some degree of *BRCA-1* loss compared with 50% of the low-grade serous carcinoma. This supports previous studies which reported that *BRCA-1* mutation is associated with HGSCs especially the papillary subtype but not with low-grade serous ones.[19] In the same study, authors reported that tumors in *BRCA-1* carriers are of higher grade, had a higher percentage of solid component and were more likely to stain strongly for P53.[19] This can be explained by the complex molecular network of *BRCA-1* inside the cell. Although *BRCA-1* is mainly involved in DNA repair, it also exerts effect on cell proliferation and apoptosis. A previous study found that tumors with hereditary *BRCA-1* mutation are characterized with higher proliferation rate and have a bigger proliferation growth fraction,[20] the association we detected with necrosis may be a result for a high proliferating tumors.

There has been a lot of debate about the effect of *BRCA-1* alteration on patient survival. In a recent meta-analysis study, it was found that although *BRCA-1* mutation is tumorigenic for ovarian cancer, however its presence is associated with better survival.[21]

The main significance of studying *BRCA-1* expression in ovarian neoplasms is to identify candidates for the PARP inhibitor therapy. At time of development of PARP inhibitors, genetic testing was first recommended to patients having family history of breast and/or ovarian carcinoma. However several studies found that *BRCA-1* mutation was detected in patients with no family history. Further more significant proportion of *BRCA-1* mutations are acquired and not germ line inherited from parents.[14] All these findings necessitate changes in the guidelines of referral for genetic testing and for offering PARP therapy in ovarian cancer patients. In 2016 it has been recommended that "All patients with invasive epithelial ovarian carcinoma (OC) (exclud-

ing borderline or mucinous), including those with fallopian tube and peritoneal cancers, should be considered as candidates for referral for BRCA genetic testing."[22] Later, PARP inhibitors have been approved for ovarian carcinomas with either somatic or germ line mutation.[23] Taken into consideration the difficulty in genetic testing that it is not available in many of the pathology laboratories, a recent study compared detection of *BRCA-1* malfunction using the IHC and mutational genetic analysis. Authors found that assessment of *BRCA-1* status by IHC is a highly reproducible and accurate modality for detecting germline, somatic, or epigenetic mechanisms of *BRCA-1* loss.[24]

In conclusion, this study found a high rate of alteration of *BRCA-1* expression in both serous and mucinous carcinoma. The rate is higher in serous carcinoma and is associated with high grade and highly proliferating tumors. Further studies are necessary to assess the cost benefit of testing all ovarian carcinoma specimens by IHC for *BRCA-1* impaired expression.

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