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Expression of Cancer-associated Fibroblasts Markers in Epithelial Ovarian Neoplasms and Its Correlation with Tumour Progression, Angiogenesis and Lymphangiogenesis: An Immunohistochemical Study

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Abstract

Background: Tumours of the ovary are common forms of neoplasia in female. The new histopathological, molecular and genetic studies provided the dualistic model of ovarian carcinogenesis: type I carcinoma with precursor lesions in the ovary forming part of a morphological and molecular continuum of adenoma carcinoma sequence. They are confined to ovaries with stable genome and without TP53 mutations. In type II carcinoma tumours develop de novo from tubal and/or ovarian surface epithelium, they are more aggressive, genetically unstable with TP53 mutations, and almost half cases show BRCA mutations. Tumour micro environment (TME) acts as an important factor of tumorogenesis and development, once recruited, activated and accumulated to tumour area, these cells are called “cancer associated fibroblasts”(CAFs).

CAFs recognition depends on their morphology and specific biomarkers as SMA, vimentin and FAP which is a cell surface glycoprotein expressed in stromal fibroblasts located in and around the tumour. The most common way of ovarian tumour metastasis is direct extension to adjacent organs, beside spread through the lymphatic and vascular system.

Aim: The aim of the present work was to study the expression of CAF markers (FAP and SMA) in epithelial ovarian neoplasms and to correlate their expression with tumour progression, MVD using CD31 immunostaining and LVD using D2-40 immunostaining.

Material and methods: Sixty formalin fixed, paraffin embedded samples of epithelial ovarian neoplasms were re-evaluated. The diagnosis was as follows: 11 cases were benign, 8 borderline and 41 cases were malignant (of which 12 cases were type I and 29 cases were type II epithelial ovarian carcinoma). All tissue specimens were subjected to primary antibodies against FAP, SMA-alpha, D2-40 and CD31 immunohistochemical stainning.

Results: CAF expression: (detected by FAP and SMA immunohistochemistry) FAP was absent in most benign cases (81.8%) and present in all borderline cases the expression was weak in (87.5%) and strong in (12.5%) of borderline cases. In malignant cases FAP was strongly expressed in (63.5%), moderate in (31.7) and weak in (4.9) of cases. SMA was absent in (54.5%) of benign cases and expressed in all borderline and malignant cases. In borderline cases the expression was weak in (87.5%) and strong in (12.5%). In malignant cases SMA expression was strong in (63.4%), moderate in (34.1) and weak in (2.4%) of cases. There was a statistically significant relation ($P<0.05$) between tumour type and expression of SMA. There was a significant increased CAFs expression from borderline to type I epithelial ovarian carcinoma. Also there was difference in their expression within the malignant group itself according to its type. As regards stage there was a significant relation ($P<0.01$) between tumour stage and presence of CAFs with increased expression in advanced stages. Assessment of MVD and LVD, as a measure of angiogenesis and lymphangiogenesis, using CD 31 and D2-40 respectively, and their correlation with CAFs showed that that there was a significant increase in MVD and LVD from benign through borderline to type I epithelial ovarian carcinoma. Also there was difference in their expression from benign through borderline to malignant epithelial tumour. Within the malignant group itself both markers showed significant difference between type I and type II ovarian carcinoma, confirming the difference in behavior and progression of each type. We found a significant relation between tumour FIGO stage and LVD but no relation with MVD.

Conclusions: TME particularly CAFs provide tumour architectural support and affect its physiology and function. CAFs play multiple roles in tumour development and progression. It is related to stage, metastasis, tumour type, LVD and MVD in human epithelial ovarian neoplasm. Their expression may be a poor prognostic indicator that indicates patients at great risk of developing metastasis.

Keywords: Ovarian neoplasms; Tumour; Cytotoxic therapies

Introduction

Epithelial ovarian carcinoma (EOC) is the most common type of ovarian cancer [1,2] representing 80-90% of all malignant ovarian tumors and it is the most lethal gynecological malignancy in the world [3]. The high mortality rate of ovarian carcinoma primarily stems from the late diagnosis of this tumor. Approximately 70% of patients are diagnosed with stage III or IV ovarian cancer, which is characterized by peritoneal or distant metastases, respectively [4]. Despite advances in cytotoxic therapies, only 30% of patients with advanced-stage ovarian cancer survive 5 years after initial diagnosis. So there is a continuous

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need for better understanding of the mechanisms involved in the spread of ovarian carcinoma [5].

Cancer tissue is considered a sophisticated construct of both malignant tumor cells and non-malignant host stromal cells [6]. However, in the past decades, the major focus of ovarian cancer researches has been the transformed tumor cell itself with a rapid progression of knowledge pertaining to the genetic and epigenetic changes they undergo and eluciding their signaling pathways in tumor cells [6,7]. Till now existing therapies remain relatively ineffective for most types of cancer, and the role of tumor stroma in tumor genesis, especially the fibroblasts which are the main component in the stroma, has not been widely explored [8].

In 1889, Paget [9] proposed a novel concept, the 'seed and soil' hypothesis, postulating that the congenial microenvironment (the 'soil') is prerequisite for the progression of tumor cells (the 'seeds'). Tumor cells are disseminated throughout the body via the blood stream, but only in congenial 'soil' can metastases develop.

Dvorak et al. proposed the theory that a "tumor is a wound that never heals" [10]. Fibroblasts in cancer tissues are similar in morphology to myofibroblasts, which are large spindle-shaped cells that are activated during the wound healing process. Over 80% of stromal fibroblasts in cancer are thought to acquire the activated phenotype [11]. Fibroblasts, [CAF] are the major components of cancer stroma. During wound healing, when the process is completed, activated fibroblasts decrease [12]. In contrast, CAFs are perpetually activated, and neither reverts to a normal phenotype nor undergoes apoptosis and elimination like normal fibroblasts [13]. To design effective therapies to target cancer, more information regarding CAFs is necessary, and novel mechanisms of CAFs are being revealed each year [14].

More researches on the role of tumor stroma in cancer progression and prognosis illustrating that a genetic alteration during cancer development, leading to a malignant cell, will consequently change the stromal host compartment to establish a permissive and supportive environment for the cancer cell [15]. During early stages of tumor development and invasion, the basement membrane is degraded, and the activated stroma, containing fibroblasts, inflammatory infiltrates, and newly formed capillaries, comes into direct contact with the tumor cells. The basement membrane matrix also modifies cytokine interactions between cancer cells and fibroblasts [16]. These cancer-induced alterations in the stroma will contribute to cancer invasion [17].

Aim of the work

To study the expression of CAF markers (FAP and SMA) in epithelial ovarian neoplasms and to correlate their expression with tumor progression, MVD using CD31 immunostaining and LVD using D2-40 immunostaining.

Material and Methods

The material of this study comprised 60 cases of epithelial ovarian neoplasms that were retrospectively retrieved from the archives of Pathology Department, Medical Research Institute and Damanhour Oncology Center between January 2015 and January 2016.

The research study was conducted in accordance with the highest scientific human and ethical principles recommended by Alexandria University, Egypt. Sixty formalin fixed, paraffin embedded samples of epithelial ovarian neoplasms were re-evaluated staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) 2014.The diagnosis was as follows:

11 cases were benign, 8 borderline and 41 cases were malignant (of which 12 cases were type I and 29 cases were type II epithelial ovarian carcinoma).

All tissue specimens were subjected to primary antibodies against FAP, SMA-alpha, D2-40 and CD31 immunohistochemical staining.

Interpretation of Immunohistochemical staining

A) Assessment of CAFs Staining: Intensity and ×400 Magnification

B) Assessment of MVD staining CD31 (5 hot spots) ×400 Magnification

C) Assessment of LVD staining D2-40 (5 hot spots) ×400 Magnification

Histopathologic Results

Out of studied cases (60 cases) 11 cases were benign (representing 18.3% of the cases), 8 cases of borderline type (13.3%), and 41 cases were malignant (68.3%) as shown in Figure 1.

Immunohistochemical Results

Assessment of presence of CAFs

Assessment of presence of CAFs (through assessment of the immunohistochemical expression of both FAP and SMA) in stroma of different ovarian neoplasms revealed that:

FAP immunohistochemistry

FAP immunostain was absent in (81.8%) of benign cases and expressed in all borderline and malignant cases. In benign group: 9 cases (81.8%) were negative, and weak immunohistochemical stain was detected in 2 cases (18.2%). In borderline cases FAP positive immunostain was detected in all cases: weak expression was detected in 7 cases (87.5%) and moderate expression in one case only (12.5%). In malignant cases FAP positive immunostain was detected in all cases: FAP were strongly expressed in 26 cases (63.4%), weak expression was detected in two cases (4.9%), and moderate in 13 cases (31.7%). The relation between tumor type (comparing benign, borderline and malignant categories) and expression of FAP revealed that there was a statistically significant relation ($p<0.05$) between tumor type and expression of FAP immunostain as shown in Figures 2 and 3.

Studying FAP immunostain in both types of ovarian carcinoma showed that in type I carcinoma cases weak expression of FAP immunostain was detected in 2 cases (16.7%), moderate expression was detected in 7 cases (58.%), and strong expression in 3 cases (25%) as shown in Figures 4 and 5.

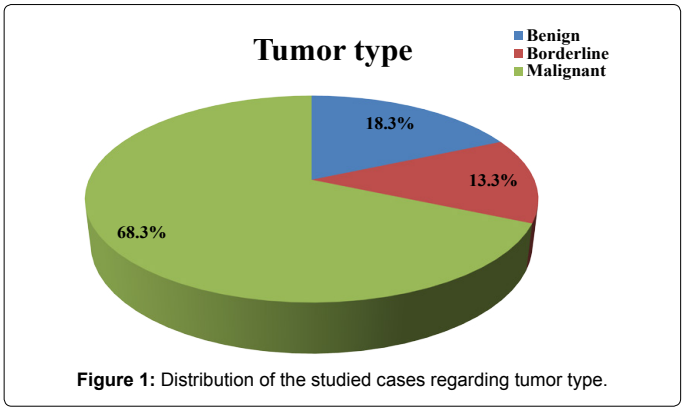

Figure 1: Distribution of the studied cases regarding tumor type.

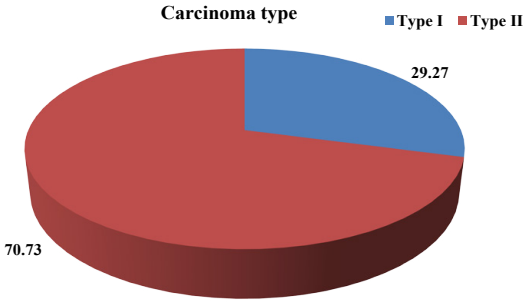

Figure 2: Distribution of the studied malignant cases regarding carcinoma type.

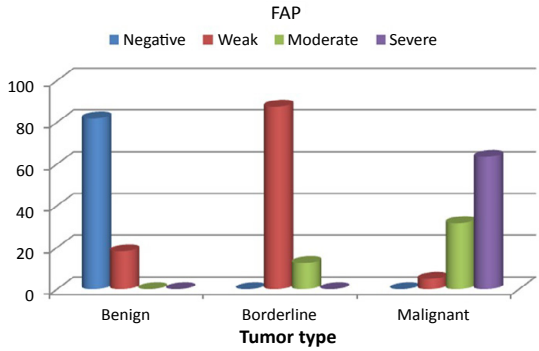

Figure 3: Relation between tumor type (benign, borderline and malignant) and FAP immunostain intensity.

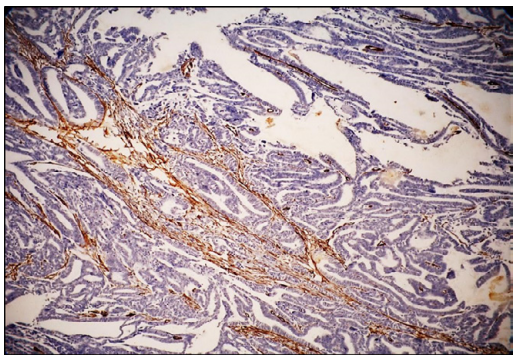

Figure 4: Moderate expression of FAP in low grade serous carcinoma (IHCx100).

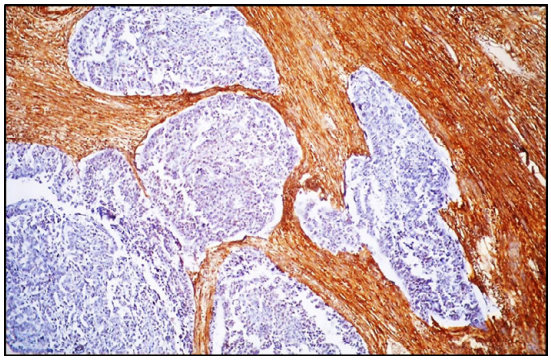

Figure 5: Strong expression of FAP in high grade serous carcinoma (IHCx100).

In Type II carcinomas strong expression of FAP immunostain was detected in 23 cases (79.3%), and moderate in 6 cases (20.7%).

The relation between tumor type (comparing the adenoma- carcinoma sequence) including benign, borderline tumors and type I ovarian carcinoma and immunohistochemical expression of FAP revealed that there was a statistically significant relation ($p<0.05$) between tumor type and FAP immunostaining intensity as shown in Figure 6.

The relation between the type of the epithelial carcinoma and presence of CAFs (detected by FAP immunohistochemistry) revealed that there was a statistically significant relation ($p<0.05$) between tumor type and FAP immunostain as shown in Figure 7.

Studying FAP immunostain in different stages of malignant cases showed that FAP immunostain were strongly expressed in all stage IV cases. In stage III cases strong expression of FAB was detected in 15 cases (83.3%) and moderate expression in 3 cases (16.7%). In stage II cases moderate FAP expression was detected in 2 cases (50%), weak expression in one case (25%) and strong expression in one case (25%). In stage I cases moderate FAP expression was detected in 8 cases (53.3%), weak expression in one case (6.7%) and strong expression in 6 cases (40%).

Study the relation between the tumor stage and presence of CAFs (detected by FAP immunohistochemistry) revealed that there was a statistically significant relation between tumor stage and FAP expression ($p<0.01$) as shown in Figure 8.

SMA immunohistochemistry

SMA immunostain were absent in (54.5%) of benign cases and expressed in all borderline and malignant cases.

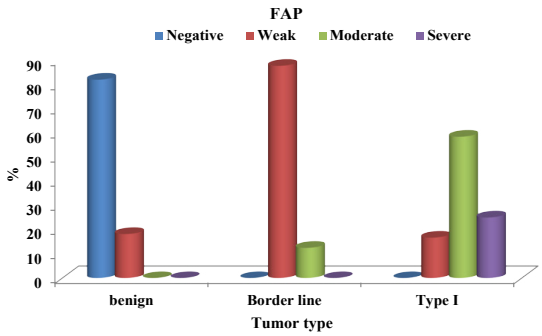

Figure 6: Relation between tumor type (benign, borderline, type I carcinoma) and FAP immunostain intensity.

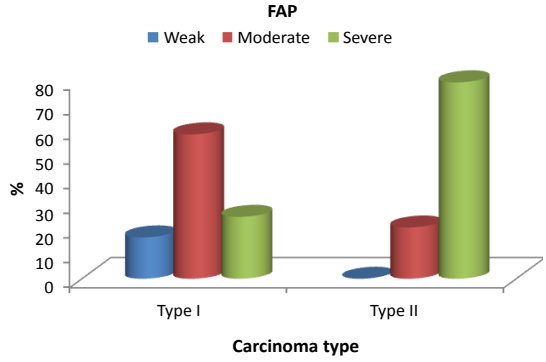

Figure 7: Relation between carcinoma type and FAP immunostain.

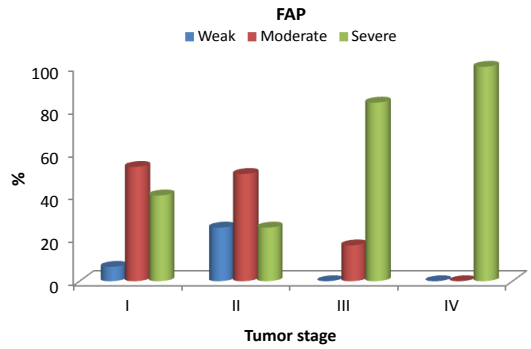

Figure 8: Relation between tumor stage and FAP immunostain intensity.

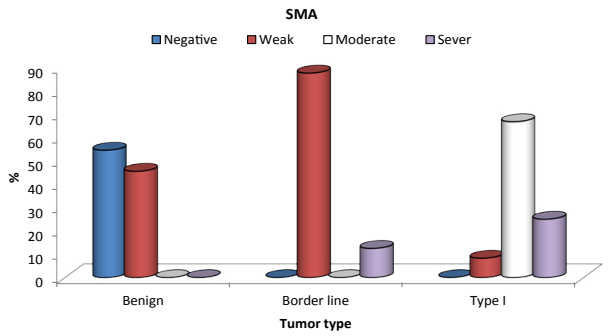

Figure 10: Relation between tumor type (benign, borderline, type I carcinoma) and SMA immunostain intensity

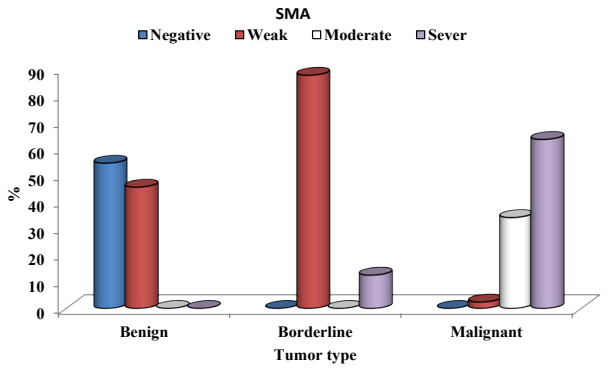

Figure 9: Relation between tumor type (benign, borderline and malignant) and SMA immunostain intensity.

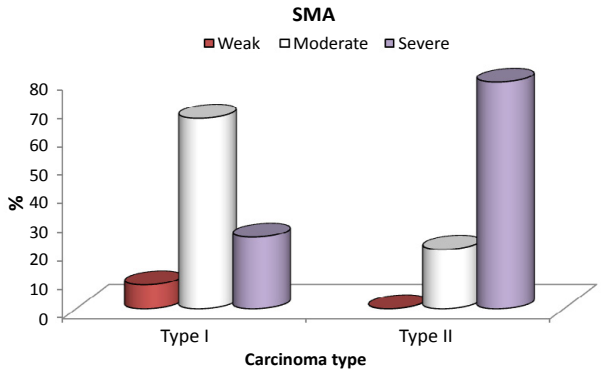

Figure 11: Relation between the carcinoma type and SMA immunostain.

In benign group: 6 cases (54.5%) were negative, and weak immunohistochemical stain was detected in 5 cases (45.5%).

In malignant cases SMA positive immunostain was detected in all cases: SMA was strongly expressed in 26cases (63.4%), weak expression was detected in one case (2.4%), and moderate expression in 14 cases (34.1%). The relation between tumor type (comparing benign, borderline and malignant categories) and expression of SMA revealed that there was a statistically significant relation ($p<0.05$) between tumor type and expression of SMA as shown in Figure 9.

Studying SMA immunostain in both types of epithelial carcinoma, showed that: In type I carcinoma cases weak expression of SMA immunostain was detected in one case (8.3%), moderate expression was detected in 8 cases (66.7.%), and strong expression in 3 cases (25%). In Type II carcinomas strong expression of SMA immunostain was detected in 23 cases (79.3%), and moderate expression in 6 cases (20.7%).

Study the relation between tumor type (comparing the adenoma-carcinoma sequence) including benign, borderline tumors and type I ovarian carcinoma and immunohistochemical expression of SMA revealed that there was a statistically significant relation ($p<0.05$) between tumor type and SMA immunostaining intensity as shown in Figure 10.

Study the relation between the type of the epithelial carcinoma and presence of CAFs (detected by SMA immunohistochemistry) revealed that there was a statistically significant relation ($p<0.05$) between tumor type and SMA immunostain as shown in Figure 11.

In borderline cases SMA positive immunostain was detected in all cases: weak expression was detected in 7 cases (87.5%) and strong expression in one case only (12.5%). Studying SMA immunostain in different stages of malignant cases showed that SMA immunostain were strongly expressed in all stage IV cases. In stage III cases strong expression of SMA was detected in 15 cases (83.3%) and moderate expression in 3 cases (16.7%). In stage II cases moderate FAP expression was detected in 2 cases (50%), weak expression in one case (25%) and strong expression in one case (25%). In stage I cases moderate FAP expression was detected in 9 cases (60%) weak expression in one case (6.7%) and strong expression in 6 cases (40%) as shown in Figures 12 and 13.

The relation between the tumor stage and presence of CAFs (detected by SMA immunohistochemistry) revealed that there was a statistically significant relation between tumor stage and SMA expression ($p<0.01$) as shown in Figure 14.

Assessment of tumor MVD

Studying of the MVD assessed by CD31immunohistochemical expression, showed that:

The mean MVD of the benign group was 8.1, ranging between 6 and 12.3, of border-line cases was 9.6, ranging between 6 and 12, and of malignant cases was 21.6 ranging between 6.3 and 30.3.

Studying the relation between tumor type and CD31 immunohistochemical expression (as a measure of MVD). There was a statistical significant difference in MVD between different types of tumor (benign, borderline and malignant) $p<0.05$, with a statistically

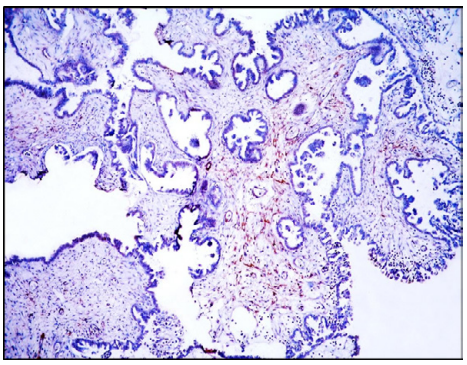

Figure 12: Weak expression of SMA in borderline serous tumor (IHCx100).

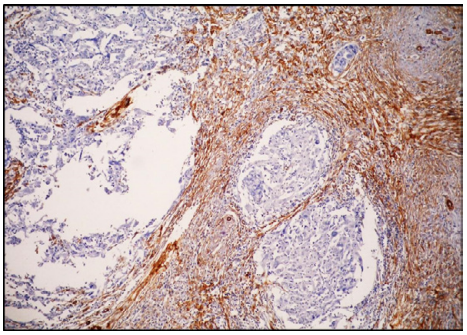

Figure 13: Strong expression of SMA in high grade serous carcinoma (IHCx100).

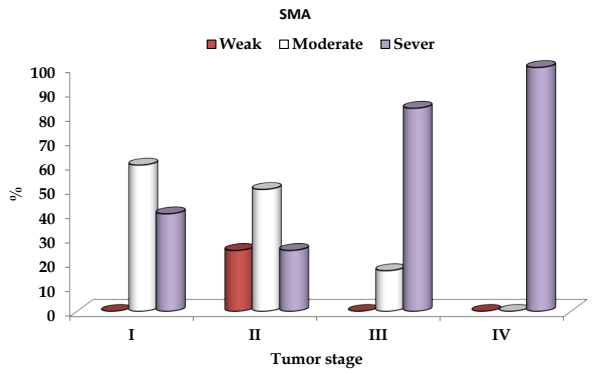

Figure 14: Relation between tumor stage and SMA immunostain.

significant increase of MVD in malignant than borderline and benign tumors as shown in Figure 15.

The mean MVD in type I carcinoma was 19.2 ranging between 6.3 and 29.6. While the mean MVD in type II carcinoma was 22.9 ranging between 9.33 and 30.33.

Studying benign, border-line and type I carcinoma cases in relation to mean CD31 immunohistochemistry. There was a statistically significant difference between benign, border-line and type I malignant tumors in relation to mean MVD, $p<0.05$ as shown in Figure 16.

Comparison between type I and II carcinoma cases regarding mean MVD (assessed by CD31 immunostain) revealed that there was a statistically significant difference between both carcinoma types regarding their mean MVD, $p<0.05$ as shown in Figure 17.

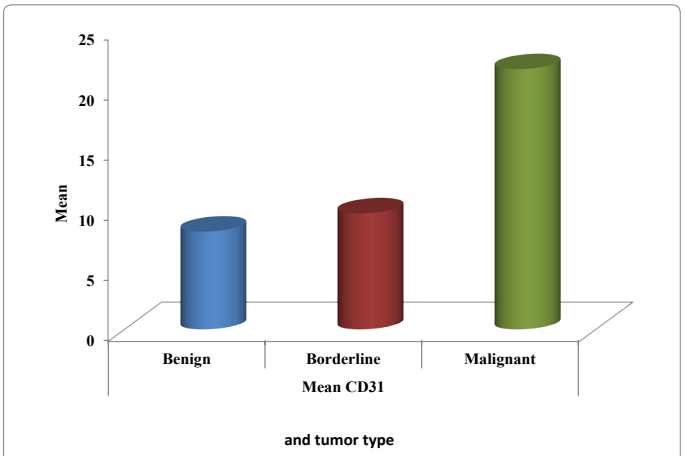

Figure 15: Relation between tumor type and CD31 IHC expression (MVD).

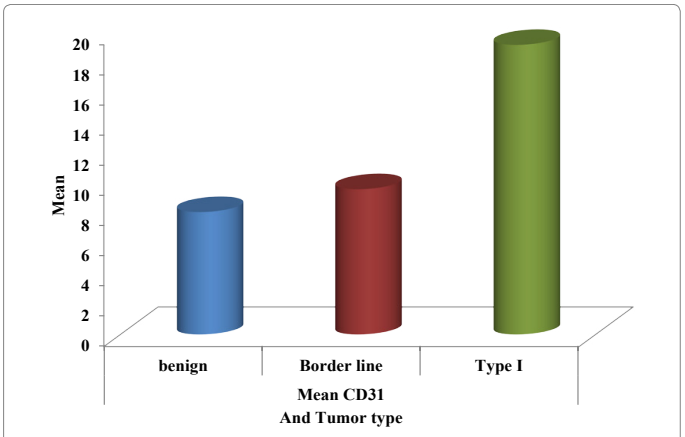

Figure 16: Relation between tumor type and CD31 IHC expression (MVD).

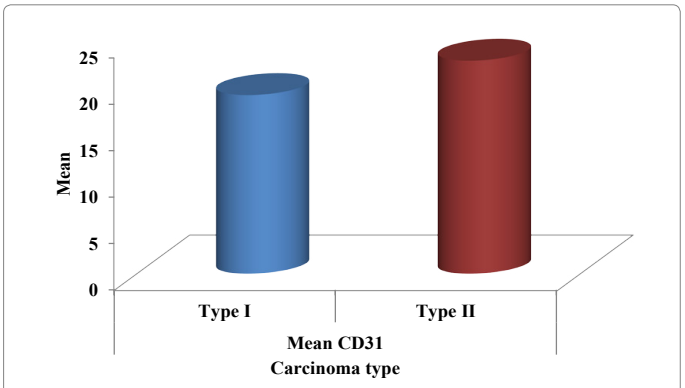

Figure 17: Relation between carcinoma type and mean MVD.

Also it was noticed that the microvessels of malignant neoplasms have reduced SMA expression and increased CD31 expression as compared to the microvessels of benign tumors as shown in Figure 18.

Studying the MVD in different stages showing: In stage I cases the MVD ranged between 6.33 and 30.33 with mean value 18.66 ± 6.96, in stage II ranged between 18 and 29.33 with mean value 22.66 ± 5.35, in stage III ranged between 9.33 and 29.60 with mean value 23.08 ± 5.13 and in stage IV ranged between 17.67 and 27.33 with mean value 23.55 ± 5.16.

The comparison between different stages of the malignant cases regarding mean MVD (assessed by CD31 immunostain) showed that there was no statistical significant difference between mean MVD (CD31 expression) and tumor stage, P > 0.05 as shown in Figures 19 and 20.

Assessment of tumor LVD

Studying of LVD assessed by D2-40 immunohistochemical expression in Figures 21 and 22 shows that:

The mean LVD of the benign group was 3.1, ranging between 2 and 3.9 of border-line cases was 3.1, ranging between 2.5 and 3.9, and of malignant cases was 4.7 ranging between 2.8 and 6.1.

Studying the relation between tumor type and D2-40 immunohistochemical expression (as a measure of LVD) showed that there was a statistically significant difference between type of tumor (benign, borderline and malignant) and D2-40 immunostain p<0.05, with a statistically significant increase of LVD in malignant than borderline and benign tumors) as shown in Figure 23.

The mean LVD in type I carcinoma was 3.9617 ranging between 2.8 and 5.54. While the mean MVD in type II carcinoma was 4.9 ranging between 2.9 and 6.1.

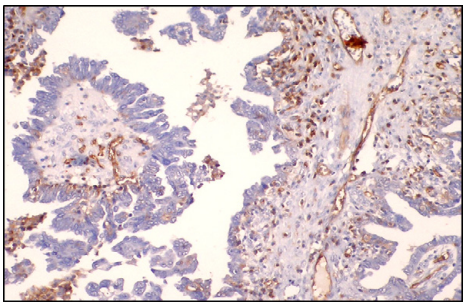

Figure 18: Moderate MVD, (CD31 IHC), in borderline serous tumor (IHCx400).

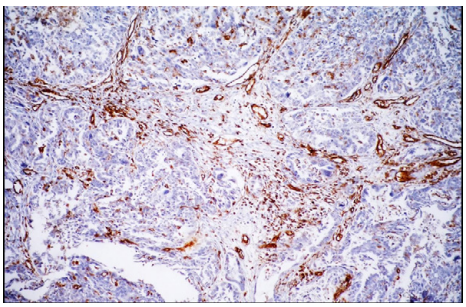

Figure 19: High MVD, (CD31 IHC), in high grade serous carcinoma (IHCx100).

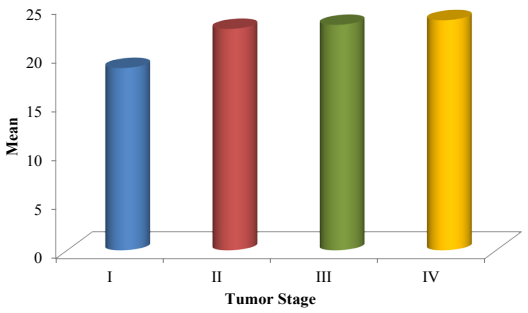

Figure 20: Relation between tumor stage and mean MVD.

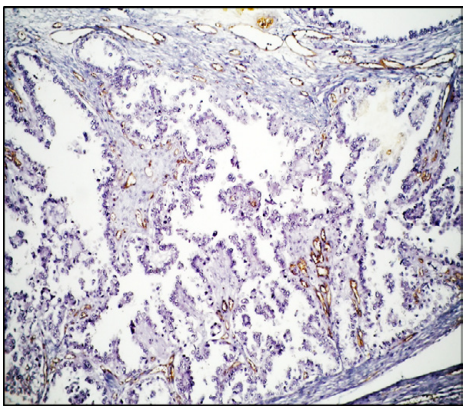

Figure 21: Low LVD (D2-40 IHC) in serous borderline ovarian tumor (IHCx100).

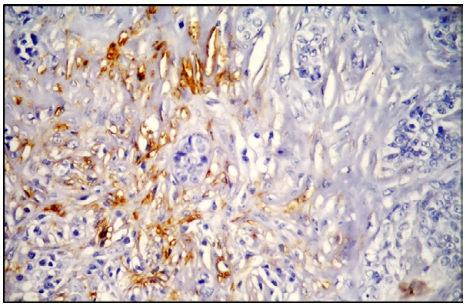

Figure 22: High LVD (D2-40 IHC) in anaplastic carcinoma (IHCx400).

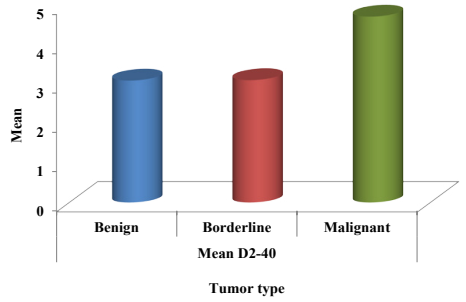

Figure 23: Relation of tumor type (benign, borderline and type I tumor) in relation to mean LVD (D2-40IHC).

Sudying the relation between tumor type (adenoma-carcinoma sequence) and D2-40 immunohistochemical expression (as a measure of LVD) showed that there was a statistically significant difference between type of tumor (benign, borderline and type I carcinoma) and D2-40 immunostain p<0.05, with a statistically significant increase of LVD in type I carcinoma than borderline and benign tumors as shown in Figure 24.

The comparison between type I and II carcinoma cases regarding mean LVD (assessed by D2-40 immunostain) showed that there was a statistically significant difference between both carcinoma types regarding mean LVD p<0.05 as shown in Figure 25.

Studying the LVD in different stages, showing: In stage I cases the LVD ranged between 2.8 and 5.6 with mean value 4.07 ± 0.99, stage II ranged between 3.5 and 5.4 with mean value 4.8 ± 0.8, stage III ranged between 2.9 and 6.1 with mean value 5.03 ± 0.76 and stage IV ranged between 5.24 and 6.05 with mean value 5.48 ± 0.38 (Table 1).

The comparison between different stages of the malignant cases regarding mean LVD in different stages of malignant cases showed that there was a statistically significant difference between the tumor stage and LVD (D2-40 expression) $p<0.05$ as shown in Figures 26-28.

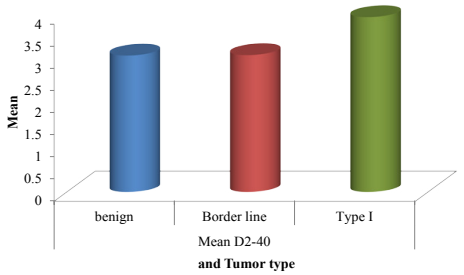

Figure 24: Relation of type of tumor (benign, border-line and type I malignant and D2-40 immunohistochemical expression.

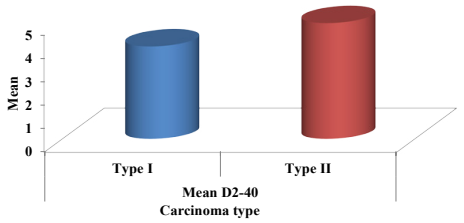

Figure 25: Relation between type I and II carcinoma with mean LVD (D2-40 IHC).

Studying the correlation between FAP immunohistochemical expression as a marker of CAFs and tumor MVD and LVD shows that the presence of stromal CAFs is correlated with tumor angiogenesis and lymphangiogenesis as shown in Table 2.

Studying the correlation between SMA immunohistochemical expression as a marker of CAFs and tumor MVD and LVD shows that the presence of stromal CAFs is correlated with tumor angiogenesis and lymphangiogenesis as shown in Table 3.

Discussion

In our study the difference in expression between different types of epithelial ovarian neoplasms showed that CAF markers expression increased significantly from benign through borderline to malignant epithelial tumors. These results suggest that CAFs probably initiate and promote tumorigenic alterations in epithelial cells in a gradual process [18,19].

The present study also examined the differences in expression within the adenoma-carcinoma sequence, regardless of the tumor histologic type, CAF markers expression increased significantly from benign through borderline to type I epithelial ovarian carcinomas. Thus, their expression could play a role in the adenoma-carcinoma sequence of these ovarian tumors.

Also the present study examined and confirmed differences in expression within the malignant group itself according to its type. There was a statistically significant difference in CAF markers expression which further supports the recent concepts that those two types are pathogenetically different with difference in molecular events

Clinicopathological Characteristics	Benign (11 cases)		Borderline (8 cases)		Malignant (41 cases)					
					Type I (12 cases)		Type II (29 cases)		Total (41)	
Age: Min-Max, (Mean)	23-80, (39.63)		30-56, (39.6)		31-68, (54.2)		35-78, (51.3)		31-78	
Menopausal state										
Pre-menopause	8	72.70%	6	75%	3	25%	13	44.80%	16	39%
Post menopause	3	27.30%	2	25%	9	75%	16	55.20%	25	71%
Laterality										
Unilateral	9	81.82%	8	100%	10	83.30%	12	41.10%	22	53.70%
Bilateral	2	18.18%	0	0%	2	16.70%	17	58.60%	19	46.30%
Gross appearance										
Cystic	11	100.00%	8	100%	7	58.30%	1	3.40%	8	19.50%
Solid + Cystic	0	0.00%	0	0%	5	41.70%	28	96.60%	33	80.50%
FIGO stage										
Stage I			8	100%	11	91.70%	4	13.80%	15	36.60%
Stage II			0	0%	1	8.30%	3	10.30%	4	9.80%
Stage III			0	0%	0	0.00%	18	62.10%	18	43.90%
Stage IV			0	0%	0	0.00%	4	13.80%	4	9.80%
Omental involvement										
Free			8	100%	11	91.70%	4	13.80%	15	35.60%
Involved			0	0%	1	8.30%	25	86.20%	26	63.40%
Nodal metastasis										
Absent			8	100%	12	100.00%	10	34.50%	22	53.70%
Present			0	0%	0	0.00%	19	63.40%	19	46.30%
Distant metastasis										
Absent					12	100.00%	25	86.20%	37	90.20%
Present					0	0.00%	4	13.80%	4	9.80%

Table 1: The clinicopathological data of the studied cases.

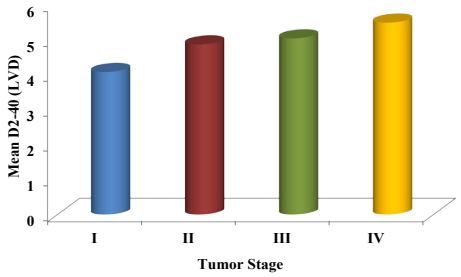

Figure 26: Relation between tumor stage and D2-40.

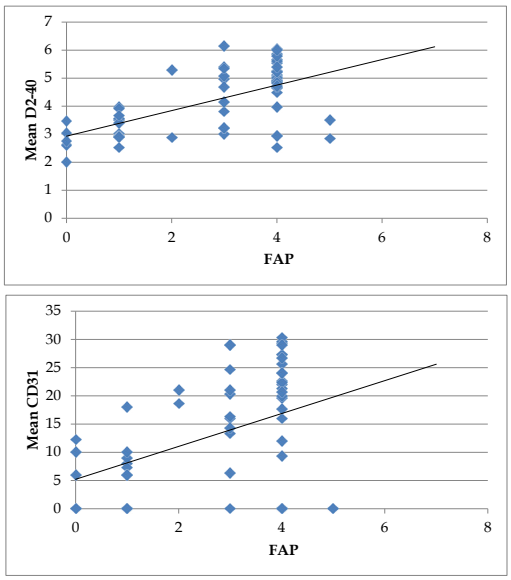

Figure 27: Correlation between FAP expression as a marker of CAFs with tumor MVD and LVD.

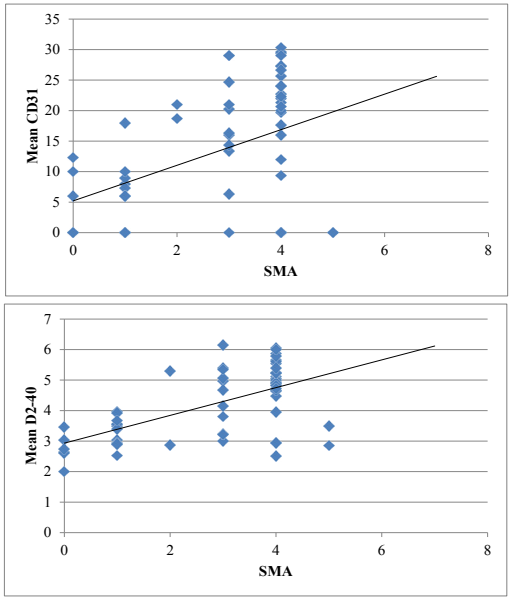

Figure 28: Correlation between SMA IHC expression as a marker of CAFs with tumor MVD and LVD.

Correlations				
		Mean CD31	**D2-40**	**FAP**
Mean CD31	Pearson Correlation	1	0.567**	0.699**
	Sig. (2-tailed)		0	0
	N	43	43	43
D2-40	Pearson Correlation	0.567**	1	0.711**
	Sig. (2-tailed)	0		0
	N	43	60	60
FAP	Pearson Correlation	0.699**	0.711**	1
	Sig. (2-tailed)	0	0	
	N	43	60	60
**Correlation is significant at the 0.01 level (2-tailed).				

Table 2: Correlation between FAP IHC expression as a marker of CAFs with tumor MVD and LVD.

		Mean CD31	**D2-40**	**SMA**
Mean CD31	Pearson Correlation	1	0.567**	0.671**
	Sig. (2-tailed)		0	0
	N	43	43	43
D2-40	Pearson Correlation	0.567**	1	0.678**
	Sig. (2-tailed)	0		0
	N	43	60	60
SMA	Pearson Correlation	0.671**	0.678**	1
	Sig. (2-tailed)	0	0	
	N	43	60	60
**Correlation is significant at the 0.01 level (2-tailed).				

Table 3: Correlation between SMA IHC expression as a marker of CAFs with tumor MVD and LVD.

and behavior, as documented in Gilks study of ovarian carcinomas as distinct diseases with different origins, genetic alterations, and clinicopathological features [20]. However, these findings need to be confirmed in large-scale patient cohorts to establish the potential correlation between CAF expressions and the evolution and progression of ovarian tumors, which might help identify individuals suitable for targeted therapy against these stromal fibroblasts.

Studies using mouse models of genetically altered fibroblasts demonstrate that over expression of TGFb and/or hepatocyte growth factor (HGF) (as markers of CAFs) in mouse fibroblasts induces the initiation of breast cancer within the normal human epithelium [21]. Olumi et al. compared the effect of CAFs isolated from primary prostatic tumor and normal fibroblasts (NFs) isolated from benign prostatic hyperplasia, found that the presence of CAFs induced the growth of tumors in mice mode, whereas NFs did not, which indicated that the CAFs may provide growth-promoting signals to epithelial cells and support epithelial transformation [22].

We studied the malignant cases for markers expression and staining score according to tumor stage and there was a significant relation between tumor stage and the presence of CAFs with increased markers expression in advanced stages, the CAFs were detected in the primary tumor and the metastatic sites (omental and lymph nodes). These results are in agreement with results in literature [18,23,24]. Zhang et al suggested that there were significant correlations between the CAFs and disease stage in ovarian cancer, and in cases with lymph node and omentum metastases the expression of α-SMA was much higher than that in cases without metastases. These findings support the opinion that perhaps CAFs facilitate tumor invasion and tumor motility that are required for metastases to occur [23].

The significant role of CAFs in ovarian cancer cell metastases was

confirmed by in vitro assay. Even though normal fibroblasts (NFs) could promote the invasion and migration of cancer cells, CAFs compared with NFs had a far more prominent potential to promote cancer cell metastases. However, neither CAFs nor NFs had influence on ovarian cancer cells proliferation [18,19].

Other studies showed that CAFs may also promote tumor progression and metastasis through communication with cancer cells such as secreting growth factors which directly affect cell motility, and proteases that can degrade extracellular matrix (ECM) [25]. Angiogenesis has a vital role in tumor progression. The degree of angiogenesis of a tumor, as assessed by MVD has emerged as a powerful candidate for prognosis and as a predictive tool [26].

In the present work assessment of MVD and LVD (as measures for angiogenesis and lymphangiogenesis, using immunohistochemical stain for CD31 and D2-40 respectively), and studying their correlation with CAFs in different cases of the research was done.

It was found that both MVD and LVD showed significant increase from benign through borderline to malignant epithelial tumors. And also from benign through borderline to Type I malignant epithelial carcinoma representing the adenoma carcinoma sequence.

Within the malignant group both markers showed significant difference between Type I and Type II ovarian carcinoma, confirming the difference in behavior and progression of both types.

In literature few studies have also tried to assess and compare MVD and LVD of benign and malignant ovarian surface epithelial neoplasms. These studies concluded that average MVD and LVD were significantly higher in malignant ovarian surface epithelial neoplasms and higher MVD was associated with transformation and acquisition of invasive phenotype of advanced epithelial ovarian cancers [18,26,27]. However, there are also few contradictory studies in literature, which have found no significant difference in MVD values of benign and malignant ovarian surface epithelial tumors [28,29].

In the present study it was noticed that vessels of malignant neoplasms have reduced SMA expression and increased CD31 expression as compared to vessels of benign tumors. Indicating that the malignant ovarian tumors showed higher production of immature blood vessels with paucity of smooth muscle support and endothelial cell proliferation as compared to benign tumors. These results suggest that tumor vessels in malignant tumors are thin-walled, which allows easier vascular invasion and dissemination. The angiogenesis in malignant neoplasms is more primitive type where there is paucity of peri-endothelial support cells and thus, higher permeability for tumor cells. This can attribute to different biological behavior of malignant tumors in form of local invasion, omental and distal metastasis. In view of these results, new therapy should target not only MVD of malignant tumors but also attempt to alter the ratio of immature/ mature vessels for successful outcomes [30]. Further larger studies on this topic are advised.

The present study, in agreement with other previous ones, showed that CAFs were significantly correlated with LVD and MVD in epithelial ovarian neoplasms. This may be because CAFs can secrete a number of important factors including growth factors, angiogenetic factors, prolymphangiogenic factors and metastasis-promoting factors such as VEGF, stromal cell-derived factor 1(SDF-1), prostaglandin E2, plasminogen activator inhibitor-1(PAI-1), insulin-like growth factor-2 (IGF-2) and hepatocyte growth factor (HGF) [30,31].

The tumor microenvironment may be a necessity in the inception of malignant tumors and is increasingly being recognized to have a vital role in their progression. Cancer-associated fibroblasts (CAFs) are the most ubiquitous element of tumor stroma and are found in numerous types of cancer.

Conclusions

- CAFs, which are the most abundant component of TME, play multiple roles in tumor development and progression.
- CAFs are related to disease stage, lymph node metastases, omentum metastases, Tumor type, LVD and MVD in human epithelial ovarian neoplasms.
- CAFs may be a poor prognostic indicator that helps to identify patients who are at high risk to metastasize.
- The angiogenesis in malignant neoplasms is more primitive type where there is paucity of periendothelial support cells and thus, higher permeability for tumor cells.

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