European Journal of Gynaecological Oncology

Original Research

Association of cancer-associated fibroblasts and survival in malignant ovarian neoplasms

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DOI:10.31083/j.ejgo4204118

Submitted: 18 April 2021 Revised: 1 June 2021 Accepted: 21 June 2021 Published: 15 August 2021

Objective: The aims of the study were to compare the stromal immunostaining of smooth muscle alpha-actin (α-SMA) and fibroblast activation protein-α (FAP) between borderline ovarian tumors and epithelial ovarian cancer, and to evaluate their association in overall survival (OS) and disease-free survival (DFS) in patients with ovarian cancer. *Methods*: Patients diagnosed with malignant (n = 28) and borderline ovarian tumors (n = 18) were evaluated. Immunohistochemical study of stromal α-SMA and FAP was carried out. The comparison of immunostaining between borderline and malignant ovarian tumors was performed using Fisher's exact test. Survival was assessed by the Kaplan-Meier method and the log-rank test. Multivariate analysis was performed by Cox regression. The differences were considered significant for $p < 0.05$. *Results*: Evaluating stromal FAP, stronger immunostaining (2 and 3) was more often found in epithelial ovarian cancer than in borderline ovarian tumors (p = 0.0331). There was no statistical significance in the assessment of α-SMA. Evaluating only patients with epithelial ovarian cancer, there was a higher OS in patients with stromal α-SMA immunostaining 3 (p = 0.017). There was no statistical significance when evaluating OS and DFS in patients with stromal FAP immunostaining, nor evaluating DFS in patients with α-SMA stromal immunostaining 3. After multivariate analysis, patients with stromal α-SMA immunostaining 3 had higher OS compared to immunostaining 0, 1 or 2 [OR (95% CI) = 0.107 (0.018–0.649), p = 0.015]. *Conclusion*: Stronger FAP immunostaining was more often found in epithelial ovarian cancer than in borderline ovarian tumors. In epithelial ovarian cancer, there was a higher OS in patients with stromal α-SMA immunostaining 3.

Keywords

Epithelial ovarian cancer; Borderline ovarian tumors; Smooth muscle alpha-actin; Fibroblast activation protein-α; Tumor microenvironment; Overall survival

1. Introduction

Epithelial ovarian cancer is a gynecological malignancy that has led to thousands of deaths among women worldwide. Risk factors that contribute to this disease are diverse, yet the most common genetic factor involves mutations in BRCA-1 and/or BRCA-2 genes [1]. Coburn *et al.* [2] have reported a stable incidence of ovarian cancer in various countries and regions of Europe, Asia, and North America. However, in the eastern and southern regions of Europe, an increased incidence of ovarian cancer has been observed, while a decreased incidence has been observed in northern Europe.

Fibroblast activation protein-α (FAP) is a cellular surface antigen which is classified as a marker of cancer-associated fibroblasts (CAFs). FAP is a type II transmembrane protein and a serine protease of the prolyl oligopeptidase family [2–4]. Expression of FAP is restricted to stromal fibroblasts and is induced in granulation and wound healing processes, fibrosis, keloids, bone sarcomas, arthritis, and epithelial carcinomas [4–7]. It is estimated that expression of FAP in the stromal compartment affects more than 90% of neoplasms of epithelial origin, and high expression of FAP has been related to poor prognosis in several types of carcinomas [4, 8–11].

Actin is a structural protein which functions in cellular motility and muscle contraction. Correspondingly, it is abundant in the cytoskeleton of eukaryotic cells and at least six isoforms have been identified [12, 13]. One of the best characterized isoforms of actin is smooth muscle alpha-actin (α-SMA) which is expressed in smooth muscle cells of the vasculature (e.g., myofibroblasts, blood vessels, and smooth muscle cells) [14]. It also exhibits significant functionality in fibrogenesis [15–17]. Immunostaining of ovarian neoplasms for α-SMA alone has not produced significant results, yet staining of α-SMA in combination with other markers has provided more accurate results [18]. Furthermore, patterns and levels of α-SMA expression can be prognostic factors and have been related to overall survival (OS) [19]. Taken together, these results support further investigations of the roles and classification of surface marker expression by CAFs.

The objectives of the present study were to compare stromal immunostaining of α-SMA and FAP between borderline ovarian tumors and ovarian epithelial malignancies, and to evaluate possible associations with OS and disease-free survival (DFS) in patients with epithelial ovarian cancer.

2. Materials and methods

2.1 Patient selection

Patients who were diagnosed with borderline or malignant ovarian tumors and treated at the Pelvic Mass Outpatient Clinic of the Discipline of Gynecology and Obstetrics/Oncology Research Institute (IPON) of the Federal University of Triângulo Mineiro (UFTM) were evaluated in this study. The inclusion criterion was a histopathological diagnosis of borderline ovarian tumor and malignant neoplasm. Patients were excluded if they received a diagnosis of torsion of the adnexal pedicle or secondary ovarian malignant neoplasm (metastasis), or if they previously received treatment or manifested recurrence.

The following medical record data were recorded in a specific database for this study: patient age, parity, hormonal status, histological type, histological grade, tumor stage according to International Federation of Gynaecology and Obstetrics (FIGO) criteria, lymph node metastases, OS, and DFS. OS was calculated from the date of diagnosis until death from any cause. DFS was calculated from the date of diagnosis until the date of first recurrence. The follow-up period was 48 months.

Informed consent was obtained from each patient included in this study. This study was approved by the Research Ethics Committee of UFTM (protocol number, 34770014.4.0000.5154).

2.2 Anatomopathological study

All cases were reviewed by an experienced pathologist from the Surgical Pathology Service of UFTM to select the best cuts for conducting an immunohistochemical study. Staging of cases was performed according to FIGO criteria [20].

2.3 Immunohistochemical study

Specimens obtained by surgical resection were processed in paraffin and reviewed by an experienced pathologist. For selected cases, new sections (4 μm) were cut in silanized sheets (ATPS—Silane, Sigma® A3648). Sections were placed on slides and kept in an oven at 56 °C for 24 h before being deparaffinized in two xylol baths (5 min each) and then dehydrated in three baths of absolute alcohol and one bath of 80% alcohol. After hydration in PBS (pH 7.2) for 5 min, antigen recovery was performed. Briefly, slides were placed in cytology tubes containing 10 mM citrate buffer solution (pH 6.0) or Tris-EDTA, according to the manufacturer's instructions. The tubes were then placed in a pot filled with distilled water at 100 °C up to the indicated limit. After 30 min, the tubes were placed on a bench to cool to an ambient temperature (22 °C).

Immunohistochemical staining was performed with the Novolink® Polymer Detection System (Leica Biosystems, Buffalo Grove, IL, USA). Briefly, the slides were first washed with distilled water and dried carefully to prevent the sections completely drying out. The slides were then incubated with Peroxidase Block (3% hydrogen peroxide) for 5 min and washed 3× in PBS buffer (5 min each). After a 5 min incubation step with Protein Block, the slides were washed 3× in PBS buffer (5 min each) and then incubated with antibodies recognizing α-SMA and FAP that were diluted in 2% bovine serum albumin (BSA, Sigma®, St. Louis, MS, USA) according to the manufacturer's indications. Stainings were performed in a humid chamber at 4 °C for durations recommended by the manufacturer. In addition, staining of positive controls was performed for each antibody, according to the manufacturer's directions. After the slides were washed with PBS buffer (5 min each), the slides were incubated with Post Primary Block for 30 min, washed with PBS buffer (5 min each), then incubated with Novolink® Polymer. After 30 min, the slides were washed with PBS buffer (5 min each) and developed with DAB Chromogen and Novolink DAB Substrate Buffer (Polymer). After 5 min, the slides were washed in running water and counterstained in Harris' hematoxylin. As a final step, the slides were immersed in three baths of absolute alcohol (10 sec each), to remove excess water; one bath of phenylated xylol; and three baths of xylol (5 min each). A coverslip was mounted on each slide with Entellan (-) and two observers evaluated immunostaining of the epithelium and stroma. Staining intensity was assessed subjectively as: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining).

2.4 Statistical analysis

Data were analyzed with GraphPad Instat software (GraphPad, San Diego, CA, USA) and SPSS Statistics 20 (IBM, NY, USA). For the immunohistochemical data, agreement between observers (n = 2) was performed according to kappa: $\kappa < 0.4$: weak agreement; $0.4 \leq \kappa < 0.8$: moderate agreement; $0.8 \leq \kappa < 1.0$: strong agreement; $\kappa = 1.0$: perfect agreement. Discordant cases were reevaluated and results were defined by consensus. Comparisons of immunostainings of borderline and malignant ovarian tumors were performed by using Fisher's exact test. Survival was assessed according to the Kaplan-Meier method and the log-rank test. Multivariate analysis was performed with Cox regression. Differences were considered significant for $p < 0.05$.

3. Results

Borderline ovarian tumors (n = 18) and malignant ovarian neoplasms (n = 28) were examined and compared in this study. Patients with the former had a median age of 48 (21–71) years and a median parity of 2 (0–7) births. In addition, 10 (55.6%) patients were at menopause and 8 (44.4%) patients were at menacme. Patients with malignant ovarian neoplasms had a median age of 56 (25–81) years and a median parity of 2 (0–12) births. The hormonal status of the patients with malignant ovarian neoplasms included 21 (75%) patients at menopause and 7 (25%) patients at menacme.

Tumor stages among the patients diagnosed with borderline ovarian tumors were: IA (n = 14, 77.8%), IB (n = 1, 5.5%), IC-2 (n = 1, 5.5%), IIB (n = 1, 5.5%), and IIIC (n = 1, 5.5%). Tumor stages among the patients diagnosed with malignant

Table 1. FAP immunostaining in borderline ovarian tumors and epithelial ovarian cancer.

	Staining 2/3	Staining 0/1	*p*
Borderline ovarian tumors (n = 17*)	5 (29.4%)	12 (70.6%)	0.0331
Malignant neoplasms (n = 28)	18 (64.3%)	10 (35.7%)	

Fisher's exact test. *In the group of borderline ovarian tumors, one of the slides was excluded for the evaluation of FAP because the cut did not have an adequate representation of the stroma.

ovarian cancer were: IA (n = 7, 25%), IB (n = 1, 3.5%), IC-2 (n = 1, 3.5%), IC-3 (n = 1, 3.5%), IIA (n = 1, 3.5%), IIB (n = 1, 3.5%), IIIA-1 (ii) (n = 1, 3.5%), IIIA-2 (n = 1, 3.5%), IIIB (n = 1, 3.5%), IIIC (n = 10, 37%), and IV-B (n = 3, 10%).

An analysis of histological types identified 12 (66.7%) mucinous borderline tumors, 5 (27.8%) serous borderline tumors, and 1 (5.5%) atypical proliferative endometrioid borderline tumor among the borderline tumors. The malignant ovarian neoplasms included: 18 (64.3%) serous cystadenocarcinomas, 3 (10.7%) mucinous cystadenocarcinomas, 5 (17.8%) adenocarcinomas, 1 (3.6%) endometrioid adenocarcinoma, and 1 (3.6%) clear cell carcinoma.

An evaluation of stromal FAP showed that stronger immunostaining (scores of 2 and 3) was more often associated with the epithelial ovarian cancers than with the borderline ovarian tumors ($p = 0.0331$) (Table 1). In contrast, there was no statistical significance observed in the assessment of α-SMA ($p = 0.7395$). A further evaluation of only the patients with epithelial ovarian cancer showed that the patients with a score of 3 for stromal α-SMA immunostaining had a higher OS ($p = 0.017$), yet no difference in DFS ($p = 0.283$). Moreover, there was no statistically significant difference in OS or DFS according to stromal FAP immunostaining ($p = 0.139$ and $p = 0.751$, respectively).

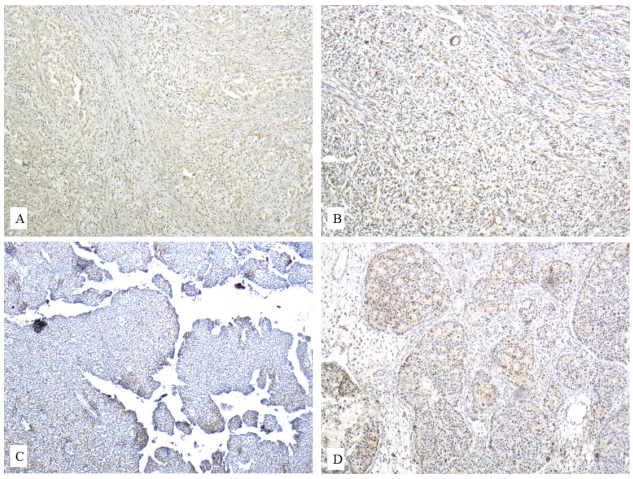

Fig. 1. Histological sections of malignant ovarian neoplasms: immunohistochemical staining of polyclonal anti-α-SMA (A,B, 100×); polyclonal anti-FAP (C,D, 100×).

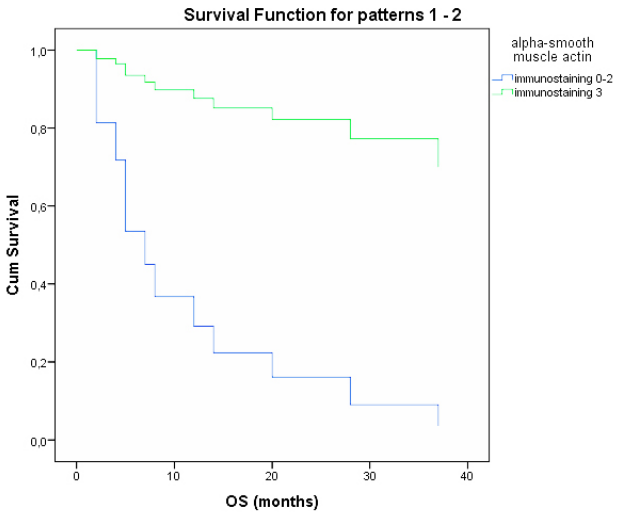

Fig. 2. OS and stromal immunostaining of α-SMA (Cox regression).

In Fig. 1, representative histological sections of malignant ovarian neoplasms stained with polyclonal anti-α-SMA and polyclonal anti-FAP antibodies are shown. A multivariate analysis (Cox regression) demonstrated that patients with a stromal α-SMA immunostaining score of 3 had a higher OS compared to patients with immunostaining scores of 0, 1, or 2 [odds ratio, 0.107; 95% confidence interval, 0.018–0.649, $p = 0.015$] (Table 2, Fig. 2).

4. Discussion

Both the tumor microenvironment and the stroma play important roles in the development of cancer and metastasis. Cancer cells in the tumor microenvironment also directly contribute to alterations in the local stroma. For example, malignant cells can manipulate the microenvironment by secreting various chemokines, cytokines, and other factors to reprogram adjacent cells and support tumor development and progression. It is also important to highlight the role of immune cells in the microenvironment which actively participate in tumor progression. Significant evidence indicates that both innate and adaptive immune cells can promote tumor progression and metastatic events.

CAFs exhibit tumorigenic characteristics in the tumor microenvironment and provide an abundant source of cytokines [21, 22]. CAFs are also characterized by high levels of FAP expression in the tumor stroma. In the absence of a pathological process, FAP expression is difficult to detect. However, significant expression has been detected in tissue immunomodulation sites in several tumors. Moreover, FAP has been identified as a protein of interest in several studies of targeted immunotherapies for certain carcinomas, mainly in pancreas, lung, prostate, and mesothelioma, yet it remains to be fully characterized [23–29]. In therapies related to ovarian cancer, stronger immunostaining has been observed in cancerous tissues than in borderline and benign tissues. Such findings reaffirm the fundamental role of CAFs in the process

Table 2. Univariate analysis (Kaplan-Meier method and the log-rank test) and multivariate analysis (Cox regression) of the variables age, histological grade, staging, lymph node metastasis and stromal immunostaining α-SMA considering epithelial ovarian cancer and overall survival.

Variable	Univariate analysis (log-rank test)	Multivariate analysis	
	p-value	OR (95% CI)	*p*-value
Age (>50 y vs ≤50 y)	0.962	0.886 (0.210–3.735)	0.870
Histological grade (3 vs 1/2)	0.717	0.494 (0.119–2.057)	0.332
Staging (III–IV vs I–II)	0.691	0.731 (0.192–2.781)	0.646
Lymph node metastasis (yes vs no)	0.131	5.954 (0.936–37.878)	0.059
Immunostaining α-SMA	0.017	0.107 (0.018–0.649)	0.015

of carcinogenesis and metastasis of ovarian cancer. They also reaffirm that high expression of FAP is present in malignant neoplasms, while low or null expression is present in non-pathological processes [30, 31]. In the current study, stronger FAP immunostaining was associated with epithelial ovarian cancers compared to borderline tumors.

Identifying other stromal markers is extremely relevant for gaining a better understanding of the mechanism(s) mediating stroma-tumor microenvironment interactions in cancer. Thus, α-SMA, a member of the actin family of proteins which plays an important role in the integrity of muscle, motility, and cellular structure, is also important in wound healing. For example, α-SMA regulates fibers which are one of the main factors in myofibroblast contractility. Consequently, α-SMA is a reference marker for the identification of CAF populations. In addition, α-SMA has been identified as a significant prognostic factor in patients with certain types of tumors [32–35]. For example, expression of α-SMA correlates with an increased risk of recurrence in patients with colon cancer, and fibroblasts with high expression of α-SMA have been strongly associated with lower OS in colon and breast cancers [35, 36]. In the present study, an increase in OS was associated with higher α-SMA expression in the ovarian cancers examined, and this result contrasts with the decrease in OS observed with α-SMA expression in colon cancer patients. These results support observations that immune responses to tumors are complex, and they manifest in different ways depending on the type of tumor present, among tumors of the same type, and according to other cofactors such as tumor stage and grade.

Myofibroblasts in cancer-associated stroma are specifically differentiated fibroblasts. Their abundance has been a useful indicator of disease recurrence after curative colorectal cancer surgery [36]. In addition, high α-SMA expression in tumor stroma has been associated with worse patient outcome in pancreatic cancer [37, 38]. It has previously been demonstrated that the area of α-SMA and FAP staining is larger in more advanced stages (III and IV) of ovarian cancer, and also in the presence of lymph node metastases [11]. Moreover, an association between immunoexpression of these markers with factors of poor prognosis has been reported. However, to the best of our knowledge, an association between α-SMA immunostaining and OS in ovarian cancer has not previously been reported. Therefore, it is significant that our multivariate analysis identified a longer OS period for the patients in this study whose tumors exhibited stronger immunostaining of stromal α-SMA.

CAFs have been shown to directly promote tumorigenesis through several mechanisms involving cell proliferation, invasion, survival, and immune suppression [39, 40]. Thus, FAP is a stromal marker that can potentially be indicative of tumor growth [41, 42] and correlate with prognostic factors. Previous studies have demonstrated that stromal FAP expression is associated with tumor progression, disease aggressiveness, and the potential for metastasis development, recurrence, and death in colon and pancreatic cancers [8–10]. High expression of FAP in ovarian cancers treated with cytoreduction followed by chemotherapy has also exhibited an association with advanced disease, lymph node metastasis, omental involvement, lymphovascular disease, and increased angiogenesis [11]. However, in the present study, no relationship between FAP immunostaining and patient survival was observed.

The immunohistochemical results of the present study demonstrate that expression FAP in the peritumoral stroma is higher in epithelial ovarian cancers compared to borderline ovarian tumors. This result suggests that FAP may have a more important role in carcinogenesis and tumor progression than previously considered. Moreover, higher OS was observed for patients with strong stromal α-SMA immunostaining, suggesting that stromal α-SMA may serve as a valuable marker in prognosis and survival. Despite the limitation of the present study that the epithelial ovarian cancers examined were not distinguished as type 1 versus type 2, the results of the present study demonstrate for the first time that α-SMA is an independent factor related to OS in malignant ovarian tumors. Thus, further study of α-SMA as a targeted therapy for ovarian epithelial cancer is warranted.

5. Conclusions

Stronger FAP immunostaining characterized epithelial ovarian cancers more often than borderline ovarian tumors. Moreover, among the patients with epithelial ovarian cancer, strong immunostaining of stromal α-SMA was associated with a higher OS.

Author contributions

RSN and EFCM designed the research study. ACS, MPJ, RME, RSN and EFCM performed the research. RME, ACS and MPJ provided help and advice on the Immunohistochemistry experiments. RSN, EFCM and MPJ analyzed the data. ACS, MPJ and RSN wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of the institution (CEP/UFTM protocol number, 34770014.4.0000.5154).

Acknowledgment

The authors wish to acknowledge the AREMG (Associação de Apoio à Residência Médica — Minas Gerais), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FUNEPU (Fundação de Ensino e Pesquisa de Uberaba), FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil) for research support.

Funding

This research received funding from the AREMG (Associação de Apoio à Residência Médica—Minas Gerais), CNPq (Conselho Nacional de De-senvolvimento Científico e Tecnológico), FUNEPU (Fun-dação de Ensino e Pesquisa de Uberaba), FAPEMIG (Fun-dação de Amparo à Pesquisa do Estado de Minas Gerais), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil).

Conflict of interest

The authors declare no conflict of interest.

References

[1] Berkenblit A, Cannistra SA. Advances in the management of epithelial ovarian cancer. Journal of Reproductive Medicine. 2005; 50: 426–438.

[2] Coburn SB, Bray F, Sherman ME, Trabert B. International patterns and trends in ovarian cancer incidence, overall and by histologic subtype. International Journal of Cancer. 2017; 140: 2451–2460.

[3] Polgár L. The prolyl oligopeptidase family. Cellular and Molecular Life Sciences. 2002; 59: 349–362.

[4] Mhawech-Fauceglia P, Yan L, Sharifian M, Ren X, Liu S, Kim G, *et al.* Stromal Expression of Fibroblast Activation Protein Alpha (FAP) Predicts Platinum Resistance and Shorter Recurrence in patients with Epithelial Ovarian Cancer. Cancer Microenvironment. 2015; 8: 23–31.

[5] Kelly T, Huang Y, Simms AE, Mazur A. Fibroblast Activation Protein-α: a key modulator of the microenvironment in multiple pathologies. International Review of Cell and Molecular Biology. 2012; 40: 83–116.

[6] Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, *et al.* Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91: 5657–5661.

[7] Bae S, Park CW, Son HK, Ju HK, Paik D, Jeon C, *et al.* Fibroblast activation protein alpha identifies mesenchymal stromal cells from human bone marrow. British Journal of Haematology. 2008; 142: 827–830.

[8] Cohen SJ, Alpaugh RK, Palazzo I, Meropol NJ, Rogatko A, Xu Z, *et al.* Fibroblast activation protein and its relationship to clinical outcome in pancreatic adenocarcinoma. Pancreas. 2008; 37: 154–158.

[9] Shi M, Yu D, Chen Y, Zhao C, Zhang J, Liu Q, *et al.* Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. World Journal of Gastroenterology. 2012; 18: 840–846.

[10] Henry LR, Lee H, Lee JS, Klein-Szanto A, Watts P, Ross EA, *et al.* Clinical implications of fibroblast activation protein in patients with colon cancer. Clinical Cancer Research. 2007; 13: 1736–1741.

[11] Zhang Y, Tang H, Cai J, Zhang T, Guo J, Feng D, *et al.* Ovarian cancer-associated fibroblasts contribute to epithelial ovarian carcinoma metastasis by promoting angiogenesis, lymphangiogenesis and tumor cell invasion. Cancer Letters. 2011; 303: 47–55.

[12] Weymouth N, Shi Z, Rockey DC. Smooth muscle α actin is specifically required for the maintenance of lactation. Developmental Biology. 2012; 363: 1–14.

[13] Vandekerckhove J, Weber K. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. Journal of Molecular Biology. 1978; 126: 783–802.

[14] Sun K, Chang Y, Reed NI, Sheppard D. A-Smooth muscle actin is an inconsistent marker of fibroblasts responsible for force-dependent TGFβ activation or collagen production across multiple models of organ fibrosis. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2016; 310: L824–L836.

[15] Anggorowati N, Ratna Kurniasari Ch, Damayanti K, Cahyanti T, Widodo I, Ghozali A, *et al.* Histochemical and Immunohistochemical Study of α-SMA, Collagen, and PCNA in Epithelial Ovarian Neoplasm. Asian Pacific journal of cancer prevention. 2017; 18: 667–671.

[16] Cherng S, Young J, Ma H. Alpha-smooth muscle actin (α-SMA). Journal of American Science. 2008; 4: 7–9

[17] Shinde AV, Humeres C, Frangogiannis NG. The role of α-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. Biochimica Et Biophysica Acta. Molecular Basis of Disease. 2017; 1863: 298–309.

[18] da Silva A, Jammal M, Etchebehere R, Murta E, Nomelini R. Role of Alpha-Smooth Muscle Actin and Fibroblast Activation Protein Alpha in Ovarian Neoplasms. Gynecologic and Obstetric Investigation. 2018; 83: 381–387.

[19] Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-Associated Fibroblasts: their Characteristics and their Roles in Tumor Growth. Cancers. 2015; 7: 2443–2458.

[20] Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Archives of Gynecology and Obstetrics. 2014; 290: 839–842.

[21] Zhang X, Hwang YS. Cancer-associated fibroblast stimulates cancer cell invasion in an interleukin-1 receptor (IL-1R)-dependent manner. Oncology Letters. 2019; 18: 4645–4650.

[22] Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Research. 2019; 79: 4557–4566.

[23] Hamson EJ, Keane FM, Tholen S, Schilling O, Gorrell MD. Understanding fibroblast activation protein (FAP): Substrates, activities, expression and targeting for cancer therapy. Clinical Applications. 2014; 8: 454–463.

[24] Ebert LM, Yu W, Gargett T, Toubia J, Kollis PM, Tea MN, *et al.* Endothelial, pericyte and tumor cell expression in glioblastoma identifies fibroblast activation protein (FAP) as an excellent target for immunotherapy. Clinical & Translational Immunology. 2020; 9: e1191.

[25] Ostermann E, Garin-Chesa P, Heider KH, Kalat M, Lamche H, Puri C, *et al.* Effective immunoconjugate therapy in cancer models targeting a serine protease of tumor fibroblasts. Clinical Cancer Research. 2008; 14: 4584–4592.

[26] Kakarla S, Chow KKH, Mata M, Shaffer DR, Song X, Wu M, *et al.* Antitumor effects of chimeric receptor engineered human T cells directed to tumor stroma. Molecular Therapy. 2013; 21: 1611–1620.

[27] Gulati P, Rühl J, Kannan A, Pircher M, Schuberth P, Nytko KJ, *et al.* Aberrant Lck Signal via CD28 Costimulation Augments Antigen-Specific Functionality and Tumor Control by Redirected T Cells with PD-1 Blockade in Humanized Mice. Clinical Cancer Research. 2018; 24: 3981–3993.

[28] Freedman JD, Duffy MR, Lei-Rossmann J, Muntzer A, Scott EM, Hagel J, *et al.* An Oncolytic Virus Expressing a T-cell Engager Simultaneously Targets Cancer and Immunosuppressive Stromal Cells. Cancer Research. 2018; 78: 6852–6865.

[29] Schuberth PC, Hagedorn C, Jensen SM, Gulati P, van den Broek M, Mischo A, *et al.* Treatment of malignant pleural mesothelioma by fibroblast activation protein-specific re-directed T cells. Journal of Translational Medicine. 2013; 11: 187.

[30] Zhang M, Xu L, Wang X, Sun B, Ding J. Expression levels of seprase/FAPα and DPPIV/CD26 in epithelial ovarian carcinoma. Oncology Letters. 2015; 10: 34–42.

[31] Nurmik M, Ullmann P, Rodriguez F, Haan S, Letellier E. In search of definitions: Cancer-associated fibroblasts and their markers. International Journal of Cancer. 2020; 146: 895–905.

[32] Micallef L, Vedrenne N, Billet F, Coulomb B, Darby IA, Desmoulière A. The myofibroblast, multiple origins for major roles in normal and pathological tissue repair. Fibrogenesis & Tissue Repair. 2012; 5: S5.

[33] Mezawa Y, Orimo A. The roles of tumor- and metastasis-promoting carcinoma-associated fibroblasts in human carcinomas. Cell and Tissue Research. 2016; 365: 675–689.

[34] Gascard P, Tlsty TD. Carcinoma-associated fibroblasts: orchestrating the composition of malignancy. Genes & Development. 2016; 30: 1002–1019.

[35] Surowiak P, Murawa D, Materna V, Maciejczyk A, Pudelko M, Ciesla S, *et al.* Occurence of stromal myofibroblasts in the invasive ductal breast cancer tissue is an unfavourable prognostic factor. Anticancer Research. 2007; 27: 2917–2924.

[36] Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I, *et al.* Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clinical Cancer Research. 2007; 13: 2082–2090.

[37] Sinn M, Denkert C, Striefler JK, Pelzer U, Stieler JM, Bahra M, *et al.* α-Smooth muscle actin expression and desmoplastic stromal reaction in pancreatic cancer: results from the CONKO-001 study. Clinical Cancer Research. 2014; 111: 1917–1923.

[38] Franco OE, Shaw AK, Strand DW, Hayward SW. Cancer associated fibroblasts in cancer pathogenesis. Seminars in Cell & Developmental Biology. 2010; 21: 33–39.

[39] Kalluri R, Zeisberg M. Fibroblasts in cancer. Nature Reviews. Cancer. 2006; 6: 392–401.

[40] Östman A, Augsten M. Cancer-associated fibroblasts and tumor growth – bystanders turning into key players. Current Opinion in Genetics & Development. 2009; 19: 67–73.

[41] Spaeth EL, Dembinski JL, Sasser AK, Watson K, Klopp A, Hall B, *et al.* Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. PLoS ONE. 2009; 4: e4992.

[42] Ge Y, Zhan F, Barlogie B, Epstein J, Shaughnessy J, Yaccoby S. Fibroblast activation protein (FAP) is upregulated in myelomatous bone and supports myeloma cell survival. British Journal of Haematology. 2006; 133: 83–92.