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STIM1/SOX2 proteins are co-expressed in the tumor and microenvironmental stromal cells of pancreatic ductal adenocarcinoma and ampullary carcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) and ampullary carcinoma (AAC) are lethal malignancies with modest benefits from surgery. SOX2 and STIM1 have been linked to anticancer activity in several human malignancies. This study included 94 tumor cases: 48 primary PDAC, 25 metastatic PDAC, and 21 primary AAC with corresponding non-tumor tissue. All cases were immunohistochemically stained for STIM1 and SOX2 and results were correlated with clinicopathologic data, patient survival, and BCL2 immunostaining results. Results revealed that STIM1 and SOX2 epithelial/stromal expressions were significantly higher in PDAC and AAC in comparison to the control groups. STIM1 and SOX2 expressions were positively correlated in the primary and metastatic PDAC ($P=0.016$ and $P=0.001$, respectively). However, their expressions were not significantly associated with BCL2 expression. SOX2 epithelial/stromal expressions were positively correlated with the large tumor size in the primary AAC group ($P=0.052$, $P=0.044$, respectively). STIM1 stromal and SOX2 epithelial over-expressions had a bad prognostic impact on the overall survival of AAC ($P=0.002$ and $P=0.001$, respectively). Therefore, STIM1 and SOX2 co-expression in tumor cells and intra-tumoral stroma could contribute to the development of PDAC and AAC. STIM1/SOX2 expression is linked to a bad prognosis in AAC.

Keywords Ampullary carcinoma, BCL2, Immunohistochemistry, Pancreatic ductal adenocarcinoma, SOX2, STIM1

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Introduction

Periampullary adenocarcinoma is a malignant tumor that develops from the ampulla of Vater either from the pancreas, duodenum, or the distal end of the common bile duct (CBD) [1]. The most common type of periampullary adenocarcinoma is pancreatic ductal adenocarcinoma (PDAC), which is the seventh largest cause of cancer mortality, whereas ampullary adenocarcinoma (AAC) is the second most prevalent type [2].

Pancreatic ductal adenocarcinoma and other non-pancreatic periampullary carcinomas are characterized by aggressive course and metastatic behavior which were demonstrated in nearly 80% of patients. In addition, pancreaticoduodenectomy (Whipple's operation), the only potentially curative intervention, is not advisable in patients presented with disseminated or advanced tumor stage [3]. Moreover, half of those who underwent surgery and adjuvant therapy developed liver metastasis [4].

Gemcitabine, the treatment of choice for advanced PDAC, has a limited role in improving the survival rate owing to the unique chemoresistance of pancreatic cancer cells [5, 6]. The mechanism of chemoresistance is multifactorial resulting from interaction among pancreatic tumor cells and tumor microenvironment, tumor stemness, the evolution of apoptosis, and the deregulation of calcium channel pathways [7]. Evasion of apoptosis might be crucial in the progression of PDAC and AAC. Additionally, apoptotic protein dysregulation is a major factor in the emergence of chemoresistance in PDAC [8]. B cell lymphoma-2 (BCL-2) is a crucial anti-apoptotic protein that has been shown to control Ca²⁺-translocation across the ER membrane via store-operated calcium channels (SOCs) [9].

In hypoxic environments, hypoxia inducible factor-1 α (HIF-1 α) increases PDAC progression by increasing stromal interaction molecule 1 (STIM1) expression in tumor tissue, potentially affecting the prognosis [10, 11]. STIM1 is a major component of SOCs [12]. STIM1 has been linked to the progression and spread of human malignancies via cell cycle arrest and anti-apoptotic activities [10, 13]. However, there has been limited research on the role of STIM1 in PDAC, and no previous studies have demonstrated STIM1's relevance in AAC.

Similarly, the expression of sex-determining region Y-box2 (SOX2) promotes prostate and breast cancer progression by inhibiting apoptosis and increasing cell proliferation [14]. SOX2 has been reported to contribute in tumor stemness and modulate epithelial-mesenchymal transition (EMT) in a subset of human pancreatic tumors [15, 16]. In addition, SOX2 could be expressed in ampullary preneoplastic mucosal epithelium and invasive carcinoma [17]. Wuebben et al. reported that the oncogenic and tumor suppressor function of SOX2 is dependent on its optimal level [18]. The limited studies on the

expression of STIM1 and SOX2 in PDAC and AAC could hinder understanding their possible pathogenic and therapeutic role in primary and metastatic PDAC and AAC. In addition, the expression of STIM1 in primary and metastatic cancer is a matter of controversy in different cancers [19, 20]. No previous studies focused on the different expression of SOX2 in primary and metastatic tumors.

The anticancer role of SOX2 and STIM1 has been reported in different human tumors [21–23]. Some anti-cancer medications have been shown to trigger cancer cell death through the SOCE pathway. The ability of SOCE to effectively slow the progression of various tumors, including breast, and liver cancer, is supported by the fact that STIM1 inhibition may have therapeutic value [24]. Similarly, clinical trials on SOX2 inhibitors give impressive results with possible medical uses in treating tumors that express SOX2 [25, 26].

The study aims to evaluate the protein expression of STIM1 and SOX2 in primary and metastatic PDAC as well as AAC. This could illustrate their potential role in advanced and metastatic patients.

Material and methods

This retrospective, case-control study used all available methods and adhered to all applicable ethical rules. It was carried out on periampullary carcinoma cases divided into 94 tumor cases: 48 primary PDAC patients, 25 metastatic PDAC cases as well and 21 primary AAC cases. Any patients who had received chemotherapy prior to surgery were excluded. There was also a control group of 35 non-tumorous tissue cases (23 pancreatic tissue and 12 intestine tissue) and 10 cases of normal pancreatic tissue. The study was according to Helinski guidelines and institutional approval.

Primary cases underwent Whipple's operation at the Hepatopancreatobiliary Department. For metastatic cases, the cases were either known cases of primary PDAC with metastatic deposits at the time of presentation or presenting as metastatic of unknown origin but later shown to be of primary pancreatic occult location using clinical, radiological, and pathological data.

The patients' relevant data, including their overall survival, were obtained from their medical files. OS statistics were calculated from the time of illness until the patient's death or last follow-up for at least a year.

All malignant cases had a histological evaluation [27, 28]. The tumor cases were divided into early (I and II) and advanced (III and IV) pathological stages, as well as two tiers of pathological grades: low (GI and GII) and high (GIII). After excluding neutrophils and macrophages, only the proportion of tumor-infiltrating mononuclear cells (TIMC) was measured for the tumor immune response [29].

Using a manual needle set (Breecher Instrument, USA) with two viable tissue cores from the tumor and one core from the matched non-tumor samples, tissue microarray construction (TMA) was carried out.

Immunohistochemical studies

Rabbit polyclonal anti-STIM1 diluted as 1:200 (Cat. No. bs-8526R) obtained from BIOSS, Woburn, Massachusetts, USA, rabbit polyclonal anti-SOX2 diluted as 1:250 (Cat. No. GB11249) obtained from Service bio, Wuhan, China, and a rabbit monoclonal anti-BCL2 antibody ready to use, (Cat. No. ab32124) obtained from Abcam, Cambridge, UK were used. After deparaffinization and rehydration of the tissues, the antigens were retrieved using a low-PH citrate solution and cooled at room temperature. Primary antibodies were applied to the slides and left incubating at 4 °C overnight. The secondary antibody, anti-polyvalent horseradish peroxidase3,3'-diaminobenzidine (DAB), was applied using Ultravision's detecting tools, and the staining was visualized with a counteract Mayer's hematoxylin. Positive and negative controls were included. Positive control for STIM1 was gastric tissue, SOX2 was esophageal tissue, and for Bcl2 the tumor-infiltrating lymphocytes (TILs) were used as an internal positive control [30].

Antibody assessment methods

STIM1 stained positively for brownish granular cytoplasmic staining, whereas SOX2 stained positively for brownish nuclear staining [10, 16]. The expression was assessed in both the epithelial and stromal components. Stromal components included fibroblasts, endothelial cells, and infiltrating inflammatory cells. BCL2 exhibited cytoplasmic and/or membrane staining [31].

The three antibodies were assessed using an H-score, calculated by determining the percentage of cells at each intensity. The following method is used to get the final score: [1 (% cells 1+)+2 (% cells 2+)+3 (% cells 3+)]. The final score is between 0 and 300 [32]. Furthermore, tumor cases were divided according to the median of the H-score into low and high expression.

Statistical analysis

Using IBM SPSS, the chi-square test, Fisher's Exact (FE), or Monte Carlo correction (MC) tests were used to ascertain the relationship between the qualitative variables. The marginal homogeneity and McNemar (McN) tests were used to determine whether there was a significant difference between two qualitatively paired data. The student t-test (t), Mann Whitney (U), and Kruskal Wallis (H) tests were employed, respectively, to compare quantitative variables that were either normally distributed or abnormally distributed, respectively. To investigate the relation between two variables, the Spearman

coefficient was utilized. When the two-tailed P-value is 0.05 or below, it is regarded as statistically significant. The OS data for the patients were assessed using Kaplan-Meier plots and the log-rank test. The factor influencing mortality was verified using univariate COX-regression analysis.

Results

The clinicopathological data of the studied groups

PDAC cases are often diagnosed at larger tumor sizes which could interfere with complete surgical resection of the tumor compared to AAC ($P < 0.001$, and $P = 0.002$, respectively). In addition, the primary PDAC group showed a significant positive perineural invasion compared to primary AAC group ($P < 0.001$).

There was no significant difference between primary and metastatic PDAC in terms of clinicopathological characteristics.

The clinicopathological data of the studied groups are summarized in Supplementary Table 1.

The expression of STIM1 and SOX2 in the studied groups

The epithelial expression of STIM1 was observed in 60% of the normal pancreatic tissue cases, 73.9% of the adjacent non-tumor pancreatic tissue, and in all primary and metastatic PDAC groups. Similarly, 75% of the non-tumor intestinal tissue group showed positive STIM1 epithelial expression while the AAC group showed positive expression, Fig. 1.

Regarding SOX2, 70% of the control pancreatic tissue groups showed SOX2 epithelial expression. 72.9% and 92% of primary and metastatic PDAC groups showed positive expression, respectively. In AAC, 50% of the control non-tumor intestinal tissue group showed positive STIM1 epithelial expression and 61.9% of the AAC group showed positive expression, Fig. 2.

Comparison of STIM1 and SOX2 expression in different groups

PDAC had significantly higher STIM1 epithelial and stromal expressions than the groups of control pancreatic tissue ($P < 0.001$ for both). STIM1 epithelial and stromal expressions did not significantly differ across the primary and metastatic PDAC groups ($P = 0.094$ and $P = 0.082$, respectively), Fig. 3a and b.

Primary PDAC had significantly higher SOX2 epithelial expression compared to non-tumor tissue ($P = 0.042$). The primary and metastatic PDAC groups showed a similar SOX2 epithelial expression ($P = 0.094$). In addition, primary PDAC had significantly increased SOX2 stromal expression in comparison to the healthy control group and nearby non-tumor pancreatic tissue ($P = 0.002$ and $P = 0.003$). When compared to the primary PDAC,

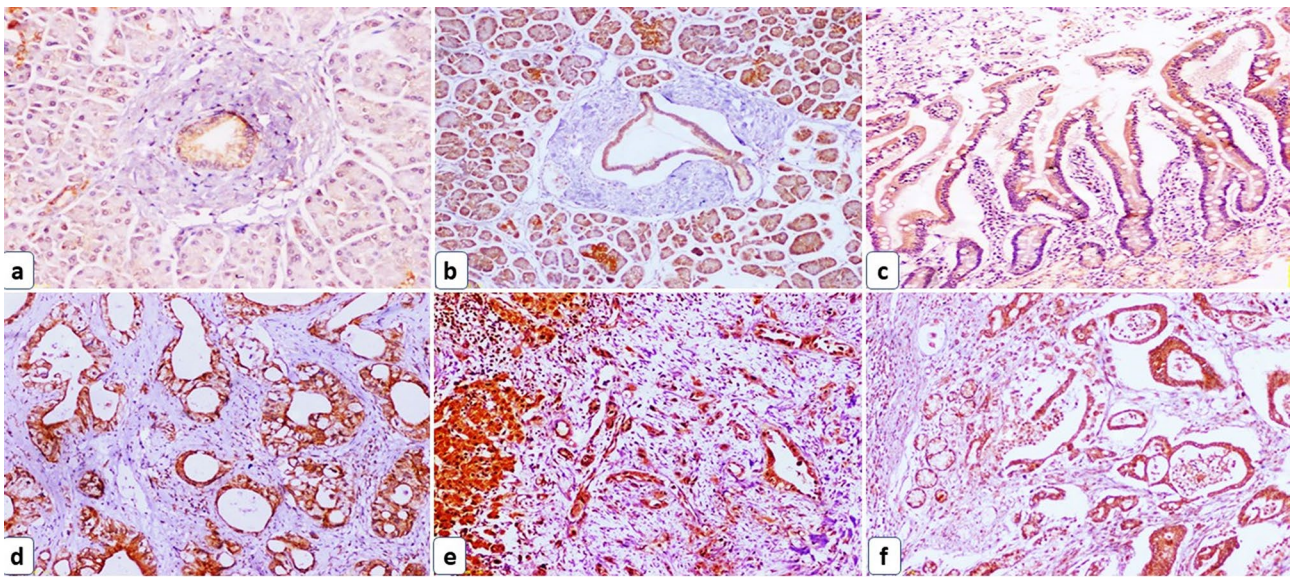


Fig. 1 STIM1 immunohistochemical expression in the studied groups. **(a)** A case of normal pancreatic tissue showed weak cytoplasmic STIM1 epithelial expression in the pancreatic duct and focal cytoplasmic STIM1 stromal expression (IHC x200), **(b)** A case of adjacent non-tumor pancreatic tissue showed moderate cytoplasmic STIM1 epithelial expression and focal STIM1 cytoplasmic stromal expression (IHC x100), **(c)** A case of control non-tumor intestinal tissue showed moderate cytoplasmic STIM1 epithelial expression and focal cytoplasmic STIM1 stromal expression (IHC x100), **(d)** A case of primary PDAC showed strong cytoplasmic STIM1 epithelial and stromal expression (IHC x100), **(e)** A case of metastatic PDAC to the liver (on the left side) showed strong cytoplasmic STIM1 epithelial and stromal expression (IHC x100), **(f)** A case of AAC showed strong cytoplasmic STIM1 epithelial and stromal expression (IHC x100)

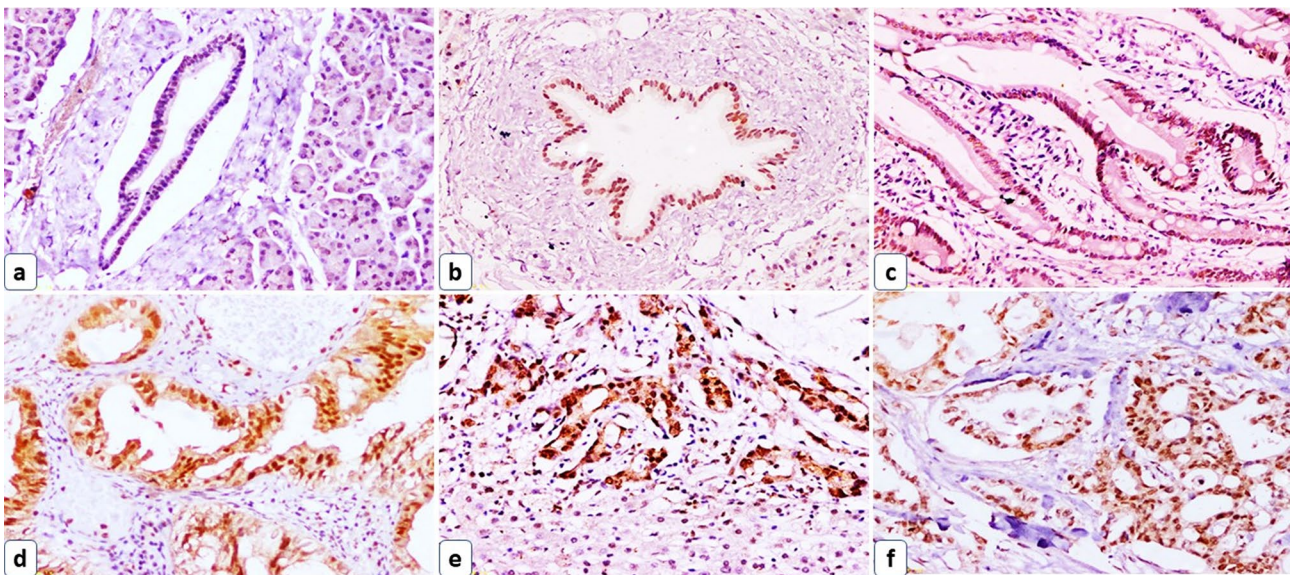


Fig. 2 SOX2 immunohistochemical expression in the studied groups. **(a)** A case of normal pancreatic tissue showed mild nuclear SOX2 epithelial expression and negative nuclear SOX2 stromal expression (IHC x200), **(b)** A case of adjacent non-tumor pancreatic tissue showed moderate nuclear SOX2 epithelial expression and negative nuclear SOX2 stromal expression (IHC x200), **(c)** A case of control non-tumor intestinal tissue showed mild nuclear SOX2 epithelial expression and negative nuclear SOX2 stromal expression (IHC x100), **(d)** A case of well-differentiated PDAC showed strong nuclear SOX2 epithelial and stromal expression (IHC x200), **(e)** A case of metastatic PDAC to the liver (down of the plate) showed strong nuclear SOX2 epithelial and stromal expression (IHC x200), **(f)** A case of AAC showed strong nuclear SOX2 epithelial and stromal expression (IHC x100)

metastatic PDAC tended to have more SOX2 stromal expression ($P=0.059$), Fig. 3c and d.

In AAC, STIM1 epithelial and stromal expressions were significantly higher compared to the control non-tumor

intestinal tissue group ($P=0.003$ and $P=0.036$), Fig. 3e and f. Primary AAC between and control non-tumor intestinal tissue groups did not significantly vary in terms of SOX2 epithelial expression ($P=0.153$). However,

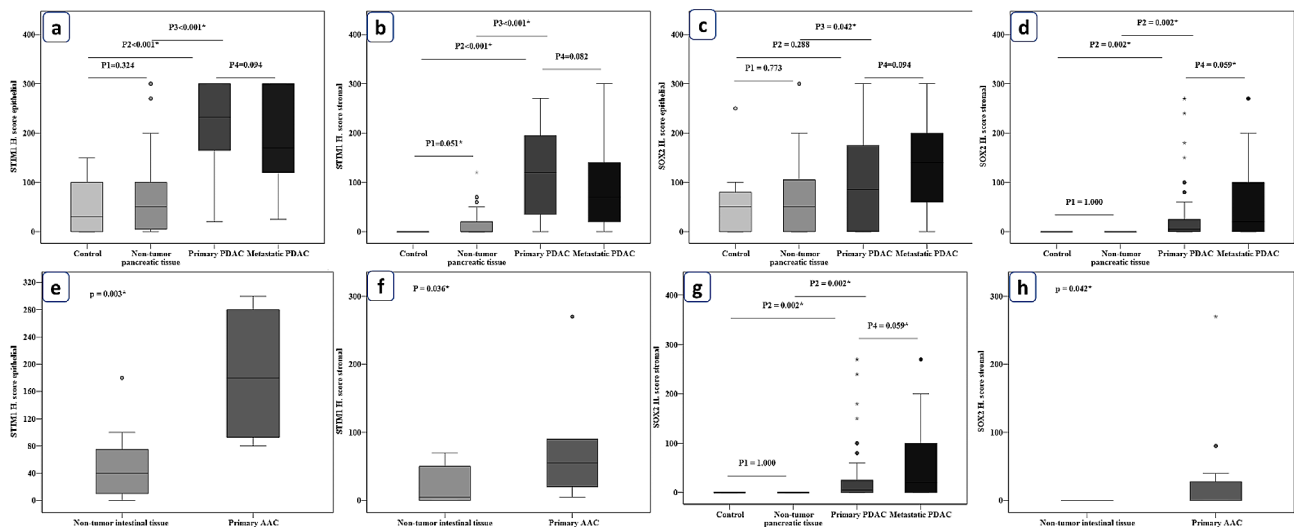


Fig. 3 Comparison between STIM1 and SOX2 expression in PDAC and the control groups. (a) Comparison between STIM1 epithelial expression in PDAC and the control groups, (b) Comparison between STIM1 stromal expression in PDAC and the control groups, (c) Comparison between SOX2 epithelial expression in PDAC and the control groups, (d) Comparison between SOX2 stromal expression in PDAC and the control groups. (e) Comparison between STIM1 epithelial expression in AAC and the control groups. (f) Comparison between STIM1 stromal expression in AAC and the control groups. (g) Comparison between SOX2 epithelial expression in AAC and the control groups. (h) Comparison between SOX2 stromal expression in AAC and the control groups

stromal expression of SOX2 was significantly increased in AAC than in the non-tumor intestinal tissue group ($P=0.042$), Fig. 3.

STIM1 and SOX2 expressions did not statistically differ between the primary PDAC and primary AAC groups.

The correlation of STIM1 and SOX2 expression in the studied groups

STIM1 and SOX2 epithelial expressions positively correlated in primary PDAC ($r=0.346$, $P=0.016$). Furthermore, STIM1 and SOX2 stromal expressions positively correlated in metastatic PDAC ($r=0.618$, $P=0.001$).

The relationship between STIM1 and SOX2 expression and the anti-apoptotic BCL2 marker

BCL2 was expressed in a range of 4-8.3% of the PDAC and AAC groups. Furthermore, no significant correlations were found between STIM1/SOX2 and BCL2 expression.

The relationship between STIM1 and SOX2 and the pathological data in PDAC and AAC

Significant associations between SOX2 epithelial and stromal expression and the primary PDAC group's well-differentiated grade have been found ($P=0.033$ and $P=0.019$). On the other hand, SOX2 epithelial and stromal expressions were positively correlated with large tumor size in the primary AAC group ($P=0.052$ and $P=0.044$), Fig. 4.

There was no significant association between STIM1 and SOX2 expression and the clinicopathological parameters of metastatic PDAC.

Tables 1 and 2 showed the detailed relationship between STIM1 and SOX2 and the pathological data in PDAC and AAC.

The OS study of primary PDAC and AAC groups categorized by clinical, pathological, and IHC marker characteristics

Survival data were available in 52.08% of the primary cases of PDAC and AAC. The mean survival time in the PDAC group was 12.66 ± 8.67 months and the median was 12 months, with 68% dying from the tumor. The mean survival time for AAC was 23.32 ± 13.33 months, with a median of 24 months, 63.6% of cases died from the tumor. In addition, the OS did not differ significantly across the primary PDAC and AAC groups ($\chi^2=0.291$, $P=0.225$). In PDAC, the univariate analysis of OS showed the adverse prognostic impact of tumor recurrence on the patient's outcome ($P=0.044$). However, none of the STIM1 or SOX2 expressions showed a significant impact on the OS of PDAC cases.

In AAC, the univariate analysis of OS showed the adverse prognostic impact of the male gender, perineural invasion, positive LNs, and late tumor stage on the patient's outcome. In addition, STIM1 stromal overexpression and SOX2 epithelial overexpression showed a bad prognostic impact on the patient's outcome, Fig. 5.

The results of a univariate COX regression analysis for the factors impacting mortality in the primary AAC group revealed that positive LN status is the most predictive parameter influencing mortality in the AAC group ($P=0.015$), Table 3.

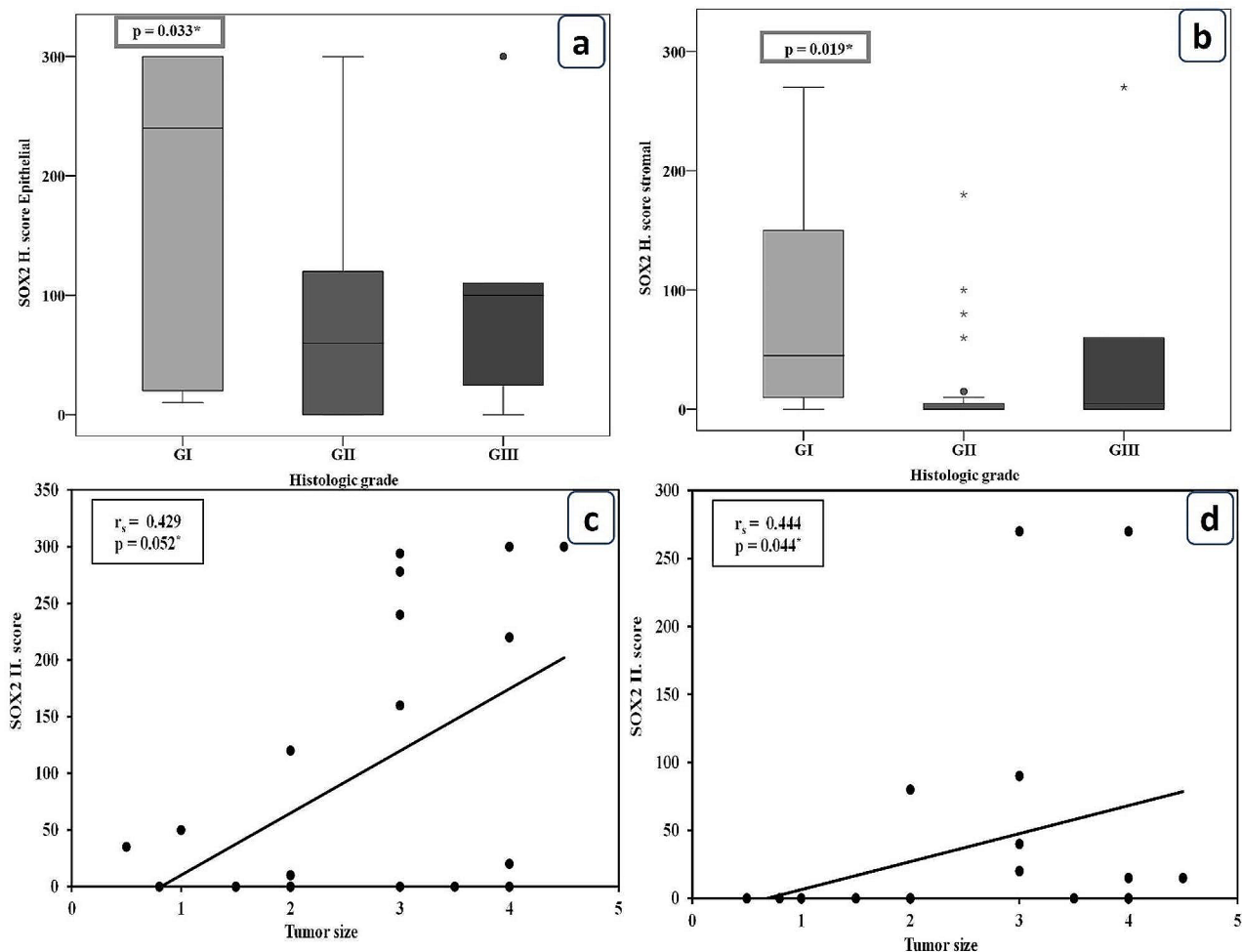


Fig. 4 The relationship of SOX2 expressions with the clinicopathological parameters of PDAC and AAC: (a) Correlation between SOX2 epithelial expression and tumor grade ($P=0.033$) in primary PDAC. (b) Correlation between SOX2 stromal expression and tumor grade ($P=0.019$) in primary PDAC. (c) Correlation between SOX2 epithelial expression and tumor size ($P=0.052$) in primary AAC. (d) Correlation between SOX2 stromal expression and tumor size ($P=0.044$) in primary AAC

Discussion

STIM1/SOX2 expression in tumor cells, as well as micro-environment cells, could contribute to PDAC and AAC tumorigenesis.

STIM1 expression increased in PDAC. STIM1 overexpression was seen in about two-thirds of PDAC cases, according to Wang et al. [10]. STIM1 overexpression was shown to be significantly higher in pancreatic tumor cell lines than in normal cell lines [33]. Despite the limited data regarding the possible oncogenic role of STIM1 in PDAC and AAC, previous studies have postulated the potential oncogenic role of STIM1 in many cancers [21, 34, 35]. The possible oncogenic role of STIM1 is through regulation of SOCE activity, a major modulator of tumor migration and invasiveness, neovascularization, anti-tumor immunity, inhibition of apoptosis, and induced hypoxia pathways [36–38].

Similarly, SOX2 overexpression was significantly observed in PDAC and AAC cases in comparison with the control groups in the present study. In previous studies, SOX2 was expressed in 20% of PDAC cases while it has a wide range (50– 89.7%) of expressions in AAC [16, 17, 39]. In addition, SOX2 was overexpressed during PDAC progression from in situ to invasive components [15, 18]. In more than 20 different malignancies, SOX2 was found to play an oncogenic function [40]. SOX2 expression was linked to aberrant cross-talks between various signaling pathways that led to the emergence of many malignant characteristics [41].

The lack of any significant difference regarding STIM1 and SOX2 expression between PDAC and AAC could indicate that these tumors may have some similarities in their molecular pathogenesis [42]. Studies have suggested that PDAC and AAC may have different pathogenesis even if both conditions have overlapping symptoms and

Table 1 Relationship between STIM1 expression and clinicopathological data in the primary PDAC group (No=48) and AAC (No=21)

| Variables | No | Epithelial STIM1 H-score in PDAC | | Test of sig. & | No | Epithelial STIM1 H-score in AAC | | Test of sig. & | |
|---------------------|----------|----------------------------------|----------------|----------------|--------------------------------------|---------------------------------|----------------|----------------|--------------------------------------|
| | | Mean ± SD. | Median | p | | Mean ± SD. | Median | p | |
| Age (years) | < 56 | 22 | 216.59 ± 77.36 | 200 | U = 261.0 | 12 | 188.75 ± 89.85 | 190 | U = 46.500 |
| | ≥ 56 | 26 | 226.54 ± 86.58 | 255 | P = 0.592 | 9 | 206.67 ± 71.94 | 220 | P = 0.602 |
| Gender | Male | 35 | 228.0 ± 85.09 | 260 | U = 182.50 | 14 | 209.29 ± 83.89 | 230 | U = 35.0 |
| | Female | 13 | 205.77 ± 72.68 | 200 | P = 0.280 | 7 | 170.71 ± 74.63 | 180 | P = 0.322 |
| Tumor recurrence | Positive | 17 | 229.12 ± 75.42 | 215 | U = 52.0 | 6 | 155.0 ± 60.25 | 155 | U = 5.500 |
| | Negative | 7 | 210.0 ± 91.47 | 200 | P = 0.664 | 5 | 240.0 ± 78.74 | 280 | P = 0.082 |
| Tumor size | | | | | r _s = -0.144 P = 0.329 | | | | r _s = -0.032 P = 0.890 |
| Histologic grade | G1 | 10 | 239.50 ± 59.28 | 237.5 | H = 1.722 | 3 | 206.67 ± 30.55 | 200 | U = 24.0 |
| | GII | 33 | 211.52 ± 89.34 | 200 | P = 0.423 | 17 | 193.24 ± 89.77 | 200 | P = 0.921 |
| | GIII | 5 | 256.0 ± 60.66 | 300 | | 1# | 220 | -- | |
| LVI | Present | 15 | 212.67 ± 91.22 | 200 | U = 231.50 | 5 | 201.0 ± 101.39 | 240 | U = 36.0 |
| | Absent | 33 | 226.21 ± 78.23 | 250 | P = 0.713 | 16 | 195.0 ± 77.72 | 200 | P = 0.780 |
| Pathological stage | Early | 29 | 234.66 ± 72.53 | 260 | U = 227.0 | 10 | 185.50 ± 84.93 | 190 | U = 47.50 |
| | Late | 19 | 202.63 ± 92.85 | 200 | P = 0.290 | 11 | 206.36 ± 80.41 | 220 | P = 0.605 |
| LNs status | Positive | 35 | 225.29 ± 84.29 | 250 | U = 199.5 | 10 | 205.0 ± 80.09 | 220 | U = 47.50 |
| | Negative | 13 | 213.08 ± 77.07 | 200 | P = 0.501 | 11 | 187.0 ± 85.64 | 200 | P = 0.605 |
| Perineural invasion | Present | 47 | 225.0 ± 79.90 | 250 | -- | 10 | 201.0 ± 79.37 | 220 | U = 52.0 |
| | Absent | 1# | 80 | | -- | 11 | 192.27 ± 86.47 | 200 | P = 0.863 |

STIM1: Stromal interaction molecule 1, PDAC: Pancreatic ductal adenocarcinoma, AAC: Ampullary adenocarcinoma, LNs: Lymph nodes, T: Tumor, SD: Standard deviation, H-score: Histo-score, U: Mann Whitney test, H: Kruskal Wallis test, r: Spearman coefficient, p: p-value for comparing between the two categories, #: Statistically significant at P ≤ 0.05

#: Excluded from the comparing due to small number of case (n=1)

Table 2 Relationship between SOX2 expression and clinicopathological data in the primary PDAC group (No=48) and AAC (No=21)

| Variables | No | Epithelial SOX2 H-score in PDAC | | Test of sig. & | No | Epithelial SOX2 H-score in AAC | | Test of sig. & | |
|---------------------|----------|---------------------------------|-----------------|----------------|-------------------------------------|--------------------------------|-----------------|----------------|--------------------------------------|
| | | Mean ± SD. | Median | p | | Mean ± SD. | Median | p | |
| Age (years) | < 56 | 22 | 110.0 ± 116.75 | 60 | U = 283.50 | 12 | 108.08 ± 130.47 | 27.5 | U = 47.0 |
| | ≥ 56 | 26 | 100.77 ± 99.69 | 95 | P = 0.958 | 9 | 82.22 ± 114.98 | 10 | P = 0.651 |
| Gender | Male | 35 | 105.86 ± 104.46 | 90 | U = 210.50 | 14 | 115.86 ± 133.79 | 35 | U = 43.0 |
| | Female | 13 | 102.69 ± 117.13 | 40 | P = 0.690 | 7 | 59.29 ± 90.20 | 10 | P = 0.689 |
| Tumor recurrence | Positive | 17 | 108.24 ± 114.47 | 90 | U = 50.0 | 6 | 17.50 ± 21.39 | 10 | U = 12.0 |
| | Negative | 7 | 55.71 ± 41.17 | 60 | P = 0.576 | 5 | 120.80 ± 160.91 | 10 | P = 0.662 |
| Tumor size | | | | | r _s = 0.180 P = 0.222 | | | | r _s = 0.429 P = 0.052* |
| Histologic grade | G1 | 10 | 191.00 ± 123.60 | 240 | H = 6.822* | 3 | 172.67 ± 150.74 | 240 | U = 20.0 |
| | GII | 33 | 78.64 ± 87.65 | 60 | | 17 | 89.35 ± 118.17 | 20 | |
| | GIII | 5 | 107.00 ± 117.77 | 100 | P = 0.033* | 1# | 0 | -- | P = 0.616 |
| LVI | Present | 15 | 83.33 ± 95.57 | 40 | U = 206.0 | 5 | 115.6 ± 112.77 | 120 | U = 32.50 |
| | Absent | 33 | 114.85 ± 111.46 | 90 | P = 0.351 | 16 | 91.19 ± 127.36 | 10 | P = 0.548 |
| Perineural invasion | Present | 47 | 105.53 ± 107.85 | 90 | -- | 10 | 146.20 ± 141.9 | 135 | U = 33.50 |
| | Absent | 1# | 80 | | -- | 11 | 52.27 ± 83.05 | 10 | P = 0.132 |
| Pathological stage | Early | 29 | 104.83 ± 113.79 | 60 | U = 275.0 | 10 | 65.80 ± 108.62 | 0.5 | U = 35.50 |
| | Late | 19 | 105.26 ± 98.07 | 100 | P = 0.991 | 11 | 125.36 ± 131.03 | 50 | P = 0.173 |
| LNs status | Positive | 35 | 95.14 ± 99.64 | 80 | U = 0.199 | 11 | 135.82 ± 135.77 | 120 | U = 40.0 |
| | Negative | 13 | 131.54 ± 124.35 | 120 | P = 0.504 | 10 | 54.30 ± 92.69 | 10 | P = 0.314 |

SOX2: Sex-determining region Y-box2, PDAC: Pancreatic ductal adenocarcinoma, AAC: Ampullary adenocarcinoma, LNs: Lymph nodes, T: Tumor, SD: Standard deviation, H-score: Histo-score, U: Mann Whitney test, H: Kruskal Wallis test, r: Spearman coefficient, p: p-value for comparing between the two categories, #: Statistically significant at P ≤ 0.05. #: Excluded from the comparison due to the small number of cases (n=1)

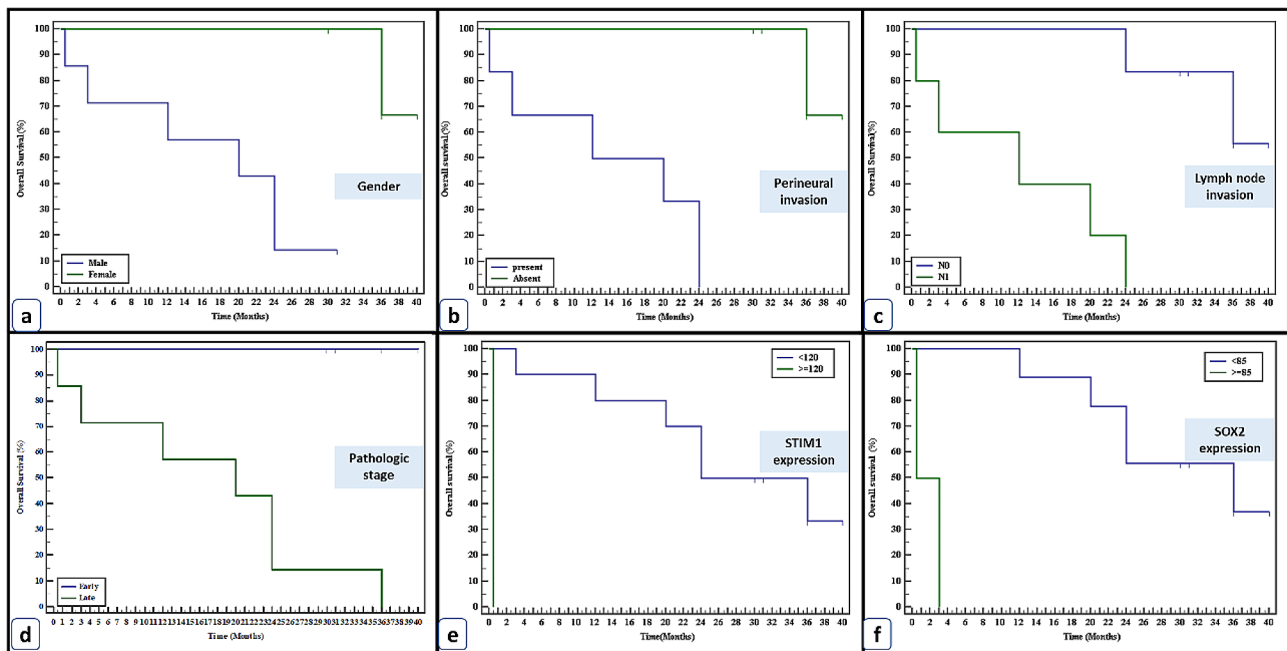


Fig. 5 Kaplan-Meier survival curve demonstrating the impact of clinicopathological and STIM1/SOX2 expressions on the overall survival of AAC cases

Table 3 Univariate and multivariate COX regression analysis for the parameters affecting mortality in the primary AAC group

| | Univariate | | #Multivariate | |
|---------------------|------------|-----------------------|---------------|-------------|
| | p | HR (95%C.I) | p | HR (95%C.I) |
| Sex (male) | 0.220 | 63.240(0.08–48065.8) | | |
| Perineural invasion | 0.175 | 116.264(0.12–112,908) | | |
| LN ^s | 0.015* | 15.396(1.70–139.70) | | |
| Staging (late) | 0.171 | 73.43(0.16–34653.2) | | |
| Stromal STIM1 | 0.823 | 0.829(0.16–4.29) | | |
| Epithelial SOX2 | 0.281 | 2.583(0.46–14.50) | | |

HR: Hazard ratio, C.I: Confidence interval, STIM1: Stromal interaction molecule1, SOX2: Sex determining region Y-box2, H-score: Histoscore, C.I: Confidence interval

#: All variables with P<0.05 was included in the multivariate

*: Statistically significant at P≤0.05

are being managed in a similar approach. Regarding clinicopathological and molecular aspects, PDAC seems to be the worst [43]. In addition, because of the rarity of AAC in the current and previous studies, there is a lack of related research and comprehension of its characteristics [44].

Furthermore, based on the term “stromal interaction molecule” of STIM1, we proposed a possible function for its expression in the tumor microenvironment cells. STIM1 was shown to be expressed in stromal cells in addition to lung cancer cells [45]. STIM1 may be involved in a wide range of biological functions in non-tumor cells, including immunological cells, endothelial cells, and fibroblasts [46, 47]. We also observed a significant overexpression of SOX2 in the intra-tumoral stromal

cells in PDAC and AAC. In some malignancies such as colorectal carcinoma, and lung, stromal SOX2 was found to be linked to a worse prognosis [47–49]. Despite the clinical significance of STIM1 and SOX2 stromal expression is not well elucidated, we could suggest their possible role in the metastatic potential of PDAC and AAC.

Primary and metastatic PDAC showed similar STIM1 and SOX2 expression. Pancreatic cancers metastasize early and thus are molecularly indistinguishable from metastatic tumors [50]. However, the expression STIM1 in metastatic cancer is a matter of controversy. STIM1 was downregulated in metastatic hepatocellular carcinoma (HCC) rather than in primary HCC cells [19]. On the other hand, STIM1 was overexpressed in metastatic gastric cancer compared to the primary sites [20]. Similarly, highly metastatic CRC cell lines have higher STIM1 expression than minimally metastatic cell lines [10]. STIM1 was overexpressed in metastatic melanoma and lung cell lines compared to primary cell line [51, 52]. Regarding SOX2, despite lacking of previous studies, SOX2 modulates several features of tumor metastasis such as EMT, migration, and invasion [53].

PDAC overexpressed epithelial STIM1 had dense desmoplastic stroma which may indicate a crosstalk between the malignant and microenvironmental cells. Desmoplastic stroma facilitates tumor cell growth, invasion, and metastasis, which mediates PDAC progression [54]. STIM1 regulates the activity of transforming growth factor-β (TGF-β) dependent Snail1 transcription genes, which are required for EMT [55]. SOX2 stromal expression in the current study tends to be higher in metastatic

PDAC than in the primary ones. SOX2 enhances mesenchymal gene activation while suppressing epithelial genes that enable EMT-induced cancer cell spread [16, 56].

Limited data regarding the correlation of STIM1 and SOX2 in cancer and their possible synergistic impact. STIM1 and SOX2 expressions were found to be positively correlated in the PDAC group. The possible mechanism is through sharing stemness and an antiapoptotic mechanism. Belotte et al. found that STIM1 overexpression influenced the activation of stemness-related genes such as SOX2 [57]. In addition, STIM1 maintained Ca²⁺ signals in CSCs in a wide range of tumors [58]. STIM1 and SOX2 were discovered to have anti-apoptotic effects in pancreatic cancer cell lines, HCC, and prostate cancer [33, 59, 60]. However, the expression of STIM1 and SOX2 did not show a significant association with the studied anti-apoptotic marker, BCL2 which could be attributed to the limited PDAC and AAC cases expressing BCL2. STIM1 has no significant impact on the apoptosis pathway in gastric cancer [52]. The current debate reflects the variety of linkages between BCL-2 proteins and Ca²⁺ signaling pathways, as not all targets or mechanisms will be active in all types of cells and situations [61].

There is disagreement regarding the prognostic significance of SOX2 and STIM1 in certain malignancies [16, 18, 57, 62–64]. This was the case in our study. SOX2 expression was linked to the well-differentiated grade of PDAC while associated with poor patients' survival. In AAC, SOX2 expression was linked to large tumor size and short patient survival. STIM1 stromal expression was linked to short survival in AAC cases. Previous studies found an association of SOX2 with worse prognostic parameters in PDAC [17, 65]. However, Herreros-Villanueva et al. reported a lack of prognostic role of SOX2 in PDAC [16]. This debate may be attributed to SOX2's ability to regulate the activity of a wide range of genes that can either accelerate or prevent tumor growth [40]. The poor prognostic impact of SOX2 has been found to promote growth, metastasis, and drug resistance in different malignancies [59, 66]. STIM1 may play a site-specific prognostic role in cancer. In pancreatic cancer, STIM1 expression was significantly linked with short survival [10]. Chong et al. reported the absence of a significant relationship between SOX2 expression with the patients' survival in periampullary cancer [39].

The limitations of this research included lack of advanced microscopes for photographs, and facilities of digital scoring, molecular and invitro experiments and lack of financial resources. Furthermore, there was difficulty obtaining the survival data of the patients.

In conclusion, the expression of STIM1 and SOX2 in PDAC and AAC could indicate a shared pathway; however, not linked to the anti-apoptotic BCL2 expression. Their role in tumorigenesis could be modulated by their

direct expression in tumor and non-tumor cells. SOX2 stromal expression could have a role in metastatic PDAC. STIM1 and SOX2 both play a negative prognostic function in AAC. Their prognostic role in PDAC, however, is unclear.

Supplementary Information

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Supplementary Material 1: **Table 1:** Comparative between PDAC and AAC regarding the clinicopathological data

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Author contributions

D.S., wrote the manuscript, contributed to the study design, and supervised the implementation of the research; S.I., implemented the research and contributed to writing the manuscript; H.A., supervised the implementation of the research; N.E., supervised implementing the research; A.H., supervised implementing the research; M.D.; contributing in study design, supervised implementing the research, revising writing process of the manuscript, and the corresponding author.

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Data availability

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Because the study was retrospective, the ethics committee/Institutional Review Board of (IRB: 2/2020PATH38) decided that informed consent was not necessary." Authors confirm that all experimental protocols were approved by the Research Ethics Committee.

Consent for publication

Not applicable.

Study design

A case-control retrospective study.

Competing interests

The authors declare that they have no competing interests.

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