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*Research Article / Araştırma Makalesi*

# A different perspective to the tumour microenvironment in periampullary cancers: a neglected ring in tumorigenesis

## *Periampuller kanserlerde tümör mikroçevresine farklı bir bakış açısı: tümörojenezde ihmal edilmiş bir halka*

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**ABSTRACT**

**Aim:** Increasing evidence shows that the microenvironment of a tumour plays a significant role in tumour development and progression and this is not only a passive observer. In this study, the immunohistochemical staining pattern of the stromal cells in the tumour microenvironment, which is rarely discussed in the literature, has been demonstrated by considering p53 and HSF1 which are important molecular proteins in the tumorigenesis.

**Material and Method:** Sixty-nine pancreatico-duodenectomy specimens that performed between 2000 and 2012 were re-evaluated in the terms of HSF1/P53 expressions for tumour microenvironment and tumoral cells. The findings were statistically analyzed.

**Results**: Significant difference was observed between tumoural microenvironment and tumoural cells in the terms of HSF1 staining ($p < 0.05$). For P53, this difference was only observed in pancreatic carcinomas ($p < 0.05$).

**Conclusion**: Significant staining of two well-known immunomarkers as P53 and HSF1 in stromal cells has further increased the importance of tumour microenvironment in tumorigenesis.

**Keywords:** Tumour microenvironment, pancreas, ampullary, P53, HSF1

**ÖZ**

**Amaç**: Tümör mikroçevresinin tümörün gelişiminde ve ilerlemesinde önemli bir rol oynadığına ve sadece pasif bir gözlemci olmadığına dair kanıtlar artmaktadır. Bu çalışmada literatürde nadiren tartışılan tümör mikroçevresindeki stromal hücrelerin immünohistokimyasal boyanma paterni, tümörogenezde önemli moleküler proteinlerden olan p53 ve HSF1 ele alınarak gösterilmiştir.

**Gereç ve Yöntem:** 2000 ve 2012 yılları arasında yapılan 69 pankreatikoduodenektomi spesmeni, tümör mikroçevresin ve tümöral hücrelerde HSF1/P53 ekspresyonu açısından tekrar değerlendirildi. Bulgular istatistiksel olarak analiz edildi.

**Bulgular**: Çalışmamızda, tümör mikroçevresi ve tümöral hücreler arasında HSF1 boyaması açısından anlamlı fark mevcuttu ($p<0.05$). P53 için anlamlı fark sadece pankreatik karsinomlarda ($p<0.05$) gözlendi.

**Sonuç**: P53 ve HSF1 gibi iki iyi bilinen immünmarkerın stromal hücrelerde de anlamlı olarak boyanması, tümör mikroçevresinin tümörogenezdeki önemini daha da artırmıştır.

**Anahtar Kelimeler**: Tümör mikroçevresi, pankreas, ampulla, P53, HSF1

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## INTRODUCTION

Progressing in the poor course of pancreatic ductal adenocarcinoma (PDC) is still limited, although there are important resources (1). The desmoplastic stromal response decreases microvascularity and drug distribution in tumoural cells, thus contributing to the difficulty of treatment by increasing tumour progression, metastasis and chemotherapy resistance (2). In fact, the reduction in tumour aggressiveness with diminished stroma clearly demonstrates the complexity of the stroma - tumour interaction (3).

Current cancer surveys focusing mainly on cancer cells and provide an increased database on deviations in genetic composition (4). However, tumours live in a microenvironment that contains complex cellular components that do not contain only cancer cells thus inflammatory cells and fibroblasts play a significant act in tumour growth (5). All these cell types can critically affect the multistage tumorigenesis process. Findings from different studies indicate that tumours can change the stroma to allow tumour progression and create a supportive environment (6). Moreover, there is a strong finding that stromal cells show a significant action in cancer development and progression (7).

Tumour protein 53 (P53) / heat shock factor-1 (HSF1) expressions in the tumour microenvironment and tumour cells in periampullary regional tumours were analyzed in this study. The main aim of our work is to draw attention to the significance of the tumour microenvironment in carcinogenesis from a different perspective, as well as to open possible clinical trials and new treatment strategies.

## MATERIAL AND METHOD

### 1. Case Selection

In this study, 69 pancreaticoduodenectomy material was included from 2000 to 2012. These cases were separate to 2 groups as pancreas and ampulla. Clinical, laboratory and radiological information of these cases were obtained from archival records. Tumoural cells and tumour microenvironment cells in both groups were examined statistically in terms of HSF1 and P53 staining.

### 2. Histological Differentiation and Staging

All the hematoxylin & eosin (H & E) painted archive preparations of the cases were re-examined. Reconstructed sections were taken from the required blocks. All cases were re-grouped according to 2016 World Health Organization's classification and staging system of the exocrine pancreas and ampullary tumours.

### 3. Immunohistochemical Study

One of the routine blocks of the specimens that best reflects the characteristics of a tumour was selected and sections were taken. HSF1 (E-4: SC-17757 (1: 30), Mouse monoclonal, cat no: Lot-A0411, Santa Cruz Biotechnology, USA) and P53 (Ab-5 (1: 200), Mouse monoclonal, cat no: MS-186-P1, Thermo Scientific, USA) antibodies were used in immunohistochemical (IHC) screening. As a positive control, invasive ductal carcinoma tissue of the breast for HSF1 and colon adenocarcinoma tissue for P53 were used. The IHC study was performed by streptavidin/avidin/biotin method. Leica Bond-Max (Leica biosystem, Germany) branded fully automated immunohistochemical staining device was used for staining.

### 4. Immunohistochemical Scoring

Nuclear staining for HSF1 and P53 was accepted as positive in the IHC screening. At least 1000 tumour nucleus were counted in each a tumour and stromal cell, and the presence of staining, staining intensity and staining prevalence in the cells were examined using a semiquantitative and subjective grading system. The prevalence of staining was semiquantitatively grouped as follows: 0: 1-5%; 1: 5-10%; 2: 10-100%. The staining intensity was grouped as strong, moderate and weak. During statistical evaluation, group 0 and group 1 case were combined.

### 5. Statistical Evaluation

In the descriptive statistics of the data, the ratio and frequency values were used. Chi-Square ($x^{2)}$ test was used for categorical data, Fischer test was used when the $x^2$ test was not available. SPSS version 21.0 (IBM institute, North Castle, New York, ABD) was used in the analyzes. Data presentation was done using numbers, ratio, the smallest and the largest values. The limit of significance was accepted as $p < 0.05$.

## RESULTS

72.4% were male and 27.6% were female (n=69) and the mean age was 61.8 (range 36 - 88). When age distribution was examined, 49.2% of the cases were between 51-80 years old.

### 1. Tumour Localization

47.8% of the tumours were located in the pancreas, and 52.2% were located in ampullary (n=69).

### 2. Tumour Size

The size of tumours was between 1 cm and 7 cm. In 34.7% of the cases, the tumour diameter was found as 2 cm or less and 65.2% was found as over than 2 cm (n=69).

### 3. Positive Lymph node

Regional lymph node metastasis was detected in 55.1% of the cases and not detected in 44.9% of the cases. The range of 1 to 13 metastatic lymph nodes was observed in the cases.

### 4. Stage

31% of the cases were detected as PT2, 56% of the cases as PT3 and 13% of the cases as PT4 (n=69).

### 5. Prognosis

68.7% of the cases dead and 31.2% of them were alive (n=48). The mean survival time was 50.5 months (range 6 to 83 months).

### 6. Histological Differentiation

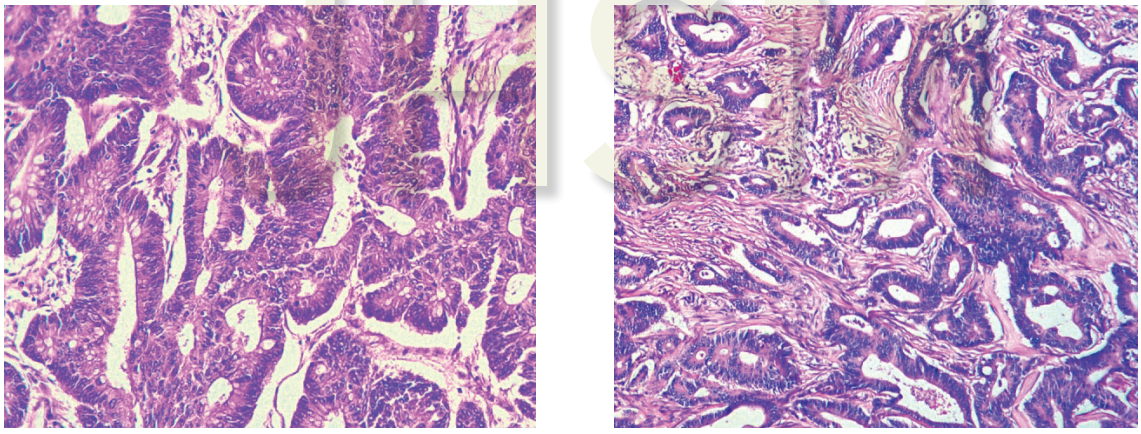
8.7% of a tumour was well differentiated, 78.2% of a tumour as moderately differentiated and 13.1% of a tumour as poor differentiated (n=69) (Picture 1).

### 7. Immunohistochemical Findings

HSF1 staining was not observed in 60.8% of the cases and 39.1% of the cases had a positive result with HSF1. In 14.8% of these staining cases, the prevalence of staining was low than 10% and in 85.2% of the cases was over than 10% (Picture 2).

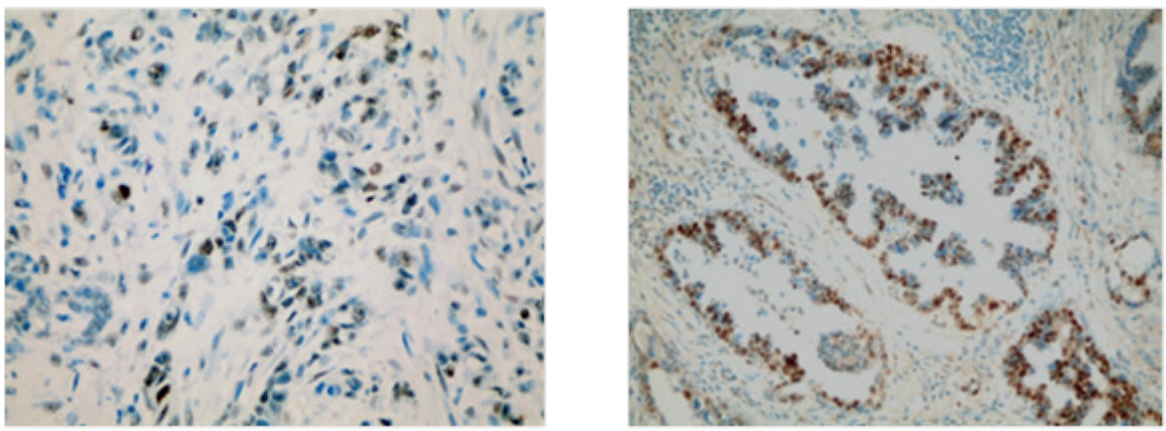
Nuclear-positive HSF1 staining is seen in PDC (right) (x20) and in ampullary region adenocarcinoma (left) (x20), over 10%. (HSF1: Heat shock factor-1, PDC: Pancreatic ductal carcinoma, arrows: nuclear positive staining with HSF1)

P53 staining was not observed in 50.4% of the cases and 49.6% of the cases had a positive result with P53. In 10.5% of these staining cases, the prevalence of staining was low than 10% and in 89.5% it was over than 10% (Picture 3).

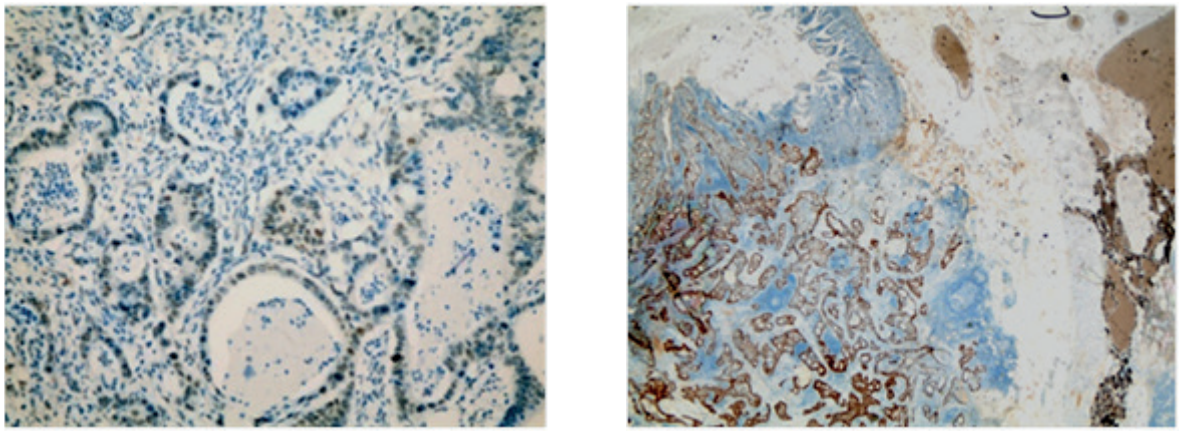


**Picture 1.** PDC (right) and ampullary region adenocarcinoma (left)

Well-differentiated PDC (right) (x20, H&E) and well-differentiated ampullary region adenocarcinoma (left) (x20, H&E) is seen. (PDC: pancreatic ductal carcinoma, H&E: Hematoxylin & Eosin staining)



**Picture 2.** HSF1 staining for the PDC (right) and ampullary region adenocarcinoma (left)



**Picture 3.** P53 staining for the PDC (right) and ampullary region adenocarcinoma (left)

Nuclear-positive P53 staining is seen in the PDC (right) (x20) and in the ampullary region adenocarcinoma (left) (x20), over 10%. (HSF1: Heat shock factor-1, PDC: Pancreatic ductal carcinoma, arrows: nuclear positive staining with P53)

### 7.1. HSF1 staining in normal pancreatic ductal cells

Normal ductal epithelial cells in the pancreas, ampullary and whole group showed significant difference with HSF1 staining ($p < 0.05$) (Table 1).

### 7.2. P53 staining in normal pancreatic ductal cells

Normal ductal epithelial cells in pancreas, ampullary and whole group showed significant difference with P53 staining ($p < 0.05$) (Table 2).

### 7.3. HSF1 staining in desmoplastic stromal cells

Desmoplastic stromal cells in pancreas, ampullary and whole group showed significant difference with P53 staining ($p < 0,05$) (Table 3 / Picture 4)

### 7.4. P53 staining in desmoplastic stromal cells

Desmoplastic stromal cells showed significant difference with P53 staining ($p < 0,05$) in pancreas and whole group but showed no differences ($p > 0,05$) in ampullary group (Table 4 / Picture 5).

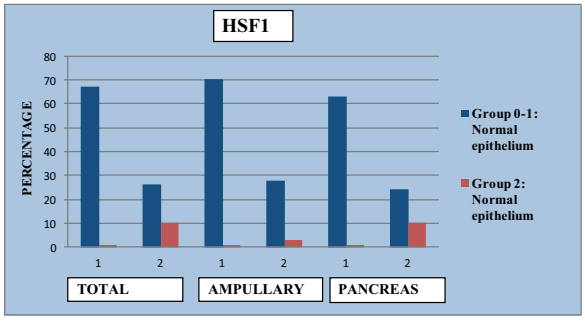



**Table 1.** Percentage of HSF1 staining in normal pancreatic ductal cells

The HSF1 staining percentage for normal pancreas duct cells was significantly higher (HSF1: Heat shock protein-1). Note 1: Column 1 shows the adjacent areas to cancer and column 2 shows the far areas from cancer.
Note 2: Group 0-1 shows HSF1 positive staining in 1-10% of cells, Group 2 shows positive staining in 10-100% cells

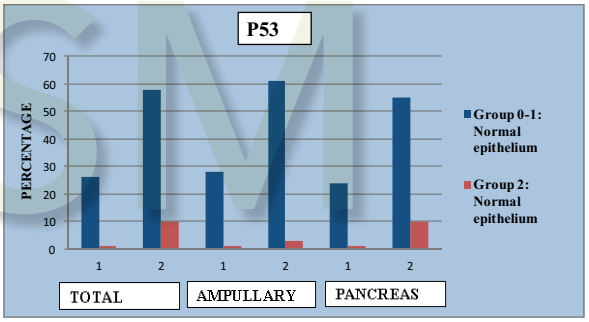



**Table 2.** Percentage of P53 staining in normal pancreatic ductal cells

P53 staining percentage for normal pancreas duct cells was significantly higher (P53: tumour protein 53).
Note 1: Column 1 shows the adjacent areas to cancer and column 2 shows the far areas from cancer
Note 2: Group 1 shows P53 positive staining in 1-10% of cells, Group 2 shows positive staining in 10-100% cells

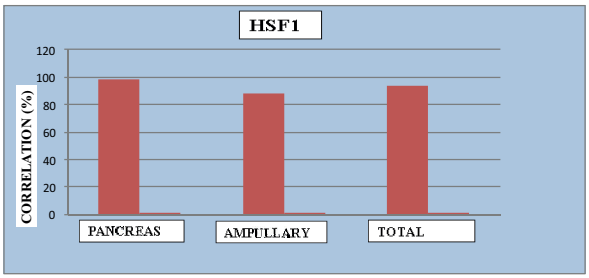
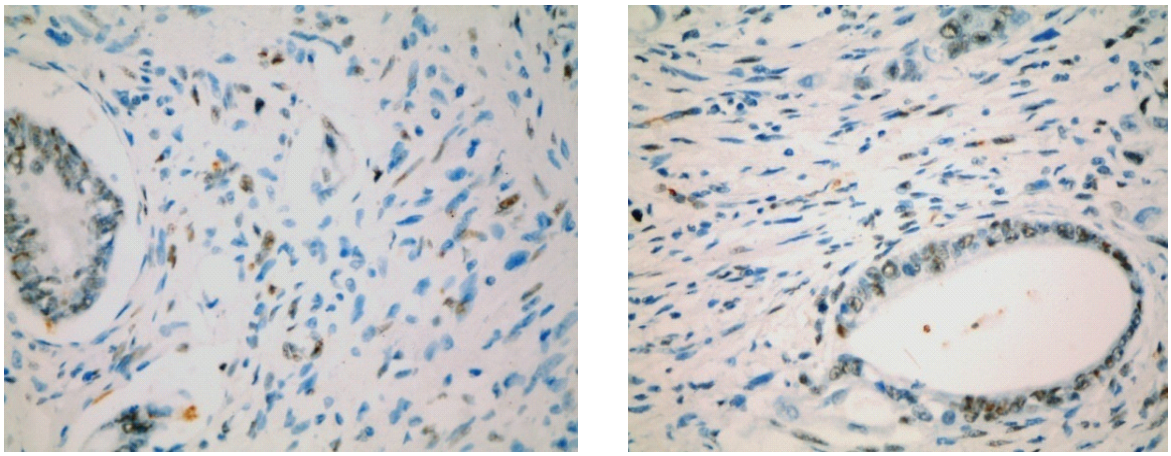



**Table 3.** Correlation of HSF1 staining between desmoplastic stromal cells and tumour cells

There was a significant correlation between desmoplastic stromal cells and cancer cells for HSF1 staining (HSF1: Heat shock protein-1)



**Picture 4.** HSF1 staining of desmoplastic stromal cells for the PDC (right) and ampullary region adenocarcinoma (left)

Nuclear-positive HSF1 staining in desmoplastic stromal cells is seen in PDC (right) (x20) and in ampullary region adenocarcinoma (left) (x20), over 10%. (HSF1: Heat shock factor-1, PDC: Pancreatic ductal carcinoma, arrows: nuclear positive staining with HSF in desmoplastic stromal cells)

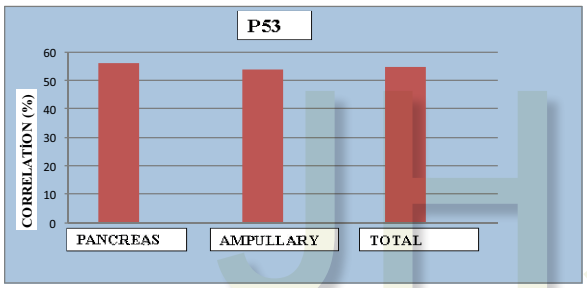
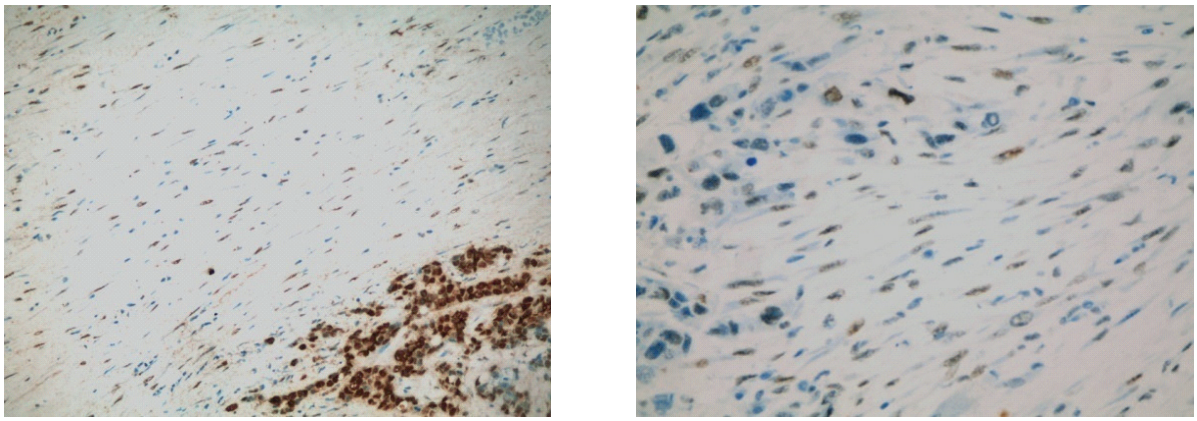



**Table 4.** Correlation of P53 staining correlation between desmoplastic stromal cells and

There was a significant correlation between desmoplastic stromal cells and cancer cells for P53 staining (P53: tumour protein 53)

## DISCUSSION

PDC is highly malignant neoplasia which is the fifth most frequent cause of death in worldwide. It is waited to take second place in Western countries in 2030 (1). The treatment option is only surgical for PDC, with overall survival of 5% to 15% even after curative resection (1). In addition to surgical resection, an adjuvant multimodal treatment that combined radiotherapy and chemotherapy is accepted. Chemotherapy that most used treatment option, has not been implemented as an active method due to various immunosuppressive effects (1,2). Several basic mechanisms that lead to therapeutic difficulties, such as desmoplastic stromal response, infiltration of T cells and the elimination of regulative cells, have been emphasized (2). Among these, the desmoplastic stromal response, especially around the



**Picture 5.** P53 staining of desmoplastic stromal cells for the PDC (right) and ampullary region adenocarcinoma (left)

Nuclear-positive P53 staining in desmoplastic stromal cells are seen in PDC (right) (x20) and in ampullary region adenocarcinoma (left) (x20), over 10%. (HSF1: Heat shock factor-1, PDC: Pancreatic ductal carcinoma, arrows: nuclear positive staining with P53 in desmoplastic stromal cells)

tumour, is also a target for new treatment strategies (3). We wanted to draw attention to this tumour microenvironment in this study. Now, let's get to know the tumour microenvironment.

Carcinomas are heterogeneous structures composed of various proportions of neoplastic epithelial and stromal cells called tumour microenvironment (4). Until now, although most of the cancer investigations have focused on the examination of carcinomatous epithelium, many shreds of evidence suggest that the tumour stromal cells play an important role in the development of tumour progression. There are important steps to be taken by cancer cells on the carcinogenesis to distant metastasis (5). In order for cancer cells to successfully complete this challenging process, they must have acquired some special abilities such as to gain epithelial/mesenchymal changeability, to trigger lymphangiogenesis, to make lymphatic metastases, to colonize by multiplying in the remote organ. All of these are related to cells in the tumour microenvironment (6,7). This microenvironment is a three-dimensional dynamic structure containing many different proteins, glycoproteins, proteoglycans and polysaccharides (6). Nowadays, investigator well known that tumour microcirculation acts a significant role in many places such as growth and feeding of cancer cells, resistance to cell death, immune system evasion, invasion / metastatic ability (8,9). Now let's look at studies dealing with the importance of the tumour environment on tumours development.

Studies have shown that a normal stromal circumference is required for the development of epithelial neoplastic lesions (10). Fibroblasts can age during environmental stress and may support to tumourigenesis by secreting metalloproteases, cytokines and growth factors (10,11). Also, tumour microenvironment fibroblasts are transformed to contractile myofibroblasts called cancer-associated fibroblasts (CAF) (11,12). Recent studies show that a strong relationship between the tumour environment and lysosomal process. These CAF's, pre-ageing cells, and autophagic images are among the factors that cause tumour progression (13).

Differences between the tumour stroma and the normal stroma have been extensively followed up by pathologists for a long time and have reported, for example, that phenotypes of CAF due to breast carcinoma are different from those of normal breast epithelium-related fibroblasts (14). DNA alterations in CAF's in experimental mouse models may lead to the ability to induce cancer (14,15). Some reports suggest that loss of heterozygosity (LOH) in human breast cancers also occurs in cancer environment, supporting the genomic instability (16). However, Qiu et al. (17), challenges the opinion that the tumour environment is affected by cancer. Some studies showed that the stromal cells affected directly the epithelium and cause epithelial dysplasia and neoplasia. In these studies on mice with fibroblast-specific ablation of the type II TGF- β (Tgfr2) gene, it has been shown that precancerous lesions develop in the prostate and stomach (18-20).

The literature information listed above suggests that the stromal component may be an important marker and target in the treatment of cancers. In this study, we focused on the stromal cells of the tumour periphery that we frequently encounter in pancreatic cancer. We decided to look at the expression of mutated tumour proto-oncogenes in stromal cells to determine the effect of these cells on tumour cells, and we chose p53 and HSF1. Now let's look at the similar study on this topic. Since the study with HSF1 is not available in the literature, we will only look at p53.

P53 is an important tumour suppressor that inactivated in the most cancer subtypes (21). Much research into the act of P53 has focused on DNA damage or oncogene activation, that is the ability of cells with a tendency for malignancy of P53 to arrest apoptosis or arrest growth. However, activation of P53 in a cell can also induce various effects in neighbouring cells through secretory factors, endocrine and paracrine mechanisms (22). For example, cancer stromal P53 may support tumour development and progression. These cancer cells under this effect gain some capacity either for silencing stromal P53 or for inhibition of stromal P53 (22, 23). For this reason, the stromal P53 activation by adjuvant therapy may be part of the explanation that causes cancer regression in these treatments.

Evaluating P53 in stromal cells around the tumour is important to confirm the association of stromal P53 with cancers. As a matter of fact, important discussions on this subject still continue. The first stromal P53 mutation report is made by Wernert et al. (24) who recognized the stromal mutant P53 in breast and colon cancers. Later, the high presence of stromal P53 mutations in breast cancer has been described (25). In addition, P53 mutations were found at high levels in leukemic bone marrow stromal cells (26). However, it has been described that P53 mutations in stromal cells of hereditary breast cancer cases are more common than sporadic breast cancer cases. Furthermore, the presence of stromal P53 mutations in breast cancers has been correlated with lymph node metastasis (27). Recently, Heisebe et al recognized that immunohistochemical staining of stromal P53 in breast cancer correlated with poor prognosis (28). Many studies have also emphasized the paracrine role of this versatile tumour suppression. Studies have indicated that activation of P53 in a cell affects the environment by modulating the exp-

ression of a number of secretory factors genes (29). However, some studies show that P53 activation in normal tissue may affect also distant tumours through endocrine mechanisms (29). In another study, an unexpected way of affecting p53 in peripheral stromal cells was described as stromal p53 inactivation may be undergone by phagocytosing DNA released from apoptotic cancer cells (30).

All these findings suggest that there is a relationship between the stromal P53 mutations and tumour progression, and stromal P53 has a capacity to affect the tumour development in many different ways. In our study, significant immunohistochemical staining of P53 and HSF1 in stromal cells was an important indicator that tumour stroma affects the tumour development for carcinogenesis. Although our study shows the contribution of stroma to tumour formation, further work is needed to support these findings at the molecular level. According to us, genetic alterations in the tumoural cells are not enough to start and progress the tumours without the tumour microenvironment. A detailed understanding of this area has critical implications for both cancer's basic biology, as well as prevention of treatment strategies.

## CONCLUSION

Carcinomas are not an individual disease of the epithelial cells, and it seems now clear that the importance of stromal microenvironment with many unanswered questions. Although the many treatment methods focus on the targeting of cancer cells treatments, effective therapeutic targeting requires a detailed demonstrating of the interaction between stroma and tumour. There is a much need for wide-ranging studies in different directions, for the detailed understanding of this topic in the tumorigenesis.

## DECLARATION OF CONFLICTING INTERESTS

The author declared no conflicts of interest with respect to the authorship and/or publication of this article

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### Compliance with ethical standards

The study was conducted under Istanbul education and research hospital and was approved by The Regional Committees on Health Research Ethics for Istanbul education and research hospital, Istanbul, Turkey. All procedures performed in studies involving human participants were in accordance with the ethical standard of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Competing interests

Author declare no conflict of interest.

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