Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Research Article Open access

Comparison Of Expression Levels Of Keratin 19 *(Krt19)* and Transmembrane Serine Protease 4 *(Tmprss4)* Genes in SU.86.86 and BxPC-3 Pancreatic Cancer Cell Lines

Seda Yavuzarslan[1], Süreyya Bozkurt[2*]

[1]*Istinye University,Institute of Graduate Education,Department of Medical Biology and Genetics, Istanbul, Turkey*
[2]*Istinye University, Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey*

DOI: 10.31383/ga.vol9iss1ga03

***Correspondence**
E-mail: sbozkurt@istinye.edu.tr

Received
March, 2025
Accepted
April, 2025
Published
May, 2025

Abstract

In this study, the expression levels of *Keratin 19 (KRT 19)* and *Transmembrane serine protease 4 (TMPRSS4)* genes in metastatic pancreatic cancer cell line SU.86.86 and nonmetastatic pancreatic cancer cell line BxPC3 cells were been determined and the capacity of these two genes to be metastatic biomarkers in pancreatic cancer is aimed to be evaluated. Genomic DNA was isolated from BxPC3 and Su8686 cell lines, which we replicated in culture medium, and then cDNA was obtained. By using gene-specific primers, expression levels of *KRT 19* and *TMPRSS4* genes were determined at the transcriptional level by real time PCR method. At the end of this study TMPRSS4 gene expression was found to be 1.31-fold decreased and KRT19 gene expression was found to be 1234-fold increased in the metastatic pancreatic cancer cell line SU8686. TMPRSS4 gene expression was found to be 2.38-fold decreased and KRT19 gene expression was found to be 183-fold increased in the non-metastatic BXPC3 cell line. In pancreatic cancers, where survival is very low, most of the patients are in the metastatic process at the time of diagnosis. For this reason, there is an urgent need for metastatic biomarkers that can provide information about the prognosis of the disease. In this study, the expression of the KRT19 gene at the transcriptional level was found to be quite high in the metastatic pancreatic cancer cell line. For this reason, it is thought that studies with large patient groups may be beneficial as a metastatic biomarker candidate in pancreatic cancer.

Keywords

Pancreatic cancer, Keratin 19 gene, TMPRSS4 gene

Introduction

Pancreatic cancer is a deadly cancer type that has been increasing in frequency in recent years. Age, obesity, long-term smoking and alcohol use, pre-existing chronic pancreatitis, and family history are among the important risk factors (Goral, 2015; Klein, 2021). The most common histological type of pancreatic cancer is pancreatic ductal adenocarcinoma, which has a very poor prognosis. Even in cases where PDAC can be surgically removed, the recurrence rate of local or widespread disease is high (De Abreu et al., 2017; Dugnani et al., 2018; Park et al., 2021; Siegel et al., 2012). In addition to inherited germline or somatic acquired mutations in cancer-associated genes, epigenetic changes in DNA and histones also contribute to the pathogenesis of PDAC. More than 85% of PDAC patients are diagnosed with metastases, usually at an advanced stage (Hidalgo, 2010; Wood et al., 2022). However, the cellular and molecular mechanisms involved in the metastasis and poor prognosis of PDAC have not yet been fully elucidated. Therefore, it is crucial to identify metastasis-associated biomarkers that will serve for early detection of PDAC.

Human keratin-19 (KRT19) is a type I keratin protein that has 40 kDa molecular weight encoded by the *KRT19* gene. Keratin proteins are intermediate filament proteins responsible for the structural integrity of epithelial cells. They play an important role in stress, cell signaling and apoptosis by participating in the formation of the cytoskeleton (Rogel et al., 2010). *KRT19* gene has been reported to be overexpressed in breast cancer, thyroid cancer, hepatocellular carcinoma, pancreatic cancer, oral squamous cell carcinoma and renal cell neoplasia (De Albuquerque et al., 2012; Govaere et al., 2014; Kabir et al., 2014; Menz et al., 2021; Okamoto et al., 2013; Paiva et al., 2011; Prasad et al., 2005; Yang et al., 2024).

Another gene whose expression level was investigated in our study is transmembrane serine protease 4 (*TMPRSS4*) gene that encodes TMPRSS4 protein. Although the oncogenic significance and molecular mechanisms of TMPRSS4 protein is not known exactly, it has been reported to be highly expressed in pancreatic, thyroid, lung and other cancer cells (Liang et al., 2013; Tazawa et al., 2022). TMPRSS4 has been reported to be overexpressed in pancreatic cancer (Wallrapp et al., 2000) and has been proposed as a diagnostic marker for malignant thyroid neoplasms (Kebebew et al., 2005). TMPRSS4 has been reported to increase the invasive and metastatic potential of human cancer cells by facilitating epithelial-mesenchymal transition (EMT) (Jung et al., 2008; Kim, 2023). To optimize the therapeutic treatment to be applied in pancreatic cancer, guiding prognostic biomarkers are urgently needed. Therefore, in this study, the expression levels of *KRT19* and *TMPRSS4* genes at the transcriptional stage in metastatic and non-metastatic pancreatic cancer cell lines were determined and their potential as biomarkers were investigated.

Material and methods

Cell culture

SU8686 metastatic and BxPC3 non-metastatic human pancreatic cancer cell lines were used in this study. The medium was prepared as 10% FBS (fetal bovine serum) and 1% Penicillin/streptomycin in RPMI-1640. The cell suspension was transferred to 25 cm^2 flasks containing 5 ml of medium and incubated at 37°C in an environment containing 5% CO_2. For passaging the cell lines,

the cell suspension was transferred to 75 cm^2 flasks and incubated at 37°C with 5% CO_2. The cells were multiplied until the desired number was reached. Cells were counted by haemocytometer.

Total RNA isolation

Total RNA was isolated from $1x10^6$ cells using an RNA isolation kit (Omega Bio-tek, R6836). Total RNA was isolated and the collected RNAs was stored at -80°C.

cDNA syntesis and Real time PCR reaction

QuantiTect Reverse Transcription Kit from Qiagen was used for cDNA synthesis. Each gene-specific primers designed to be used in quantitative PCR reaction for gene expression analysis are shown in Table 1. Corbett Research Real-time PCR Thermal Cycler was used for amplification. 12 µl of Sybr Green 2X (HibriGen brand, catalogue number mg-sybr-01-400) was added to the reaction tube, 0.5 µl of each of the reverse and forward primers were added, 3 µl of cDNA sample and 4 µl of nuclease-free water were added. The reaction steps consist of 36 cycles; 95 °C for 30 seconds for denaturation, 58 °C for 30 seconds for annealing and 72 °C for 30 seconds for elongation.

Results and discussion

As a result, Ct changes in *TMPRSS4* and *KRT19* gene expressions in metastatic and non-metastatic cell lines are given in table 2 and table 3.

SU8686 and BXPC3 cell lines were used as experimental groups and human epithelial cell line BEAS-2B was used as the control group. $2^{-\Delta\Delta Ct}$ calculation for each gene were performed independently in 3 replicates and the average of these three experiments was used for gene expressions analysis. As a result of gene expression levels compared to BEAS-2B control cell line, we found that expression of *TMPRSS4* gene decreased 1.31-fold and a expression of *KRT19* gene increased 1234-fold in metastatic SU8686 cell line (Table 2 and Figure 1A), expression of *TMPRSS4* gene decreased 2.38-fold and expression of *KRT19* gene increased 183-fold in non-metastatic BXPC3 cell line (Table 3 and Figure 1B).

Pancreatic cancer is a dangerous type of cancer that has been increasing in recent years and biological function of the TMPRSS4 gene in pancreatic ductal adenocarcinoma (PDAC) remains unclear. In this study, in contrast to previous studies in the literature, we found that

Table 1. Desinged primer sequences

Gene name		Primer Sequence
GAPDH	Forward	CATGGCCTTCCGTGTTCCTA
GAPDH	Reverse	TACTTGGCAGGTTTCTCCAGG
TMPRSS4	Forward	CCTGGCGAGTATCATCATTGTG
TMPRSS4	Reverse	GATCGGTCCTTGGAGAGGCG
KRT19	Forward	CGGGACAAATTCTTGGTGCC
KRT19	Reverse	ATCCAGCACCCTGCGCAGGCC

TMPRSS4 gene expression is low in both a metastatic pancreatic cancer SU8686 cell line and a non-metastatic pancreatic cancer BXPC3 cell line. Kim et al. (2012) and Cheng et al. (2019) reported that TMPRSS4 affected the proliferability and invasiveness of pancreatic cancer cells and overexpressed TMPRSS4 indicated poor prognosis in pancreatic cancer. Moreover, especially the expressions of TMPRSS4 and ECT2 genes were reported to be high and both genes may be involved in the development of PDAC and may be a potential therapeutic target in patients with PDAC (Bhasin et al., 2016). Contrary to our study results, another study found a high transcriptional expression level of the TMPRSS4 gene was done by the Gu et al. (2021). These researchers were reported that expression levels of the *TMPRSS4* gene were high in pancreatic cancer cell lines, including AsPC-1, BxPC-3, Capan-1, CFPAC-1, Hs766t, PANC-1, and SW1990. The researchers also reported that the expression of TMPRSS4 was significantly elevated in the cancerous tissues of 97 patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) compared to normal pancreatic tissue, and this elevation was correlated with shorter survival times in the patients. Hereby, they suggested that TMPRSS4 expression could be prognostic biomarker and a potential therapeutic target in PDAC. Similar to this study, in a study conducted by Tazuma et al. (2024) in 81 pancreatic ductal adenocarcinoma patients, the five-year overall and recurrence-free survival rates were significantly lower in the TMPRSS4-positive group than in the TMPRSS4-negative group.

Table 2. Results of fold change calculation with average Ct values in SU8686 cell line

	Average Ct Value (BEAS-2B)	**Average Ct Value (SU8686)**	**ΔCt (BEAS-2B)**	**ΔCt (SU8686)**	**Expression Change (Fold) $2^{-\Delta\Delta Ct}$**
PRSS4	31,92	33,33	-11,16	-11,74	0,76
KRT19	30,78	21,54	-10,22	0,05	1234,74
GAPDH	20,56	21,59			

Table 3. Results of fold change calculation with average Ct values in BXPC3 cell line

	Average Ct Value (BEAS-2B)	**Average Ct Value (BXPC3)**	**ΔCt (BEAS-2B)**	**ΔCt (BXPC3)**	**Expression Change (Fold) $2^{-\Delta\Delta Ct}$**
TMPRSS4	31,92	33,75	-11,36	-12,59	0,42
KRT19	21,16	23,86	-10,22	-2,7	183,54
GAPDH	20,56	21,16			

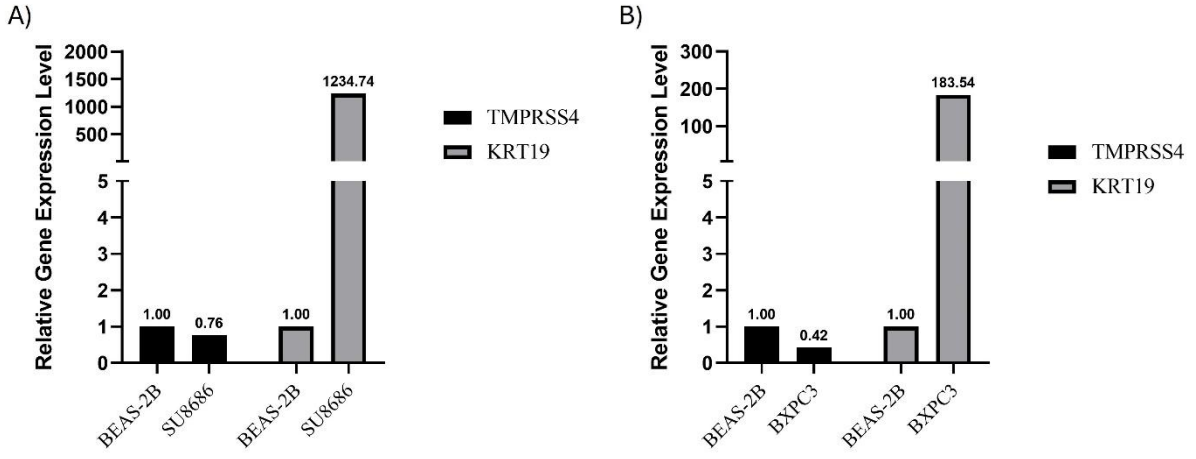

Figure 1. Relative gene expression of *TMPRSS4* and *KRT19* genes. BEAS-2B is normal cell line. (A) TMPRSS4 gene expression was found to be 1.31-fold decreased and KRT19 gene expression was found to be 1234-fold increased in the metastatic pancreatic cancer cell line SU8686. (B) TMPRSS4 gene expression was found to be 2.38-fold decreased and KRT19 gene expression was found to be 183-fold increased in the non-metastatic BXPC3 cell line.

Another gene we investigated in our study was *Keratin 19* (*KRT19*). Studies have shown that *Keratin19* has the potential to be a reliable prognostic marker in different tumors. In these studies, high expression of Keratin19 has been associated with poor survival (Barak et al., 2004; Karantza, 2011). Despite clinical data showing a positive correlation between increased *KRT19* expression and short survival rates among patients with various cancer types, the role of KRT19 in some cancer types, such as breast cancer, remains unclear. On the other hand, findings from studies with hepatocellular carcinoma (Tang et al., 2014), oral squamous cell carcinoma (Crowe et al., 1999), and lung cancer (Ohtsuka et al., 2016) cell lines have shown that KRT19 may have an effect on cell proliferation and migration (Sharma et al., 2019).

Although various studies have been conducted on *KRT19* in different solid tumor types, studies on pancreatic cancer are limited. In this study, the *KRT19* gene was found to be high at the transcriptional level in metastatic and non-metastatic SU8686 and BXPC3 cell lines, respectively. In addition, the fold change increase in the expression level of the *KRT19* gene was found to be much higher in the metastatic pancreatic cancer cell line SU8686 than in the non-metastatic BXPC3. These findings regarding the *KRT19* gene are parallel to the limited number of studies in the literature. For example; in a study conducted by Yao et al. (2017) it was observed that *KRT19* gene expression was higher in pancreatic ductal adenocarcinoma than in pancreatic tissues with benign lesions. In addition, in a study conducted by Brembeck and Rutsgi (2000), it was found that although pancreatic acinar cells do not normally express *KRT19*, they overexpress *KRT19* under the influence of gut-enriched Krüppel-like factor (GKLF/KLF4) and Sp1 transcription factors. In a study conducted by Dugnani et al. (2018), mRNA levels of various genes, including *KRT19*, were examined in 17 pancreatic ductal carcinoma (PDAC) cell lines and 41 PDAC patient tumor samples. At the end of the study, the expression of the *KRT* gene was found to be high and it was stated that this was correlated with low survival and early disease recurrence. In

a meta-analysis study including pancreatic endocrine tumors (PNETs), which constitute 1-2% of all pancreatic tumors, a relationship was found between *KRT19* expression and short-term survival, and it was stated that KRT19 could be used as a poor prognosis marker in PNETs (Cen et al., 2017). Another study conducted in 2024 supports the study results summarized above. In this study Bekker et al. (2024) was found that expression of glypican-3 (GPC3) and cytokeratin-19 (CK19) determined by immunohistochemistry to be associated with higher stage and grade disease, with a more adverse prognosis. We believe that we have contributed to the very few studies in the literature with this study.

Conclusion

In pancreatic cancer, where survival is quite low, it is extremely important to elucidate the cellular and molecular mechanisms involved in the metastatic process. Therefore, more studies are needed to identify biomolecules associated with metastasis, which will serve for early diagnosis of pancreatic cancer. In this study, which was planned based on this, the expression of the *KRT19* gene was found to be high in both metastatic and non-metastatic cell lines, with a much higher expression in the metastatic pancreatic cell line. It is thought that the *KRT19* gene has the potential to be a metastatic biomarker in pancreatic cancer, and it is recommended to study it in large patient groups.

Authors' contributions

S. Yavuzarslan:

Conceptualization; Formal analysis; Investigation; Methodology.

S. Bozkurt:

Conceptualization; Investigation; Methodology; Supervision; Validation; Writing – review and editing.

Conflict of interest

Authors declare no conflict of interest.

References

Barak V, Goike H, Panaretakis KW, Einarsson R (2004) Clinical utility of cytokeratins as tumor markers. Clin Biochem 37:529–540.

Bekkers C, Ramjeesingh R, Arnason T (2024) Glypican-3 and Cytokeratin-19 Expression in Pancreatic Cancer in a Canadian Population. J Clin Med 13.

Bhasin MK, Ndebele K, Bucur O, Yee EU, Otu HH, Plati J, Bullock A, Gu X, Castan E, Zhang P, Najarian R, Muraru MS, Miksad R, Khosravi-Far R, Libermann TA (2016) Meta-analysis of transcriptome data identifies a novel 5-gene pancreatic adenocarcinoma classifier. Oncotarget 7:23263–23281.

Brembeck FH, Rustgi AK (2000) The tissue-dependent keratin 19 gene transcription is regulated by GKLF/KLF4 and Sp1. J Biol Chem 275:28230–28239.

Cen D, Chen J, Li Z, Zhao J, Cai X (2017) Prognostic significance of cytokeratin 19 expression in pancreatic neuroendocrine tumor: A meta-analysis. PLoS ONE 12.

Cheng Y, Wang K, Geng L, Sun J, Xu W, Liu D, Gong S, Zhu Y (2019) Identification of candidate diagnostic and prognostic biomarkers for pancreatic carcinoma. EbioMedicine 40:382–393.

Crowe DL, Milo GE, Shuler CF (1999) Keratin 19 Downregulation by Oral Squamous Cell Carcinoma Lines Increases Invasive Potential. J Dent Res 78:1256–1263.

De Abreu FB, Liu X, Tsongalis GJ (2017) MiRNA analysis in pancreatic cancer: The Dartmouth experience. *Clin Chem Lab Med* 55:755–762.

De Albuquerque A, Kubisch I, Breier G, Stamminger G, Fersis N, Eichler A, Kaul S, Stölzel U (2012) Multimarker gene analysis of circulating tumor cells in pancreatic cancer patients: A feasibility study. Oncology *82*:3–10.

Dugnani E, Sordi V, Pellegrini S, Chimienti R, Marzinotto I., Pasquale V, Liberati D, Balzano G, Doglioni C, Reni M, Gandolfi A, Falconi M, Lampasona V, Piemonti L (2018) Gene expression analysis of embryonic pancreas development master regulators and terminal cell fate markers in resected pancreatic cancer: A correlation with clinical outcome. Pancreatology 18:945–953.

Goral V (2015) Pancreatic cancer: Pathogenesis and diagnosis. Asian Pac J Cancer Prev 16:5619–5624.

Govaere O, Komuta M, Berkers J, Spee B, Janssen C, De Luca F, Katoonizadeh A, Wouters J, Van Kempen LC, Durnez A, Verslype C, De Kock J, Rogiers V, Van Grunsven LA, Topal B, Pirenne J, Vankelecom H, Nevens F, Van Den Oord J, … Roskams T (2014) Keratin 19: A key role player in the invasion of human hepatocellular carcinomas. Gut 63:674–685.

Gu J, Huang W, Zhang J, Wang X, Tao T, Yang L, Zheng Y, Liu S, Yang J, Zhu L, Wang H, Fan Y (2021) TMPRSS4 Promotes Cell Proliferation and Inhibits Apoptosis in Pancreatic Ductal Adenocarcinoma by Activating ERK1/2 Signaling Pathway. Frontiers in Oncology, 11:628353.

Hidalgo M (2010) Pancreatic Cancer. N Engl J Med 362:1605–1617.

Jung H, Lee KP, Park SJ, Park JH, Jang YS, Choi SY, Jung JG, Jo K, Park DY, Yoon JH, Park JH, Lim DS, Hong GR, Choi C, Park YK, Lee JW, Hong HJ, Kim S, Park YW (2008) TMPRSS4 promotes invasion, migration and metastasis of human tumor cells by facilitating an epithelial-mesenchymal transition. Oncogene 27:2635–2647.

Kabir NN, Rönnstrand L, Kazi JU (2014) Keratin 19 expression correlates with poor prognosis in breast cancer. Mol Biol Rep 41:7729–7735.

Karantza V (2011) Keratins in health and cancer: More than mere epithelial cell markers. Oncogene 30:127–138.

Kebebew E, Peng M, Reiff E, Duh QY, Clark OH, McMillan A, Prinz RA, Shaha AR, McHenry CR, Chen H (2005) ECM1 and TMPRSS4 are diagnostic markers of malignant thyroid neoplasms and improve the accuracy of fine needle aspiration biopsy. Ann Surg 242:353–363.

Kim MS, Kuppireddy SV, Sakamuri S, Singal M, Getnet D, Harsha HC, Goel R, Balakrishnan L, Jacob HKC, Kashyap MK, Tankala SG, Maitra A, Iacobuzio-Donahue CA, Jaffee E, Goggins MG, Velculescu VE, Hruban RH, Pandey A (2012) Rapid characterization of candidate biomarkers for pancreatic cancer using cell microarrays (CMAs). J Proteome Res 11:5556–5563.

Kim S (2023) TMPRSS4, a type II transmembrane serine protease, as a potential therapeutic target in cancer. Exp Mol Med 55:16–724.

Klein AP (2021) Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol 18:493–502.

Liang B, Wu M, Bu Y, Zhao A, Xie F (2013) Prognostic value of TMPRSS4 expression in patients with breast cancer. Med Oncol 30.

Menz A, Bauer R, Kluth M, Marie von Bargen C, Gorbokon N, Viehweger F, Lennartz M, Völkl C, Fraune C, Uhlig R, Hube-Magg C, De Wispelaere N, Minner S, Sauter G, Kind S, Simon R, Burandt E, Clauditz T, Lebok P, … Bernreuther C (2021) Diagnostic and prognostic impact of cytokeratin 19 expression analysis in human tumors: a tissue microarray study of 13,172 tumors. Hum Pathol 115:19–36.

Ohtsuka T, Sakaguchi M, Yamamoto H, Tomida S, Takata K, Shien K, Hashida S, Miyata-Takata T, Watanabe M, Suzawa K, Soh J, Youyi C, Sato H, Namba K, Torigoe H, Tsukuda K, Yoshino T, Miyoshi S, Toyooka S (2016) Interaction of cytokeratin 19 head domain and HER2 in the cytoplasm leads to activation of HER2-Erk pathway. Sci Rep 6.

Okamoto S, Niikura H, Nakabayashi K, Hiyama K, Matoda M, Takeshima N, Watanabe M, Nagase S, Otsuki T, Yaegashi N (2013) Detection of sentinel lymph node metastases in cervical cancer: Assessment of KRT19 mRNA in the one-step nucleic acid amplification (OSNA) method. Gynecol Oncol 130:530–536.

Paiva F, Duarte-Pereira S, Costa VL, Ramalho-Carvalho J, Patrício P, Ribeiro FR, Lobo F, Oliveira J, Jerónimo C, Henrique R (2011) Functional and epigenetic characterization of the KRT19 gene in renal cell neoplasms. DNA Cell Biol 30:85–90.

Park W, Chawla A, O'Reilly EM (2021) Pancreatic Cancer: A Review. *JAMA - J Am Med Assoc* 26:851–862.

Prasad ML, Pellegata NS, Huang Y, Nagaraja HN, De La Chapelle A, Kloos RT (2005) Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19 immunohistochemistry is useful for the differential diagnosis of thyroid tumors. Mod Pathol 18:48–57.

Rogel MR, Jaitovich A, Ridge KM (2010) The role of the ubiquitin proteasome pathway in keratin intermediate filament protein degradation. Proc Am Thorac Soc 7:71–76.

Sharma P, Alsharif S, Bursch K, Parvathaneni S, Anastasakis DG, Chahine J, Fallatah A, Nicolas K, Sharma S, Hafner M, Kallakury B, Chung BM (2019) Keratin 19 regulates cell cycle pathway and sensitivity of breast cancer cells to CDK inhibitors. Sci Rep 9.

Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA: Cancer J Clin 62:10–29.

Tang F, Li W, Chen Y, Wang D, Han J, Liu D (2014) Downregulation of hnRNP K by RNAi inhibits growth of human lung carcinoma cells. Oncol Lett 7:1073–1077.

Tazawa H, Suzuki T, Saito A, Ishikawa A, Komo T, Sada H, Shimada N, Hadano N, Onoe T, Sudo T, Shimizu Y, Kuraoka K, Tashiro H (2022) Utility of TMPRSS4 as a Prognostic Biomarker and Potential Therapeutic Target in Patients with Gastric Cancer. J Gastrointest Surg 26:305–313.

Tazuma S, Sudo T, Ishikawa A, Yamaguchi A, Shibata Y, Ishida Y, Kuraoka K, Uemura K, Takahashi S, Tashiro H (2024) Effects of transmembrane serine protease 4 on the survival in patients with pancreatic ductal adenocarcinoma undergoing surgery followed by adjuvant chemotherapy. Surg Today.

Wallrapp C, Hähnel S, Müller-Pillasch F, Burghardt B, Iwamura T, Ruthenbürger M, Lerch MM, Adler G, Gress TM (2000) A novel transmembrane serine protease (TMPRSS3) overexpressed in pancreatic cancer. Canc Res 60:2602–2606.

Wood LD, Canto MI, Jaffee EM, Simeone DM (2022) Pancreatic Cancer: Pathogenesis, Screening, Diagnosis, and Treatment. Gastroenterology 163:386-402.e1

Yang CL, Song R, Hu JW, Huang JT, Li NN, Ni HH, Li YK., Zhang J, Lu Z, Zhou M, Wang JD, Li MJ, Zhan GH, Peng T, Yu HP, Qi LN, Wang QY, Xiang BDe (2024) Integrating single-cell and bulk RNA sequencing reveals CK19 + cancer stem cells and their specific SPP1 + tumor-associated macrophage niche in HBV-related hepatocellular carcinoma. Hepatol Int 18:73–90.

Yao H, Yang Z, Liu Z, Miao X, Yang L, Li D, Zou Q, Yuan Y (2017) Glypican-3 and KRT19 are markers associating with metastasis and poor prognosis of pancreatic ductal adenocarcinoma. Cancer Biomarkers 17:397–404.