ORIGINAL ARTICLE

rs10273639 C/C Genotype of *PRSS1*: How Can It Affect Management of Pancreatic Cancer?

Tianming Zhang[1], Feng Gao[1], Ling Guo[2], Yan Wang[1], Zheng Guo[3], Chengfei Zhao[4]

Department of [1]Pathology, The First Affiliated Hospital, [2]Pharmaceutical Analysis, and [3]Bioinformatics, Fujian Medical University, Fuzhou 350005, People's Republic of China

[4]Pharmaceutical and Medical Technology College, Putian University, Putian 351100, China

ABSTRACT

Background Pancreatic cancer is a rapidly progressive and usually fatal disorder that has risen to be the 6th leading cause of cancer deaths in China. Trypsin is believed to play an important role in the development of pancreatic cancer. The aim of the present study was to determine whether serum trypsin levels and cationic trypsinogen (*PRSS1*) genotypes could be served as prognostic indicators in pancreatic cancer. **Methods** A total of 140 patients with pancreatic cancer were included in this study. The serum trypsin levels were determined by enzyme linked immunosorbent assay. The genotypes of *PRSS1* gene were analyzed by polymerase chain reaction -direct sequencing. And the clinicopathologic parameters of patients were also evaluated. **Results** It showed that the median survivals of patients with high serum trypsin level (6.4 months) were significantly shorter than that who with low serum trypsin level (7.6 months; *P*=0.0192). The median survivals were also found significantly shorter in patients who had rs10273639 CC genotype of *PRSS1* (4.1 months) compared to those who had TT (8.4 months) or TC (6.6 months) genotypes (*P*=0.0058 for TT *vs.* CC, *P*=0.0377 for TC *vs.* CC). However, there were no significant differences in the median survivals between patients with TT and TC genotypes of *PRSS1*. **Conclusions** Serum trypsin levels and the rs10273639 CC genotype of *PRSS1* may be used as novel prognostic indicators in pancreatic cancer.

INTRODUCTION

Pancreatic cancer is responsible for one of the most life-threatening malignancies all over the world [1]. Although the progress of surgical and intensive inspect techniques, the outcome of pancreatic cancer remains disappointing and the overall 5-year survival rate is only 6% [2]. And it has risen to be the 6th leading cause of cancer deaths in China [3]. The poor prognosis is mainly attributed to late diagnosis, early local invasiveness and distant organs metastasis [4]. And the exact molecular mechanisms underlying pancreatic cancer remain to be clarified [5]. Therefore, it is urgent to find novel useful diagnosis and prognostic biomarkers for early diagnosis and effective treatment of pancreatic cancer [6].

The abnormal activation of trypsinogen may cause an imbalance between enzymes and antienzymes, which may result in inflammatory injury and microenvironment damage in the pancreas [7]. These changes contribute to the development of pancreatitis and cell carcinogensis [7, 8]. Trypsin can act as a signaling factor to promote the proliferation of cancer cells, and digest the matrix to accelerate the invasion and metastasis of cancer cells [9, 10]. Moreover, trypsin may also serve as a stimulator of lymphocytes [11], and play a vital role in the immune tolerance for mutated cells, which provides a selective advantage environment for the growth of cancer cells [12, 13, 14]. Therefore, the abnormal of trypsin is close related to pancreatic cancer, and may be an early diagnostic or prognostic maker for pancreatic cancer. Several studies have found that the prognosis is positively associated with the genetic background of patients [15, 16, 17]. Our previous study found that different rs10273639 genotypes in the promoter of cationic trypsinogen (*PRSS1*) gene could affect the quantity of trypsin [18], which lead to the abnormal activation of PAR-2 and subsequently changed the distribution of cell cycle and induced the onset of pancreatic cancer [19, 20]. Our previous results showed that rs10273639 genotypes of *PRSS1* gene in peripheral blood are positively correlated with the risk of pancreatic cancer [21]. Researchers also found that the prognosis of hereditary pancreatitis could be poor with the presence of *PRSS1* mutations [22, 23]. To date, most of the studies showed that the mutations of *PRSS1* were related the onset of hereditary pancreatitis [24]. However, the relation between *PRSS1* variations and the prognosis of pancreatic cancer has not yet been well established.

Received April 29th, 2017 - Accepted June 12th, 2017
Keywords Biomarkers; Pancreatic Neoplasms; Prognosis; PRSS1 protein, human; Trypsin
Correspondence Chengfei Zhao
Pharmaceutical and Medical Technology College
Putian University, Putian 351100, China
Tel +86 594 2787689
Fax +86 594 2787689
E-mail zhaochengfei8798@163.com
Zheng Guo
Department of Bioinformatics
Fujian Medical University
Fuzhou 350005, People's Republic of China
E-mail markzguo@163.com

In this study, we investigated the serum trypsin levels and rs10273639 genotypes of *PRSS1* in patients with pancreatic cancer, assessed their correlations with the clinicopathologic parameters as well as the survivals of patients.

MATERIALS AND METHODS

Study Population

A total of 140 patients with pancreatic cancer who had not undergone any form of antitumor therapy prior to admission at the 1st Affiliated Hospital of Fujian Medical University between May 2013 and January 2016 were consecutively enrolled in our study. The diagnosis of pancreatic cancer was based on the 7th edition of the American Joint Committee on Cancer (AJCC) staging system [25]. Patients who did not satisfy with the indication for operation, or did not undergo surgical treatment were excluded from the study. For the purpose of this study, the clinic-pathological parameters including age, sex, histological information laboratory parameters were also collected. Venous blood samples were obtained before surgery from each patient at the time of diagnosis for detecting trypsin level and *PRSS1* genotyping. Sera were separated immediately and stored at −80°C until trypsin enzyme-linked immunosorbent assay was done. DNA was isolated from blood samples and was kept at −20°C. The study protocol was approved by the Ethics Committee of Fujian Medical University. Written informed consents were obtained from all the patients.

Serum Trypsin Measurement

The serum trypsin was tested with ELISA kits (Human trypsin ELISA Kit; Ameko, Shanghai, China) according to the manufacturer's protocol.

DNA Isolation and *PRSS1* Genotyping

Genomic DNA was isolated from blood sample using TIANamp Blood DNA Kit (Tiangen Biotect, PR China). *PRSS1* genotypes were detected using PCR-direct sequencing, as previously described [18].

Statistical Analysis

Statistical analysis was performed using the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA). Normal variables were expressed as mean ± SD and compared by ANOVA test. Abnormal continuous variables were expressed as median (min-max) and compared by the Kruskal - Wallis H test. Comparisons were made using $\chi 2$ test for categorical variables. Survival data were analyzed by using Kaplan-Meier survival curves and log-rank test. For all tests, a two-sided P value <0.05 was considered to be statistically significant.

RESULTS

Patient Characteristics

A total of 140 patients with pancreatic cancer were recruited into this study. Their clinicopathological characteristics including age, survival and pathological types were summarized in **Table 1**.

The relationship between PRSS1 genotypes and clinicopathologic features in pancreatic cancer: There were three *PRSS1* genotypes of rs10273639 (TT, TC and CC). The correlation between these genotypes and clinicopathological features in pancreatic cancer was illustrated in **Table 2**. We found the *PRSS1 genotype* was obviously associated with the length of survival (P=0.043). However, the other clinical factors, such as age, pathological types, serum trypsin levels, liver functions, serum tumor marker levels and lipid levels, suggested no significant association with *PRSS1* genotypes. In order to further investigate the correlation between *PRSS1* genotypes and the prognoses of pancreatic cancer, Kaplan-Meier analysis and log-rank test were performed to analyze the association between *PRSS1* genotypes and 140 patients' survival. Patients with rs10273639 CC genotype had a significantly shorter overall survival than who with TT (P=0.0058) and TC genotype (P=0.0377) **(Figure 1)**, though there were no obvious differences between TT and TC carriers. These findings implicated that CC genotype of *PRSS1* gene is positive with poor outcome in pancreatic.

The relationship between serum trypsin and clinicopathologic features in pancreatic cancer: In order to evaluate the relationship between serum trypsin concentration and clinicopathological characteristics in pancreatic cancer, the patients were divided into low serum concentration (≤16.0 ng/mL) and high serum concentration (>16.0 ng/mL) groups, according to the mediun serum concentration of all the patients. As shown in **Table 3**, the serum glucose in low serum trypsin group was significant higher compared to high serum trypsin group (P=0.029). Importantly, Kaplan-Meier analysis revealed that patients with high serum trypsin concentration had a significantly worsened survival (6.4 months) than who with low serum trypsin concentration (7.6 months) (P=0.0192) **(Figure 2)**, which reflects that high serum trypsin concentration could be a valuable a prognostic indicator for pancreatic cancer. But no statistically significant differences were found in other clinical factors between the two groups.

DISCUSSION

This study was conducted to explore the relationship of trypsin and *PRSS1* genotypes with various clinical and pathological parameters as well as the prognosis of pancreatic cancer. Our findings showed that high serum trypsin level and rs10273639 CC genotype of *PRSS1* were positive related to the short survival of pancreatic

Table 1. Patient characteristics.

Clinicopathological features	Case (n=140)
Age (year)	60.61±12.13
Male (n%)	94(67.14%)
Survival (Month)	6.9(0.5-47.5)
Pathological types	
Pancreatic papillary carcinoma (n%)	4(2.86%)
Pancreatic ductal adenocarcinoma (n%)	127(90.71%)
Pancreatic neuroendocrine carcinoma (n%)	9(6.43%)

Table 2. Correlation between *PRSS1* genotypes and clinicopathologic variables in patients with PC.

Clinicopathological Features	rs10273639 genotypes of *PRSS1* gene			P value
	TT (n=72)	TC (n=60)	CC (n=8)	
Age (year)	60.75±11.69	60.35±12.75	64.13±11.47	0.707
Male (n%)	52 (72.2%)	38 (52.8%)	4 (50.0%)	0.316
Survival(Month)	8.4(0.5-36.7)	6.6(1.0-47.5)	4.1(1.8-9.0)	0.043
Pathological types				0.557
Pancreatic papillary carcinoma (n%)	4(5.6%)	0(0)	0(0)	
Pancreatic ductal adenocarcinoma (n%)	62(86.1%)	57(95.0%)	8(100%)	
Pancreatic neuroendocrine carcinoma (n%)	6(8.3%)	3(5.0%)	0(0)	
Trypsin	16.9 (0.1-105.2)	15.1(1.2-211.7)	12.6(1.7-75.7)	0.467
TCR	512.90(104.5-812.6)	520.4 (139.0-802.3)	523.2(210.0-785.4)	0.99
Liver function				
5-NT	12.3(1.8-219.0)	15.3(2.1-272.4)	15.6(2.9-91.1)	0.946
Albumin/globulin	1.37±0.36	1.34±0.30	1.33±0.23	0.839
ADA	13.40(3.00-28.00)	13.10(4.30-32.90)	14.25(8.50-34.30)	0.621
ALP	109.0(41.0-1248.0)	170.5(38.0-1442.0)	115.5(62.0-490.0)	0.748
ALT	37(4-678)	40(9-772)	73(6-321)	0.808
TBA	5.8(0.3-420.5)	6.6(0.1-330.4)	9.1(3.0-84.2)	0.551
AST	33(12-722)	42(1-514)	66(16-150)	0.769
CHE	6554±2356	5843±2657	6017±1617	0.254
GGT	67(5-1772)	113(9-6016)	219(15-1436)	0.664
Tumor markers				
CA125	20.31 (6.24-839.00)	20.37 (0.68-3339.00)	14.01 (5.26-182.50)	0.737
CA199	89.44 (0.60-2708.00)	86.50(0.60-1000)	210.25 (3.69-1000.00)	0.631
CEA	2.95 (0.51-1000.00)	3.80 (0.37-363.10)	2.11 (1.40-173.60)	0.265
AFP	3.07 (0.90-75.05)	2.56 (0.49-22.00)	2.98 (1.19-4.15)	0.161
Lipid levels				
GLU	6.40±2.16	6.56±3.17	7.39±3.92	0.621
TCHO	4.63 (1.59-11.84)	4.81 (2.12-11.34)	4.22 (3.46-9.86)	0.93
TG	1.33 (0.25-7.23)	1.25 (0.31-4.31)	1.06 (0.59-3.58)	0.622
APO-A1	0.96±0.36	0.98±0.4	1.05±0.15	0.794
APO-B	0.99±0.40	0.94±0.33	0.98±0.39	0.765
HDL-C	1.00 (0.13-2.20)	0.95 (0.09-8.07)	1.07 (0.42-1.94)	0.506
LDL-C	2.45±1.06	2.31±1.03	2.39±0.70	0.711
LP(A)	81.15 (6.40-779.50)	76.70 (7.50-649.10)	52.00 (34.40-546.90)	0.989

ADA Adenosinedeaminase; AFP α-fetoprotein; ALP Alkaline phosphatase; ALT Alanine aminotransferase; APO-A1 Apolipoprotein A1; APO-B Apolipoprotein B; AST Aspartate aminotransferase; CA125 Carbohydrate antigen 125; CA199 Carbohydrate antigen 19-9; CEA Carcino-embryonic antigen; CHE Cholinesterase; GGT Glutamyl transferase; GLU Glucose; HDL-C High density lipoprotein - cholesterol; LDL-C Low density lipoprotein – cholesterol; LP(A) Lipoprotein A; TBA Total bile acid; TCHO Total cholesterol; TCR T-cell receptor; TG Triglyceride

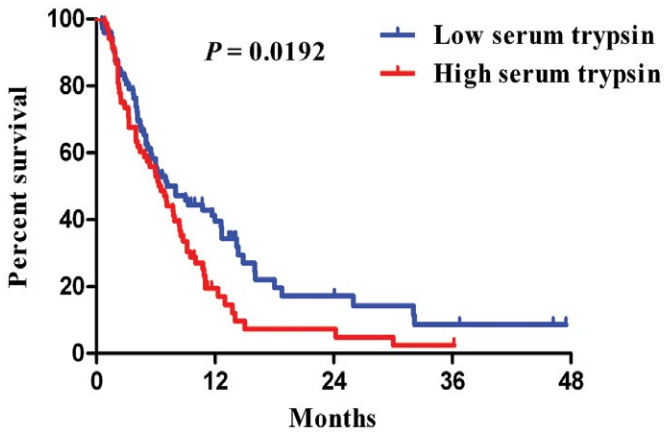

Figure 1. The relation between *PRSS1* genotypes and survival. Kaplan-Meier analysis and log-rank test were performed to analyze the association between *PRSS1* genotypes and 140 patients' survival. Patients with rs10273639 CC genotype had a significantly shorter overall survival than who with TT (P = 0.0058) and TC genotype (P = 0.0377).

cancer, which inflects that serum trypsin levels and the CC genotype of *PRSS1* may serve as novel prognostic indicators for pancreatic cancer.

Our previous study had demonstrated that the medium serum trypsin concentration in patients with pancreatic cancer was 1.68 times higher than that in healthy controls [7]. High serum trypsin not only causes an imbalance between enzymes and antienzymes, which may result in inflammatory injury and microenvironment damage in the pancreas [21] but also serves as a risk factor for pancreatic cancer, inducing pancreatic cells malignant transformation through proteinase-activated receptor-2 (PAR-2)- p-ERKI/2- proliferating cell nuclear antigen (PCNA) signal pathway [26, 27, 28]. Moreover, trypsin may digest the extracellular matrix and basement membranes, and contribute to the invasion and migration of pancreatic

Table 3. Correlation between serum trypsin and clinicopathologic features in patients with PC.

Clinicopathological features	Serum Trypsin Concentration		P value
	low (n=72)	high (n=68)	
Age (year)	59.99±12.28	61.53±11.93	0.452
Male (n%)	46 (63.9%)	50 (73.5%)	0.219
Survival (Month)	7.6(0.5-47.5)	6.4 (0.8-36.1)	0.0192
Pathological types			0.591
Pancreatic papillary carcinoma (n%)	3 (4.2%)	1 (1.5%)	
Pancreatic ductal adenocarcinoma (n%)	64 (88.9%)	63 (92.6%)	
Pancreatic neuroendocrine carcinoma (n%)	5 (6.9%)	4 (5.9%)	
TCR	514.3 (139.0-782.3)	515.6 (104.5-812.6)	0.717
Liver function			
5-NT	16.0(2.1-272.4)	10.2(1.8-241.7)	0.117
Albumin / globulin	1.37±0.34	1.34±0.31	0.549
ADA	13.50(6.80-34.30)	12.55(3.00-32.90)	0.31
ALP	142(38-1442)	116(40-1108)	0.692
ALT	40(6-772)	37(4-678)	0.429
TBA	6.1(0.3-291.7)	6.9(0.10-420.5)	0.614
AST	37(1-722)	34(12-422)	0.614
CHE	6181±2566	6249±2358	0.871
GGT	88(10-6016)	56(5-1772)	0.402
Tumor markers			
CA125	24.26 (0.68-839.00)	16.31 (3.11-3339.00)	0.137
CA199	87.00 (0.60-2708.00)	91.87(0.60-1000)	0.935
CEA	3.30 (0.51-1000.00)	2.88 (0.37-1000.00)	0.993
AFP	2.90 (0.56-75.05)	2.85 (0.49-50.37)	0.796
GLU	7.01±3.24	6.01 1.97	0.029
lipid levels			
TCHO	4.51 (1.59-11.34)	4.78 (2.2-11.84)	0.955
TG	1.25 (0.26-7.23)	1.32 (0.25-4.70)	0.703
APO-A1	0.99±0.35	0.96±0.39	0.717
APO-B	0.94±0.36	1±0.38	0.39
HDL-C	0.99 (0.09-8.07)	0.99 (0.13-2.14)	0.934
LDL-C	2.33±1.03	2.44±1.03	0.524
LP(A)	86.5 (6.40-779.5)	65.4 (10.2-649.1)	0.518

ADA Adenosinedeaminase; AFP α-fetoprotein; ALP Alkaline phosphatase; ALT Alanine aminotransferase; APO-A1 Apolipoprotein A1; APO-B Apolipoprotein B; AST Aspartate aminotransferase; CA125 Carbohydrate antigen 125; CA199 Carbohydrate antigen 19-9; CEA Carcino-embryonic antigen; CHE Cholinesterase; GGT Glutamyl transferase; GLU Glucose; HDL-C High density lipoprotein - cholesterol; LDL-C Low density lipoprotein – cholesterol; LP(A) Lipoprotein A; TBA Total bile acid; TCHO Total cholesterol; TCR T-cell receptor; TG Triglyceride

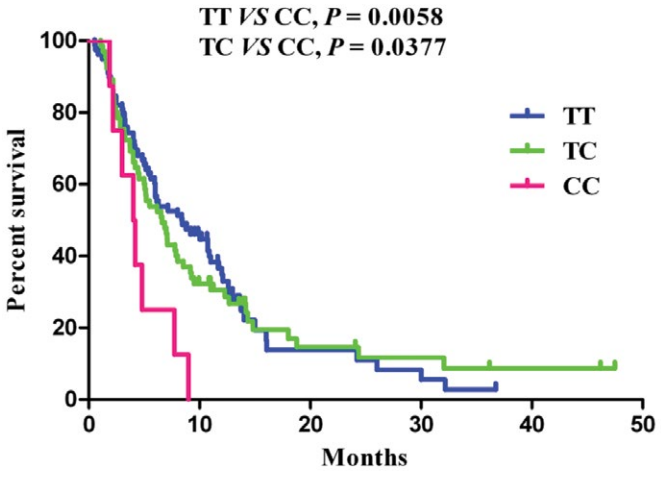

Figure 2. The relation between serum trypsin concentration and survival. Kaplan-Meier survival analysis of 140 patients with PC based on serum trypsin levels.

According to the medium of serum trypsin concentration, patients were separated into low serum concentration (≤16 .0ng/ml) and high serum concentration (＞16.0 ng/ml) groups. Patients in the high serum trypsin concentration group had a significantly shorter overall survival than who in the low serum trypsin concentration group (P = 0.0192).

cancer cells [29, 30, 31]. Therefore, the abnormal of trypsin may be related to the outcome of pancreatic cancer. Our results showed that high serum trypsin level was positive related to the short survival of pancreatic cancer, which is not only well in line with these theories, but also indicates that serum trypsin may be a useful prognostic maker for pancreatic cancer.

Serum trypsin levels may be affected by several factors including *PRSS1* variations. Our previous studies found that trypsin was highly expressed in the pancreatic tissues in patients with *PRSS1* mutation [7] and was associated with the rs10273639 genotypes in neonates with sepsis [18]. These studies showed that the genetic variations in the *PRSS1* gene may increase the transcriptional activity of promoters or enhance the stability of abnormal trypsin to increase the quantity of trypsin. But in this study, we didn't find any differences among rs10273639 TT, TC and CC genotypes. This case may be caused by different subjects and diseases. As for pancreatic cancer, the pancreatic microenvironment, especially the pancreatic cells damage, may also trigger the release of more trypsin. In this study,

we found that the serum glucose in low serum trypsin group was significant higher compared to high serum trypsin group, which may also reflect that the trypin levels positively related to the pancreatic microenvironment change. Additionally, we also found that patients with rs10273639 CC genotype had a significantly shorter survival than whom with TT and TC genotypes. Our results implicated that the CC genotype of *PRSS1* was positive with poor outcome in pancreatic cancer and may be act as a prognostic maker for pancreatic cancer.

CONCLUSION

Our results demonstrated that high serum trypsin level and the CC genotype of PRSS1 were positive related to the short survival of patients with pancreatic cancer. Thus, high serum trypsin level and rs10273639 CC genotype of PRSS1 may serve as novel prognostic indicators for pancreatic cancer.

Acknowledgments

This work was supported by a grant from the National Natural Science Foundation of China (no.81571613, no.8152442) and Joint Fund for Program of Science innovation of Fujian Province (No.2016Y9011). The authors also thank Dr. Qingquan Chen, Fujian Medical University, China, for his help with formatting the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. CA Cancer J Clin 2015; 65:5-29. [PMID: 28055103]

2. Grant RC, Selander I, Connor AA, Selvarajah S, Borgida A, Briollais L, et al. Prevalence of germline mutations in cancer predisposition genes in patients with pancreatic cancer. Gastroenterology 2015; 148:556–64. [PMID: 25479140]

3. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66:115-32. [PMID: 26808342]

4. Pang L, Zhang N, Xia Y, Wang D, Wang G, Meng X. Serum APN/CD13 as a novel diagnostic and prognostic biomarker of pancreatic cancer. Oncotarget 2016; 7:77854-77864. [PMID: 27788483]

5. Zhou L, Wang MY, Liang ZY, Zhou WX, You L, Pan BJ, et al. G-protein-coupled receptor kinase 2 in pancreatic cancer: clinicopathologic and prognostic significances. Hum Pathol 2016; 56:171-7. [PMID: 27346572]

6. Li X, Xiao Y, Fan S, Xiao M, Wang X, Zhu X, et al. Overexpression of DIXDC1 correlates with enhanced cell growth and poor prognosis in human pancreatic ductal adenocarcinoma. Hum Pathol 2016; 57:182-192. [PMID: 27498060]

7. Yi Q, Dong F, Lin L, Liu Q, Chen S, Gao F, He Q. PRSS1 mutations and the proteinase/antiproteinase imbalance in the pathogenesis of pancreatic cancer. Tumour Biol 2016; 37:5805-10. [PMID: 26546433]

8. Yu C, Merza M, Luo L, Thorlacius H. Inhibition of Ras signalling reduces neutrophil infiltration and tissue damage in severe acute pancreatitis. Eur J Pharmacol 2015; 746:245-51. [PMID: 25460024]

9. Koskensalo S, Hagstrom J, Louhimo J, Stenman UH, Haglund C. Tumour-associated trypsin inhibitor TATI is a prognostic marker in colorectal cancer. Oncology 2012; 82:234–41. [PMID: 22508321]

10. Williams SJ, Gotley DC, Antalis TM. Human trypsinogen in colorectal cancer. Int J Cancer 2001; 93:67-73. [PMID: 11391623]

11. Mohammed A, Janakiram NB, Pant S, Rao CV. Molecular targeted intervention for pancreatic cancer. Cancers (Basel) 2015; 7:1499–542. [PMID: 26266422]

12. Hunt JM, Tuder R. Alpha 1 anti-trypsin: one protein, many functions. Curr Mol Med 2012; 12:827–35. [PMID: 22697349]

13. Costello E, Greenhalf W, Neoptolemos JP. New biomarkers and targets in pancreaticcancer and their application to treatment. Nat Rev Gastroenterol Hepatol 2012; 9:435-44. [PMID: 22733351]

14. Prassas I, Eissa A, Poda G, Diamandis EP. Unleashing the therapeutic potential of human kallikrein-related serine proteases. Nat Rev Drug Discov 2015; 14:183-202. [PMID: 25698643]

15. Wu C, Miao X, Huang L, Che X, Jiang G, Yu D, et al. Genome-wide association study identifies five loci associated with susceptibility to pancreatic cancer in Chinese populations. Nat Genet 2011; 44:62-6. [PMID: 22158540]

16. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015; 518:495-50. [PMID: 25719666]

17. Whitcomb DC. Genetic aspects of pancreatitis. Annu Rev Med 2010; 61:413–424. [PMID: 20059346]

18. Chen QQ, Xue H, Chen M, Gao F, Xu J, Liu Q, et al. High Serum Trypsin Levels and the -409 T/T Genotype of PRSS1 Gene Are Susceptible to Neonatal Sepsis. Inflammation 2014; 37:1751-6. [PMID: 24777884]

19. Caruso R, Pallone F, Fina D, Gioia V, Peluso I, Caprioli F, et al. Protease-activated receptor-2 activation in gastric cancer cells promotes epidermal growth factor trans-activation and proliferation. Am J Pathol 2006; 169:268–278. [PMID: 16816379]

20. Nakanuma S, Tajima H, Okamoto K, Hayashi H, Nakagawara H, Onishi I, et al. Tumor-derived trypsin enhances proliferation of intrahepatic cholangiocarcinoma cells by activating protease-activated receptor-2. Int J Oncol 2010; 36:793-800. [PMID: 20198321]

21. Liu Q, Lin X, Liu J, Liu A, Gao F. The - 409C/T Genotype of PRSS1 Protects Against Pancreatic Cancer in the Han Chinese Population. Dig Dis Sci 2012 ; 57: 573-9. [PMID: 21922221]

22. Rebours V, Boutron-RuaultMC, Schnee M, Férec C, Maire F, Hammel P, et al. Risk of pancreatic adenocarcinoma in patients with hereditary pancreatitis: a national exhaustive series. Am J Gastroenterol 2008; 103:111–119. [PMID: 18184119]

23. Chen JM, Fe´rec C. Chronic pancreatitis: genetics and pathogenesis. Annu Rev Genomics Hum Genet 2009; 10:63–87. [PMID: 19453252]

24. Weiss FU, Simon P, Bogdanova N, Mayerle J, Dworniczak B, Horst J, et al. Complete cystic fibrosis transmembrane conductance regulator gene sequencing in patients with idiopathic chronic pancreatitis and controls. Gut 2005; 54:1456-1460. [PMID: 17539902]

25. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th Edition of the AJCC Cancer Staging Manual and the Future of TNM. Ann Surg Oncol 2010; 17:1471-4. [PMID: 20180029]

26. Tawara I, Sun Y, Lewis EC, Toubai T, Evers R, Nieves E, et al. Alpha-1-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proc Natl Acad Sci U S A 2012;109: 564–9. [PMID: 22203983]

27. Johansen D, Manjer J, Regner S, Lindkvist B. Pre-diagnostic levels of anionic trypsinogen, cationic trypsinogen, and pancreatic secretory trypsin inhibitor in relation to pancreatic cancer risk. Pancreatology 2010; 10:229–37. [PMID: 20484960]

28. Tang D, Gao J, Wang S, Yuan Z, Ye N, Chong Y, et al. Apoptosis and anergy of T cell induced by pancreatic stellate cells-derived galectin-1 in pancreatic cancer. Tumour Biol 2015; 36:5617–26. [PMID: 25725585]

29. Koskensalo S, Hagstrom J, Louhimo J, Stenman UH, Haglund C. Tumour-associated trypsin inhibitor TATI is a prognostic marker in colorectal cancer. Oncology 2012; 82:234–41. [PMID: 22508321]

30. Cenac N, Cellars L, Steinhoff M, Andrade-Gordon P, Hollenberg MD, Wallace JL, et al. Proteinase-activated receptor- 1 is an anti-inflammatory signal for colitis mediated by a type 2 immune response. Inflamm Bowel Dis 2005; 11:792–8. [PMID: 16116312]

31. Williams SJ, Gotley DC, Antalis TM. Human trypsinogen in colorectal cancer. Int J Cancer 2001; 93:67-73. [PMID: 11391623]