Journal of Genetic Syndromes & Gene Therapy
ISSN: 2157-7412

Qing-quan et al., J Genet Syndr Gene Ther 2013, 4:11
DOI: 10.4172/2157-7412.1000200

Review Article Open Access

Trypsin-antitrypsin Imbalance in Immune Escape and Clonal Proliferation of Pancreatic Cancer

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Abstract

So far it has been failed to confirm whether trypsinogen (PRSS1) besides its homologous genes and *TCRVß* gene which are co-localized is a gene linkage, though the process of trypsinogen activation has been loaded into textbooks as a classical theory. At present, researchers mainly focuse on *PRSS1/PRSS2* genes mutations themselves without considering the particularities of genes structures and changes, therefore, cannot explain the immune mechanism of pancreatic cancer. It has been verified that trypsin can serve as a signal factor in stimulating tumor cells proliferation and destroying matrix to promote cell transfer and as a stimulator of lymphocytes to combat with its inhibitor (α1-antitrypsin, α1-AT/AAT), so to play an important role in tumor immune surveillance. Therefore, in order to illuminate the pancreatic cancer immune escape, it is necessary to clarify the correlative spatiotemporal expression of *PRSS1/PRSS2/AAT* genes. It may provide a theoretical basis for *TCRVß* gene modification or *AAT* gene treatment of pancreatic cancer to expound the process of trypsinogen gene mutation or abnormal activation leading to *AAT* gene's over expression which induced body immune tolerance to cause mutant cells immune escape and clonal proliferation.

Keywords: Trypsinogen; α1- antitrypsin; Gene co-localization; Micro environment; Pancreatic cancer; Immune escape; Clonal proliferation

Introduction

Various gene mutations are closely associated with pancreatic cancer. K-ras gene mutation is commonly restricted to codon 12 or 13, so it is regarded as a 'signature' of pancreatic cancer [1-3]. Thus, Computed tomography (CT)-guided pancreatic puncture is required and it was difficult to carry out in clinical routine because of its costs and the risks of tumor peritoneal or pancrea leakage. Most of the reported results from exon sequencing technology is base of the study of cancer tissue samples, that it is concerned about the variation of the tumor cells themselves; but it was elaborated in this paper is due to the germline mutations caused enzyme expression abnormalities and cause tumors. It has been identified that mutations (p.R122H, p.A121T, p.C139S and p.T137M) or rs10273639 T/T of *PRSS1* gene were high risk factors of pancreatic cancer, and the clinical outcomes in patients were closely related to the serum level of trypsin. What is more, the mutations of *PRSS1* gene can be found in the exfoliated cells. it is servers as a noninvasive method and offers the more humanistic care to the potential of pancreatic cancer patients [4-9].

Trypsinogen gene (*PRSS1*) located in the 7q35 has been proven to be a pathogenic gene of hereditary pancreatitis for nearly 20 years [4-6]. For a long time trypsin has been only used as a tool of biomedical research, while how the balance between trypsin and AAT will influence the cellular microenvironment has rarely been studied. So far it remains unclear that how the co-localization of *TCRVß* gene with *PRSS1, PRSS2* and their homologous genes [5-7] (Figure 1) affect the maturation and function of lymphocytes. The relationship between trypsin, α1-antitypsin (AAT) and *TCRVß* genes' spatiotemporal expression and tumor besides tumor lymphatic invasion has not been clear [9-11]. Problems such as how *PRSS1* gene mutations affect calcium binding loop or autolysis loop and the auto activation mechanism of trypsinogen being triggered are still

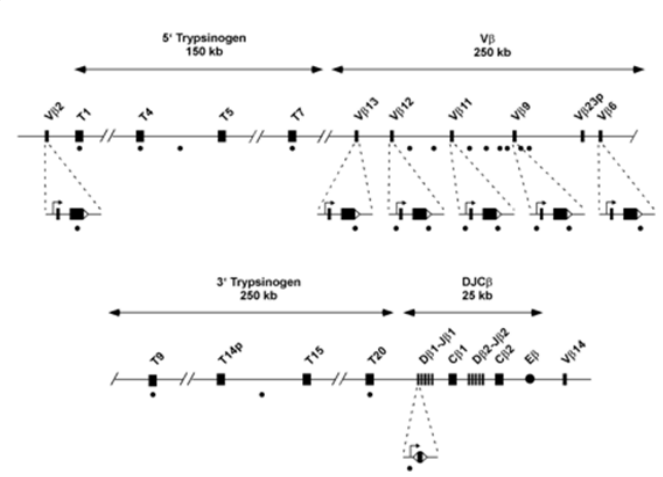

Figure 1: Location Map of Trypsinogen Homologous Genes and *TCR Vß* Gene [6].

Trypsin gene families T1 ~ T20 are mosaic arrangement with *TCR Vß* gene family. Of trypsin gene families, only T4, T8 have functions and named *PRSS1* and *PRSS2* respectively, and the others are silent in the long river of human evolution.

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Received April 30, 2013; **Accepted** October 14, 2013; **Published** October 24, 2013

Citation: Qing-quan C, Feng G, Ze-hao Z, Zhi-bo Z, Qi-cai L, et al. (2013) Trypsin-antitrypsin Imbalance in Immune Escape and Clonal Proliferation of Pancreatic Cancer. J Genet Syndr Gene Ther 4: 200. doi:10.4172/2157-7412.1000200

intense controversial issues [12]. Therefore, the abnormal quality and quantity of trypsin and AAT in the pathogenesis of pancreatic cancer is not following the classic mode that traditional disease-causing gene mutations which lead to coding protein variation, which has been hot research point of the pancreas epidemiological and biological engineering.

A-1 anti-trypsin (AAT) is the most abundant circulating serine protease inhibitor and an acute phase reactant. Systemic deficiency in AAT due to genetic mutations can result in liver failure and chronic lung disease such as emphysema. Evidence now suggests that AAT plays an important role in modulating immunity, inflammation, proteostasis, apoptosis, and possibly cellular senescence programs [12]. Trypsinogen genes in mammals and birds constitute a multigene family whose members are found within the beta T cell receptor (TCRβ) locus. In the fully characterized TCRβ loci of human and mouse, clusters of trypsinogen (T) or trypsinogen-like (TL) genes flank a region spanning hundreds of kilobases containing the TCRβ variable gene segments. Analysis of the human TCRβ locus on 7q35 revealed genes coding for the functional trypsinogen proteins PRSS1 and PRSS2, also known, respectively, as the cationic and anionic trypsinogens. The 3.6-kb five-exon genes coding for PRSS1 and PRSS2 are embedded within the first and last units, respectively, of a tandem array of five 10.6-kbduplication units located near the 3' end of the TCRβ locus.

Micro Environmental Destruction and Pancreatic Cancer

The occurrence of pancreatic cancer can be concluded that changes of microenvironment cause gene mutations following with immune tolerance and clonal proliferation, and abnormal activation of trypsinogen leads to less or over activation of other zymogens and destroys the microenvironment of pancreatic cells, together with gene mutations such as k–ras, causing the formation of early tumor cells. Subsequently, trypsin serves as a signal factor in stimulating tumor cells proliferation and destroying matrix to promote cell transfer [13-15] and as a stimulator of lymphocytes to combat with AAT, so to play an important role in mutant cells immune tolerance. This process also conferred cells advantageous selection and made the pancreatic tumor cells become dominant in cell populations. Tumors become more malignant and grow more quickly [16-18] through clonal selection (Figure 2).

New Interpretation of Enzymes and Anti-enzyme Imbalance Causing Tumors

Pancreatic secretory trypsin inhibitor (PSTI) also known as serine protease inhibitor Kazal-type 1 (SPINK1) or tumor-associated trypsin inhibitor (TATI) (Account for 20%) and *AAT* (Account for about 80%) can antagonize against the activity of trypsin in the physiological state, but over expression of AAT can cause immune tolerance of mutant cells [19-24]. Therefore, it is necessary to analysis the relation between chain expression of *PRSS1, PRSS2, AAT* besides *TCRVβ* and the occurrence, invasion and clinical outcome of pancreatic cancer. Understanding the effect of space-time chain expression of *PRSS1, PRSS2, AAT* and *TCRVβ* on transcriptional activation of 7q35 regional genes is an effective ways of clearing the effecter genes and cells of abnormal quality and quantity of PRSS1 / PRSS2 enzyme. But, there are no reports on the systemic research of these two relationships.

There are exogenous and endogenous proteases *in vivo*, such as bacterial toxins and white blood cells disintegration protease, which can damage the microenvironment of liver, pancreas and other organs.

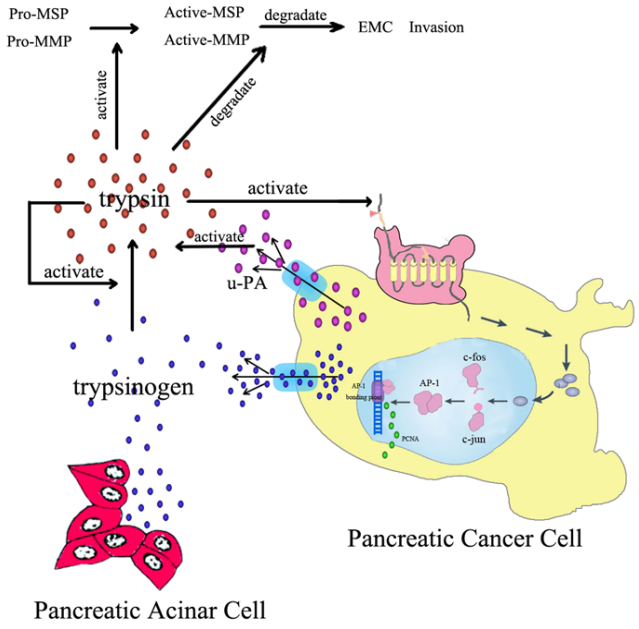

Figure 2: Trypsinogen Activation and the Mechanism of Pancreatic Cancer Occurrence and Invasion [19] .

But these enzymes can be inhibited by AAT to maintain the integrity of tissue cells. In addition, AAT also can regulate immune response, affect the removal of antigen-antibody immune complex, and activate the complement and inflammatory response [25-29]. AAT can be synthesized by mononuclear cells, alveolar macrophages and epithelial cells and those synthesized by extrahepatic cells play an important role in the regulation of local tissue injury. Our previous work has demonstrated that trypsin can degrade extracellular matrix and basement membrane to promote the occurrence, invasion and metastasis of pancreatic cancer [15,17,21]. The principle of trypsin induces normal cells turning into cancerous ones is that; trypsin activates cell surface transmembrane receptor PAR-2 which promotes the formation of p-ERKI/2. And p-ERKI/2 leads to the increases of p-ERK going into nucleus, which raises the expression of p-ERK downstream factor c-fos, then promotes c-fos and c-jun to dimerize into AP-1. Binding to the AP-1 site of PCNA gene, AP-1 regulates the mRNA of PCNA gene on the transcription affected the synthesis of PCNA protein, and then induces carcinogenesis. Corresponding research results were published in Digestive Diseases & Science and Chinese Medical Journal [30,31]. Meanwhile, it also has been confirmed that the theory of AAT compensatory increase is to neutralize and inhibit the activation of trypsin and other proteolytic enzymes so to inhibit the proliferation and spread of tumor cells [23-27]. And another hot issue is that trypsin is a kind of lymphocyte stimulation and its inhibitor-AAT has the function of immune suppression, and the confrontation of them plays an important role in immune surveillance of mutant cells. Recently, our research group also has confirmed that the abnormal activation of trypsinogen led to immune abnormalities. Mutation p.T81M of *PRSS1* gene was found in autoimmune pancreatitis family as a pathogenic gene, which causes trypsinogen ectopic activation and results in all trypsin expressed organs injury. So far, this is a relative direct evidence to support that trypsinogen gene mutations are correlated with abnormality of immune system. And the results of the study have been published in World J Gastroenterology [32] (Figure 3).

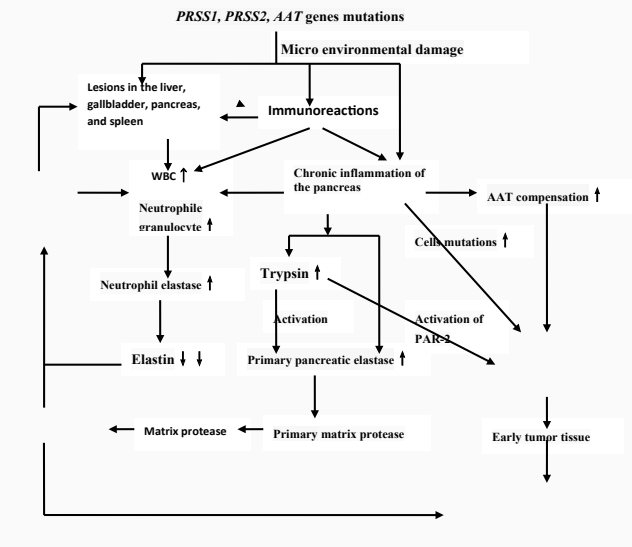

Figure 3: Mechanism of Trypsin and Trypsin Imbalance Leads to Multiple Organs Lesions and Pancreatic Cancer.

Trypsin and Pancreatic Cancer

It has been identified that hot mutation spots (p.R122H, p.A121T, p.C139S and p.T137M) of *PRSS1* gene were high risk factors of pancreatic cancer, and the clinical outcomes in patients were closely related to the serum level of trypsin [18,20]. Besides, trypsin is a highly specific expression in the tissue of the pancreas (the expression of trypsin in pancreas is 1000 times more than other tissues). Visibly, the abnormal quality and quantity of trypsin caused by trypsinogen gene mutations plays a significant role in the occurrence, invasion and metastasis of pancreatic cancer through trypsin and PAR-2 signal pathway [33-36].

Prospect of Gene Therapy for Pancreatic Cancer

The body's anti-tumor immunity is mainly mediated by activated T lymphocytes, and the confrontation of trypsin and AAT plays an important role in tumor immunity. Therefore, basing on the relationship between mutations of *PRSS1, K-ras* besides other genes and the pancreatic cancer, it can lay foundations of early diagnosis and treatment for pancreatic cancer to solve practical problems of looking for specific genotypes of trypsinogen and AAT, which chained with its pathogenesis and the balance of trypsin and antitrypsin.

Acknowledgments

Financial supported by the project of Outstanding Youth Foundation of Fujian Provincial Higher Education (JA12133), National Natural Science Foundation of China (81201362, 81201590, 21275028), Fujian Medical Innovations (2012-CXB-21), Foundation of Fujian Education Department (JA12143), National High Technology Investigation Project Foundation of China (2012AA022604) and Natural Science Foundation of Fujian Province (2013J01302).

References

1. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, et al. (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature 496: 101-105.
2. Cogoi S, Paramasivam M, Filichev V, Géci I, Pedersen EB, et al. (2009) Identification of a new G-quadruplex motif in the KRAS promoter and design of pyrene-modified G4-decoys with antiproliferative activity in pancreatic cancer cells. J Med Chem 52: 564-568.
3. Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, et al. (2012) Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell 149: 656-670.
4. Whitcomb DC, LaRusch J, Krasinskas AM, Klei L, Smith JP, et al. (2012) Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. Nat Genet 44: 1349-1354.
5. Rowen L, Koop BF, Hood L (1996) The complete 685-kilobase DNA sequence of the human beta T cell receptor locus. Science 272: 1755-1762.
6. Tripathi R, Jackson A, Krangel MS (2002) A change in the structure of Vbeta chromatin associated with TCR beta allelic exclusion. J Immunol 168: 2316-2324.
7. Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, et al. (1996) Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 14: 141-145.
8. Lamontagne J, Pinkerton M, Block TM, Lu X (2010) Hepatitis B and hepatitis C virus replication upregulates serine protease inhibitor Kazal, results in cellular resistance to serine protease-dependent apoptosis. J Virol 84: 907-917.
9. Johansen D, Manjer J, Regner S, Lindkvist B (2010) Pre-diagnostic levels of anionic trypsinogen, cationic trypsinogen, and pancreatic secretory trypsin inhibitor in relation to pancreatic cancer risk. Pancreatology 10: 229-237.
10. Gao J, Zhu F, Lv S, Li Z, Ling Z, et al. (2010) Identification of pancreatic juice proteins as biomarkers of pancreatic cancer. Oncol Rep 23: 1683-1692.
11. Itkonen O (2010) Human trypsinogens in the pancreas and in cancer. Scand J Clin Lab Invest 70: 136-143.
12. Hunt JM, Tuder R (2012) Alpha 1 anti-trypsin: one protein, many functions. Curr Mol Med 12: 827-835.
13. Hanas JS, Hocker JR, Cheung JY, Larabee JL, Lerner MR, et al. (2008) Biomarker identification in human pancreatic cancer sera. Pancreas 36: 61-69.
14. Gouyer V, Fontaine D, Dumont P, de Wever O, Fontayne-Devaud H, et al. (2008) Autocrine induction of invasion and metastasis by tumor-associated trypsin inhibitor in human colon cancer cells. Oncogene 27: 4024-4033.
15. Jiang G, Cao F, Ren G, Gao D, Bhakta V, et al. (2010) PRSS3 promotes tumour growth and metastasis of human pancreatic cancer. Gut 59: 1535-1544.
16. Ji B, Gaiser S, Chen X, Ernst SA, Logsdon CD (2009) Intracellular trypsin induces pancreatic acinar cell death but not NF-kappaB activation. J Biol Chem 284: 17488-17498.
17. Gaber A, Nodin B, Hotakainen K, Nilsson E, Stenman UH, et al. (2010) Increased serum levels of tumour-associated trypsin inhibitor independently predict a poor prognosis in colorectal cancer patients. BMC Cancer 10: 498.
18. Koskensalo S, Hagström J, Louhimo J, Stenman UH, Haglund C (2012) Tumour-associated trypsin inhibitor TATI is a prognostic marker in colorectal cancer. Oncology 82: 234-241.
19. Yamashita K, Mimori K, Inoue H, Mori M, Sidransky D (2003) A tumor-suppressive role for trypsin in human cancer progression. Cancer Res 63: 6575-6578.
20. Gaber A, Johansson M, Stenman UH, Hotakainen K, Pontén F, et al. (2009) High expression of tumour-associated trypsin inhibitor correlates with liver metastasis and poor prognosis in colorectal cancer. Br J Cancer 100: 1540-1548.
21. Itkonen O (2010) Human trypsinogens in the pancreas and in cancer. Scand J Clin Lab Invest 70: 136-143.
22. Koskensalo S, Hagström J, Louhimo J, Stenman UH, Haglund C (2012) Tumour-associated trypsin inhibitor TATI is a prognostic marker in colorectal cancer. Oncology 82: 234-241.
23. Pardo J, Aguilo JI, Anel A, Martin P, Joeckel L, et al. (2009) The biology of cytotoxic cell granule exocytosis pathway: granzymes have evolved to induce cell death and inflammation. Microbes Infect 11: 452-459.
24. Chen N, Zou J, Wang S, Ye Y, Huang Y, et al. (2009) Designing protease sensors for real-time imaging of trypsin activation in pancreatic cancer cells. Biochemistry 48: 3519-3526.
25. Tawara I, Sun Y, Lewis EC, Toubai T, Evers R, et al. (2012) Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proc Natl Acad Sci U S A 109: 564-569.
26. Solakidi S, Dessypris A, Stathopoulos GP, Androulakis G, Sekeris CE (2004)

Tumour-associated trypsin inhibitor, carcinoembryonic antigen and acute-phase reactant proteins CRP and alpha1-antitrypsin in patients with gastrointestinal malignancies. Clin Biochem 37: 56-60.

27. Stenman UH (2002) Tumor-associated trypsin inhibitor. Clin Chem 48: 1206-1209.

28. Malecki M (2012) Frontiers in Suicide Gene Therapy of Cancer. J Genet Syndr Gene Ther 2012.

29. Cullen SP, Brunet M, Martin SJ (2010) Granzymes in cancer and immunity. Cell Death Differ 17: 616-623.

30. Liu Q, Lin X, Liu J, Liu A, Gao F (2012) The -409 C/T genotype of PRSS1 protects against pancreatic cancer in the Han Chinese population. Dig Dis Sci 57: 573-579.

31. Liu QC, Gao F, Ou QS, Zhuang ZH, Lin SR, et al. (2008) Novel mutation and polymorphism of PRSS1 gene in the Chinese patients with hereditary pancreatitis and chronic pancreatitis. Chin Med J (Engl) 121: 108-111.

32. Gao F, Li YM, Hong GL, Xu ZF, Liu QC, et al. (2013) PRSS1_p.Leu81Met mutation results in autoimmune pancreatitis. World J Gastroenterol 19: 3332-3338.

33. Kereszturi E, Sahin-Tóth M (2009) Intracellular autoactivation of human cationic trypsinogen mutants causes reduced trypsinogen secretion and acinar cell death. J Biol Chem 284: 33392-33399.

34. Kume K, Masamune A, Takagi Y, Kikuta K, Watanabe T, et al. (2009) A loss-of-function p.G191R variant in the anionic trypsinogen (PRSS2) gene in Japanese patients with pancreatic disorders. Gut 58: 820-824.

35. Olbe L (2007) Strong activation of PAR-2 receptors: a common trigger for the development of gastrointestinal adenocarcinomas? Scand J Gastroenterol 42: 1133-1137.

36. Ikeda O, Egami H, Ishiko T, Ishikawa S, Kamohara H, et al. (2006) Signal of proteinase-activated receptor-2 contributes to highly malignant potential of human pancreatic cancer by up-regulation of interleukin-8 release. Int J Oncol 28: 939-946.

This article was originally published in a special issue, **Suicide Gene Therapy of Cancer** handled by Editor(s). Dr. Marek Malecki, Genetic and Biomolecular Engineering, PBMEF, USA