

A Brief Review of SPINK1 Studies

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Abstract: SPINK1, a trypsin inhibitor secreted by the pancreas, is closely associated with the development of pancreatitis. Additionally, SPINK1 exhibits multiple biological functions, acting as a trypsin inhibitor, a growth factor-like protein, and a negative regulator of autophagy. This paper reviews the role of the SPINK1 gene and its variants in pancreatitis development, its expression as TATI in cancer, and its role in oncogenesis.

Keywords: Human Serine Protease Inhibitor; Kazal Type 1; Inhibitor; Pancreatitis.

1. Summary of SPINK1:

The serine protease inhibitor Kazal type 1 (SPINK1) is a prominent member of the Kazal family of serine protease inhibitors and is expressed by pancreatic mucous cells. It is secreted by these cells and inhibits trypsin and other serine proteases[1]. The human gene is located on chromosome 5, consisting of four exons, and encodes a 56-amino acid protein[3]. The mature peptide comprises 56 amino acids, with a reactive site at position 18, which serves as a specific target substrate for trypsin[4]. SPINK1's primary function is to maintain the integrity of the digestive tract mucosa. It achieves this by inhibiting the premature activation of pancreatic trypsin, thereby preventing excessive digestion of the gastrointestinal (GI) mucosa by trypsin[2,5]. Thus, SPINK1 is closely related to gastrointestinal diseases. SPINK1 has diverse biological functions and its expression has been detected in various cancer tissues, including pancreatic, bladder, breast, liver, and colon cancers.

2. SPINK1 Gene Mutations and Pancreatitis:

Recent advancements in clinical genetics and extensive genetic testing in the general population have significantly enhanced our understanding of the inflammatory processes in the pancreas, particularly the etiology of pancreatitis. The SPINK1, which regulates trypsin activation in the pancreas to safeguard the digestive tract mucosa, is deemed a crucial inhibitor of trypsin activation. Mutations in the SPINK1 gene could alter its function, potentially leading to the onset of pancreatitis[6]. Specifically, mutations in the SPINK-1 gene elevate the risk of developing chronic pancreatitis (CP) twelve-fold[7].

To date, over 30 mutations in the SPINK1 gene have been identified, with the p.N34S variant being the most commonly reported. Witt et al.[8] initially reported the link between SPINK1 and pancreatitis, observing that the p.N34S variant was present in 21% of children with idiopathic pancreatitis. Since then, there has been a steady rise in clinical reports associating SPINK1 gene mutations with pancreatitis. For instance, Pfützner RH et al. [9] conducted a genetic linkage study involving five familial pancreatitis families and sequenced the full SPINK1 gene from DNA samples of 112 familial pancreatitis patients and 95 controls. Their findings suggested that SPINK1 mutations are disease modifiers, potentially increasing susceptibility to pancreatitis via genetic

or environmental factors, but are not intrinsically pathogenic. A study[10] by Threadgold et al. indicated that the N34S mutation in the SPINK1 gene is linked to a familial pattern of idiopathic chronic pancreatitis (ICP) but is not causative. A descriptive study involving pancreatic tissue sections from 28 patients with SPINK1-associated CP characterized SPINK1-associated chronic pancreatitis by parenchymal fibrosis, indicating a distinct pathophysiological mechanism compared to PRSS1- and CFTR-associated[11] pancreatitis. In a study of 134 patients with acute recurrent pancreatitis (ARP) or CP from the United States, 88 pathogenic gene variants were identified, including SPINK1, PRSS1, CFTR, and [12]CTRC. A genome-wide association studies (GWAS) meta-analysis of acute pancreatitis (AP) identified three known (ABCG5/G8, SPINK1, and PRSS2) and two potentially novel AP-susceptibility loci (TWIST2 and MORC4)[13].

To investigate the association between SPINK1 gene variants and alcoholic chronic pancreatitis, Atsushi Masamune and Kiyoshi Kume[14] et al. conducted polymerase chain reaction amplification on genomic DNA from 96 pancreatitis patients and 165 healthy controls in Japan. This process aimed to acquire all exons and promoter regions of the SPINK1 gene for direct sequencing. The corresponding SPINK1 gene data were also gathered by reviewing the patients' clinical courses. The findings revealed a higher prevalence of [N34S; IVS1-37T>C] and [-215G>A; IVS3+2T>C] mutations in familial CP (55.6% and 11.1%, respectively) and idiopathic CP (15.2% and 18.1%, respectively) compared to controls (0.6% and 0%, respectively). The mutation rates of SPINK1 N34S and IVS3+2T>C genes in patients with alcoholic chronic pancreatitis were 0% and 4.5%, respectively (P<0.05). SPINK1 mutations were correlated with idiopathic and familial chronic pancreatitis, but their role in alcoholic chronic pancreatitis appeared less clear.

In conclusion, the N34S mutation in the SPINK1 gene exhibited a strong association with ICP, while its association with alcoholic pancreatitis was significantly less pronounced compared to ICP. Patients with the N34S mutation in the SPINK1 gene experienced a more severe clinical course, suggesting that clinical risk[14] assessment is valuable for identifying susceptibility to chronic pancreatitis and assessing the risk of alcoholic chronic pancreatitis. Genetic risk assessment can aid in pinpointing individuals at risk of severe CP, enabling targeted therapeutic or preventive measures to either avert the disease or decelerate its progression. While the mechanisms leading to pancreatitis remain elusive,

SPINK1 variants are implicated in the disease's development, playing a contributory role.

3. Role of SPINK1 in Cancer

The SPINK1, also known as the tumor-associated trypsin inhibitor (TATI), inhibits trypsin and other serine proteases and is found in various tissues. TATI was first identified in the urine of ovarian cancer patients in 1982 by U.H. Stenman, M.L.[15] Huhtala et al. That same year, it was discovered that pancreatic secretory trypsin inhibitor (PSTI) is identical to TATI. TATI expression has been detected in several cancer tissues, such as bladder, kidney, pancreas, colorectal, prostate, lung, breast, and liver cancers, and elevated expression levels may correlate with a poor prognosis[16].

The relationship between SPINK1/TATI and tumorigenesis has garnered attention as studies delve deeper into its association with tumor development and diagnosis. In prostate cancer (PCa) patients, SPINK1 over-expression and ETS gene fusion occur independently, with SPINK1 over-expression indicating a poorer clinical outcome. The 22RV1 cell line, known for its invasive nature, serves as a model for aberrant[17] SPINK1 expression. SPINK1 has been linked to the prognosis of castration-resistant PCa, with one study revealing a significant association between SPINK1 and biochemical recurrence (BCR), CRPC-disease-free survival, and overall survival[18]. Androgen-deprivation therapy (ADT) is a prominent systemic palliative treatment for prostate cancer (PCa)[19]. Post-ADT treatment, the inhibition of the invasive androgen receptor (AR) is lifted, leading to increased SPINK1 transcription and expression, potentially inducing epithelial-mesenchymal transition, stemness, and [20]cellular plasticity.

SPINK1 has been demonstrated to bear structural similarity to epidermal growth factor (EGF). Structural analyses reveal that SPINK1 shares 50% amino acid sequence homology with EGF and interacts with the epidermal growth factor receptor[21] (EGFR). EGFR can be aberrantly activated through various mechanisms, such as receptor over-expression, mutation, ligand-dependent receptor dimerization, and ligand-independent activation. It is implicated in the development of numerous human tumors and has become a prominent target for cancer[22] treatment. The growth factor-like function of SPINK1 is hypothesized to be associated with this property. A study by Ateeq B. et al. showed that SPINK1 mediates some of its tumorigenic effects by interacting with EGFR. Treatment of mice bearing 22RV1 xenografts with either SPINK1 or EGFR antibodies (cetuximab) resulted in tumor growth reductions by over 60% and 40%, respectively. When administered concurrently, tumor growth was inhibited by approximately 75% without impacting the growth of PC3 xenografts (SPINK1-/ETS-). This suggests that SPINK1 may be a potential therapeutic target for SPINK1+/ETS-prostate cancer[23] patients.

4. Conclusion and Outlook

In conclusion, while research into the relationship between SPINK1 and pancreatitis is still in its early stages, a link is evident.

Further investigation is required to determine whether specific mutations in the SPINK1 gene are invariably linked to various forms of pancreatitis and to understand the underlying mechanisms. Exploring the association between SPINK1 and cancer may offer novel insights and approaches

for cancer treatment. While SPINK1 could potentially serve as a therapeutic target, the complexity and multifaceted nature of its functions need to be considered. The exact role of SPINK1 in cancer development remains to be elucidated. It is postulated that SPINK1's growth factor function is structurally like EGF and that it can interact with EGFR. The potential of SPINK1/TATI as a diagnostic marker for various cancers warrants further exploration. Advances in testing technologies and methodologies may solidify the position of SPINK1/TATI in the diagnostic criteria for cancer.

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