Emerging Roles of SPINK1 in Cancer
Kati Räsänen,1 Outi Itkonen,1,2 Hannu Koistinen,1 and Ulf-Håkan Stenman1,2*

BACKGROUND: Tumor-associated trypsin inhibitor (TATI) was originally isolated from the urine of a patient with ovarian cancer. It was later shown to be produced by many other tumors and several normal tissues. It had earlier been isolated from the pancreas and was hence called pancreatic secretory trypsin inhibitor (PSTI). It belongs to a family of protease inhibitors presently called serine peptidase inhibitor Kazal type (SPINK). In the SPINK family TATI/PSTI is SPINK1, which is the name used in this review.

CONTENT: In addition to being a protease inhibitor, SPINK1 also acts as an acute-phase reactant and a growth factor. Furthermore, it has been shown to modulate apoptosis. Overexpression of SPINK1 predicts an unfavorable outcome in several cancers and determination of SPINK1 in serum can be used to identify patients at increased risk of aggressive disease. Thus serum SPINK1 can be used as a prognostic tumor marker. Because SPINK1 acts as a growth factor and an inhibitor of apoptosis in some cancers, it has also been suggested that it can be a therapeutic target in cancer. However, because SPINK1 is the major physiological inhibitor of trypsin, inhibition of SPINK1 may increase the risk of pancreatitis.

SUMMARY: Taking into account the many functions of SPINK1, assessing the role of SPINK1 in cancer has several potentially important clinical applications ranging from a biomarker to a potential new target for cancer therapy.

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Tumor-associated trypsin inhibitor (TATI), originally identified in urine from patients with ovarian cancer, was later shown to be identical to pancreatic secretory trypsin inhibitor (PSTI). PSTI was originally purified from bovine pancreatic tissue by Kazal et al. and was therefore also called the Kazal inhibitor. TATI/PSTI belongs to the family of serine peptidase inhibitors Kazal type (SPINK). Within this family, TATI/PSTI is SPINK1, which is the official name for the gene and thus the name used in this review. The serine peptidase inhibitor, Kazal type 1 (SPINK1) gene is located in chromosomal region 5q32. It encodes a 79 amino acid peptide that includes a 23 amino acid signal peptide. Mature SPINK1 consists of 56 amino acids and has 3 disulfide bonds and a molecular weight of 6242 Da (1).

Physiologically SPINK1 is secreted by pancreatic acinar cells serving as a first line of defense against premature trypsinogen activation in the acini and the pancreatic ducts. SPINK1 is an efficient inhibitor of (cationic) trypsin-1 and (anionic) trypsin-2, but not of trypsin-3 (mesotrypsin) (2). However, the inhibition is reversible and during prolonged incubation SPINK1 is gradually degraded by cleavage of the peptide bonds between Arg42-Lys43 and Arg44-Glu45, resulting in dissociation of the protease inhibitor complex. In addition to trypsin, SPINK1 inhibits plasmin, urokinase type plasminogen activator, and acrosin, but the inhibition of the first 2 is much less efficient than that of trypsin. The inhibition of acrosin is similar to that of trypsin (3).

Serum concentrations of SPINK1 are markedly increased in patients with acute pancreatitis (4). This is most likely mainly caused by leakage from the pancreas, but SPINK1 may also behave as an acute-phase reactant that is probably produced by the liver (1). Supporting this, a 40-bp interleukin 6–responsive element that is conserved among various genes for acute-phase proteins has been found in the SPINK1 gene and is functional in hepatoma (5) and colorectal cancer cells (our own unpublished data).

Comparison of the amino acid sequences of SPINK1 and epidermal growth factor (EGF) revealed similarity (6), and on the basis of this finding it has been proposed that SPINK1 may function as an autocrine or paracrine growth factor. Early studies showed that SPINK1 stimulates the growth of human fibroblasts and human endothelial cells (7, 8), and the rat ortholog of transition; HCC, hepatocellular carcinoma; CRC, colorectal carcinoma; RCC, renal cell carcinoma; TR-FIKA, time-resolved immunofluorometric assay.
SPINK1 competed with rat EGF for binding to the EGF receptor (EGFR) in murine NIH 3T3 cells (9). However, binding of human SPINK1 to mouse NIH 3T3 fibroblasts was not inhibited by human EGF (7). This may be explained by differences in SPINK1 binding to EGF receptors from different species. These contradicting results may also be due to the complex regulation of EGFR signaling. After ligand binding to the extracellular domain, EGF receptors form homo- or heterodimers and become phosphorylated in several tyrosine residues residing in the cytoplasmic domain and are finally internalized (10). Therefore, it is possible that the reported differences between EGF and SPINK1 stimulation arise from their ability to induce different members of the EGF receptor family to form heterodimers. Findings regarding SPINK1–EGFR interaction in pancreatic, bladder, and prostate cancer are detailed in their respective sections below.

Inhibition of prematurely activated trypsin in the pancreas is thought to be the main function of SPINK1. In this review, we highlight recent evidence indicating that SPINK1 also exerts other functions, i.e., stimulation or inhibition of cancer growth, inhibition of apoptosis, and action as an acute-phase reactant.

SPINK1 in Cancer

Since its original characterization as a secreted factor in the urine of ovarian cancer patients, expression of SPINK1 in other cancers has been widely studied and found to be present in serum, urine, and tumor tissues. The expression levels of SPINK1 mRNA in normal and cancerous tissues are presented in Fig. 1. Under normal conditions, SPINK1 is expressed by liver, pancreas, colon, and other gastrointestinal (GI) organs. In cancer, increased expression of SPINK1 is observed in GI tumors and also in cancers of the lung, bladder, kidney, prostate, testis, ovary, cervix, and breast. Below we review the current literature of the role of SPINK1 in various types of cancer. The information is summarized in Table 1.

OVARIAN CANCER

Very high SPINK1 concentrations have been measured in the cyst fluid of patients with ovarian tumors. Interestingly, this has been shown to be associated with similar molar concentrations of tumor-associated trypsinogen-1 and -2 (11), which led to the assumption that SPINK1 plays a similar protective role against trypsinogen activation in ovarian tumors as it does in the pancreas (12). Early studies showed that SPINK1 in urine is a promising marker for diagnosis of ovarian cancer (13, 14). As a diagnostic marker for ovarian cancer, serum SPINK1 was shown to be inferior to cancer antigen 125 (CA125) but superior as a prognostic marker (15). CA125 is the most widely studied marker for ovarian cancer. However, CA125-based screening for ovarian cancer is not currently recommended as its concentrations can also be increased in other pathological and physiological conditions, like endometriosis and menstruation, respectively. Furthermore, not all ovarian cancers express CA125. In a study comparing SPINK1 with several other markers, including CA125, SPINK1 was found to be particularly...
Table 1. Summary of the recent findings on the role of SPINK1 in cancer.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Prognostic value*</th>
<th>Tissue expression in advanced stage</th>
<th>Proliferation</th>
<th>Migration</th>
<th>Invasion</th>
<th>Angiogenesis</th>
<th>Metastasis</th>
<th>Apoptosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian</td>
<td>Serum</td>
<td>NDb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Venesmaa et al. (15); Taccone et al. (16)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>NA</td>
<td>x</td>
<td></td>
<td>▲</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hedstrom et al. (21); Freeman et al. (26)</td>
</tr>
<tr>
<td>Prostate</td>
<td>Tissue</td>
<td>x</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td></td>
<td></td>
<td></td>
<td>Tomlins et al. (31); Ateeq et al. (35); Wang et al. (36)</td>
</tr>
<tr>
<td>HCC</td>
<td>Tissue, serum</td>
<td>x</td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Lee et al. (38); Lu et al. (42); Lu et al. (43)</td>
</tr>
<tr>
<td>Breast</td>
<td>Tissue, serum</td>
<td>x</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>▼</td>
<td>El-mezyen et al. (49); Soon et al. (50); Gouyer et al. (51)</td>
</tr>
<tr>
<td>CRC</td>
<td>Serum</td>
<td>x</td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Gouyer et al. (51); Gaber et al. (53); Marchbank et al. (58)</td>
</tr>
<tr>
<td>Bladder</td>
<td>Urine, serum</td>
<td>Lost</td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Patschan et al. (61); Pectasides et al. (65); Marchbank et al. (66)</td>
</tr>
<tr>
<td>Gastric</td>
<td>Serum</td>
<td>Lost</td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Kemik et al. (67); Wiksten et al. (69)</td>
</tr>
<tr>
<td>RCC</td>
<td>Serum</td>
<td>Lost</td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Lukkonen et al. (72)</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>x</td>
<td></td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Bohe et al. (73)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>x</td>
<td></td>
<td></td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▲</td>
<td>▼</td>
<td>Hedstrom et al. (21); Tonouchi et al. (74)</td>
</tr>
<tr>
<td>Esophageal</td>
<td>Serum</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Taccone et al. (16); Gion et al. (75)</td>
</tr>
</tbody>
</table>

* Increased SPINK1 levels correlating to adverse prognosis.

b ND, not detected; NA, not applicable as SPINK1 concentrations are increased also in pancreatitis; x, •••; ▲, increased proliferation, migration, invasion, angiogenesis and metastasis; ▼, inhibition of apoptosis; ?, •••.
useful for diagnosis of ovarian cancer (16). During patient monitoring, the serum concentrations of SPINK1 reflect the development of recurrent disease (17). Compared to serum concentrations, up to 1000-fold higher concentrations of SPINK1 have been found in cyst fluid from both benign and malignant ovarian cysts, but increased serum concentrations are rare in patients with benign cysts (18). A recent study (19) of mucinous borderline ovarian tumors showed that increased serum concentrations of SPINK1 were found in these patients and a significant correlation was observed with tissue expression of claudin-5. However, a stronger correlation was observed with serum CA125 and claudin-5 expression, supporting previous findings (15). Claudin-5 is a tight junction protein and its expression is associated with poor prognosis; it was suggested that claudin-5 could influence the expression of SPINK1 and CA125 but this remains to be investigated. Patients with ovarian cancer have higher SPINK1 concentrations in urine than in serum, but they correlate strongly if the urine concentrations are normalized according to urine creatinine or density (13, 17). The interindividual variation in serum concentrations of SPINK1 are smaller and therefore serum measurements are preferred for follow-up of cancer patients (20).

PANCREATIC CANCER

Tissue expression of SPINK1 can be demonstrated by immunohistochemistry in the pancreas and in patients with pancreatic cancer, and pretreatment serum concentrations of SPINK1 are clearly increased in most patients with pancreatic cancer (21). In a study comparing several serum and urine biomarkers, SPINK1 was a good marker for diagnosis of pancreatic cancer (16). However, the clinical utility of SPINK1 for diagnosis of pancreatic cancer is limited because the concentrations are also increased in most patients with pancreatitis and biliary disease (22). The presence of high concentrations of SPINK1 in pancreatic cyst fluid and pancreatic fluid is a strong indicator of malignant disease (23). In population studies with a median follow-up time of 25 years, serum SPINK1 did not predict later development of pancreatic cancer (24). In a recent study focusing on pancreatitis-predisposing mutations in pancreatic cancer patients, the frequency of SPINK1 mutations in exon 3 and adjacent introns was significantly decreased in patients with pancreatic cancer in comparison with patients with idiopathic pancreatitis. However, the mutation frequency did not vary significantly in comparison with healthy controls (25).

In vitro studies SPINK1 has been shown to stimulate proliferation and colony formation of cells derived from a rat pancreatic acinar cell tumor (26). In human pancreatic cancer cell lines SPINK1 was shown to promote proliferation by inducing EGFR phosphorylation and subsequent downstream activation of the mitogen-activated protein kinase (MAPK) pathway, as both EGFR and MAPK/extracellular-signal–regulated kinase (ERK) inhibitors inhibited cell proliferation induced by SPINK1 (27). The binding affinity of SPINK1 to EGFR was half that of EGF binding affinity, and SPINK1 did not bind to the other members of the family, i.e., HER2, HER3, and HER4 (27). Further evidence for the role of SPINK1 and EGFR in pancreatic cancer comes from studies showing coexpression in pancreatic adenocarcinoma cells and in early-stage precancerous pancreatic intraepithelial neoplasms (27).

PROSTATE CANCER

About 10% of prostate cancers show strong staining for SPINK1 (28–31) and the serum concentrations of SPINK1 are increased in more than 40% of patients with advanced prostate cancer (28). A correlation between high tissue expression and shorter time to recurrence has been observed in some studies (31), but in others a correlation between SPINK1 expression and adverse prognosis was not observed (32, 33). Importantly, a majority of these studies reported SPINK1 overexpression mainly in androgen-induced E26 transformation-specific (ETS) gene fusion–negative tumors (31, 32). In a recent study an inverse correlation between SPINK1 and trefoil factor 3 (TFF3) positivity and ETS negativity was shown, and in this TFF3+ subgroup SPINK1 staining was associated with adverse clinical outcome (34). The findings that SPINK1 expression is confined to androgen-independent tumors and is higher in the metastases of castration-resistant prostate cancers than in primary tumors (32) suggest that SPINK1 may be involved in the metastatic processes.

Further support for a role of SPINK1 in metastatic dissemination comes from functional studies conducted in prostate cancer cell lines. SPINK1 has been found to stimulate cell proliferation and invasion in ETS rearrangement–negative prostate cancer cell lines (31, 35). Furthermore, in the benign immortalized prostate cell line RWPE, recombinant SPINK1 increased invasiveness and intravasation, supporting the role of SPINK1 as an oncogene (35). However, SPINK1 was not able to restore invasion of EGFR-knockdown prostate cancer cells. This indicates that the oncogenic effects of SPINK1 were only partly mediated through EGFR. Furthermore, direct binding was demonstrated only with exogenous SPINK1 (35). A more recent study conducted with RWPE cells showed that SPINK1-induced increase in motility was associated with epithelial–mesenchymal transition (EMT) (36). Correlating with the results from pancreatic cancer studies, EGFR and MAPK inhibitors were able to reverse the invasive capacity of prostate cancer cells, suggesting that SPINK1-induced EMT was mediated by the EGFR–MAPK signaling pathway (36).
This study also showed that SPINK1 and EGFR were coexpressed in prostate cancer tissue and that SPINK1 overexpression was associated with poor prognosis.

HEPATOCELLULAR CANCER
SPINK1 mRNA is expressed in hepatocellular carcinoma (HCC) associated with hepatitis (37) and overexpression of SPINK1 is associated with adverse prognosis (38). Global gene expression profiling and immunohistochemistry have shown that SPINK1 is the most strongly upregulated gene in hereditary hemochromatosis–related HCC (39). A recent study by Hass et al. (40) confirmed significant overexpression of SPINK1 mRNA in HCC. Notably in this cohort, SPINK1 upregulation was observed in 80% of the samples in hepatitis C virus–induced HCC cases compared to nonmalignant liver. Thus SPINK1 expression in tumor cells is independent of etiology. Furthermore, these results suggest that SPINK1 overexpression could be used as a diagnostic marker for HCC. The plasma concentrations of SPINK1 are significantly increased in HCC patients and the concentrations correlated with tumor size (37). Recently, serum SPINK1 has been found to be a strong prognostic factor in HCC (41).

In functional studies, overexpression of SPINK1 decreased the susceptibility of Huh7 HCC cells to serine protease–dependent apoptosis (42). SPINK1 expression was shown to be upregulated by hepatitis B and C viruses in Huh7 cells, correlating with the recent findings in HCC patients (40). In these cells SPINK1 inhibited granzyme A and thus suppressed apoptosis, resulting in attenuated immune response and evasion of apoptotic death (43). This is thought to facilitate persistent virus replication and eventual development of liver cancer. Inhibition of granzyme A by SPINK1 has also been demonstrated by using affinity-purified granzyme A from rat small-intestinal cells and a recombinant rat ortholog of SPINK1 (44). Granzyme A and granzyme B play important roles as mediators of granule-mediated apoptosis (45). Taken together these data suggest an antiapoptotic role of SPINK1 in HCC. SPINK1 production by hepatocellular cancer cells was increased by lipopolysaccharide, suggesting a role as an acute-phase reactant in HCC (46). A more recent study using HepG2 and PLC cells showed that a liver-protective drug, silibilin, reduced SPINK1 expression together with a panel of other genes (47). These results suggest that SPINK1 is a potential target for cancer therapy.

BREAST CANCER
Serum concentrations of SPINK1 have been shown to be increased in 33% of patients with advanced breast cancer but SPINK1 in serum did not reliably predict response to chemotherapy (48). In a more recent study, serum concentrations of SPINK1 were significantly increased in patients with metastatic breast cancer, together with increased trypsin concentrations, indicating diagnostic value (49). With the use of genome-wide profiling, SPINK1 was found to be a driver of aggressiveness and associated with poor survival outcome in estrogen receptor–positive breast cancer. This was also shown at the protein level in a panel of clinically annotated breast tumors (50).

In vitro studies, breast cancer cells overexpressing SPINK1 were resistant to drug-induced caspase-dependent apoptosis due to reduced expression of caspase-3 and high levels of Bcl-2 and its phosphorylated form. Interestingly, this mechanism required protease inhibitor activity of SPINK1 (50). Forced expression of SPINK1 induced in vitro migration and invasion but not proliferation (50, 51). Furthermore, in an in vivo experiment, SPINK1 was shown to induce metastasis in the mouse tail-vein model (50). These results support recent clinical findings that show increased serum concentrations of SPINK1 in metastatic breast cancer patients (49) and thus indicate a role for SPINK1 in metastatic dissemination in breast cancer.

COLORECTAL CANCER
Increased serum concentrations of SPINK1 have been observed in 50% of patients with colorectal cancer (CRC), and SPINK1 in serum has been found to be a useful marker for distinguishing between patients with or without liver metastasis (16, 52, 53). Increased serum SPINK1 has been found to be an independent prognostic factor in CRC (53). In some studies strong tissue staining for SPINK1 was found to be associated with adverse prognosis (52, 54), but in another study strong expression correlated with good prognosis, especially if the tumor also showed positive EGFR staining (55). Interestingly, the recent study by Chen et al. (54) showed a positive correlation between SPINK1 and EGFR, contradicting previously published results. In another recent study, SPINK1 was shown to be expressed in around 55% of colorectal tumors and significantly higher protein levels were observed in advanced tumors. However, no correlation between SPINK1 expression and overall survival was found (56). Hence the prognostic value of tissue expression of SPINK1 in colon cancer remains controversial.

Overexpression of SPINK1 in the HT-29 colon cancer cell line induced invasion, angiogenesis, and lung metastases in mouse xenographs (51). This required an intact trypsin interaction site, as an inactive form of SPINK1 did not exert these effects. In another study, SPINK1 was shown to increase migration of HT-29 colon cancer cells, but only in the copresence of EGF (57). In these cells, an antiapoptotic effect of SPINK1 was also observed, as the effect of indomethacin was markedly reduced by the copresence of SPINK1 and EGF. Notably, in this model,
Further, in the study by Chen and colleagues (54) on oncogene and an acute-phase reactant in CRC. Between colitis and subsequent tumor burden, suggesting a link, the mouse ortholog of SPINK3 and colon cancer mouse model, expression levels of proteins have been suggested to function as tumor suppressors in CRC and their expression is downregulated in advanced tumors. In a carcinogen-induced colitis and colon cancer mouse model, expression levels of SPINK3, the mouse ortholog of SPINK1, correlated with colitis and subsequent tumor burden, suggesting a link between SPINK3 and inflammation-induced colon cancer (56). These data support the role of SPINK1 as an oncogene and an acute-phase reactant in CRC.

**BLADDER CANCER**
In most cancers, increased tissue expression of SPINK1 is associated with adverse prognosis. The opposite appears to be the case in bladder and gastric cancer, in which SPINK1 expression has been found to be decreased in advanced tumors (60–62). In spite of this, increased urine concentrations of SPINK1 are significantly associated with adverse prognosis (61, 63). In particular, SPINK1 has been shown to be a useful marker for high-grade bladder cancer (64, 65), and serum SPINK1 has been shown to predict the response to chemotherapy and to be useful for monitoring of treatment (65).

Marchbank and coworkers (66) found that SPINK1 stimulates the migration and invasion of bladder carcinoma cell lines, and this was inhibited by an EGFR-blocking antibody. The effect was synergistic with that of EGF. Furthermore, knockdown of endogenous SPINK1 was found to suppress migration and invasion. However, unlike EGF, SPINK1 did not affect proliferation of the cells. Mechanistically SPINK1 induced phosphorylation of EGFR, ERK1/2, and Akt2 and Akt3, but not Akt1, correlating with the results from migration and proliferation experiments. These data support the hypothesis that SPINK1 mediates its promigratory effects through the EGFR signaling cascade in bladder cancer.

**GASTRIC CANCER**
Increased SPINK1 concentrations have been observed in serum from patients with gastric cancer and this has been associated with advanced stage (67). However, strong expression of SPINK1 at the tissue level was shown to be a sign of favorable outcome (68), whereas loss of SPINK1 immunoreactivity was associated with adverse prognosis (69). The loss of SPINK1 expression in gastric cancer tissue has also been observed on the mRNA level (70). Thus the mechanism causing increased serum concentrations of SPINK1 in gastric cancer remains to be elucidated.

**RENAL CANCER**
Increased SPINK1 concentrations have been found in the serum of renal cell carcinoma (RCC) patients and this has been shown to correlate with stage (71). Further, serum SPINK1 has been shown to be an independent prognostic factor in RCC (72). SPINK1 expression has been detected in renal cancer cell lines but not in renal cell cancer tissue (72). Therefore, as in the case of gastric cancer, the source of SPINK1 in renal cancer patients is currently unclear.

**OTHER CANCERS**
SPINK1 expression has been detected by immunohistochemistry in the gallbladder (73) and in cholangiocarcinoma (21). In intrahepatic cholangiocarcinoma, overexpression of SPINK1 has been detected by DNA microarray analysis. High expression of SPINK1 in tumor tissue is associated with recurrence of intrahepatic cholangiocarcinoma (74).

In a study comparing SPINK1 with several other markers, serum SPINK1 was found to be especially useful for diagnosis of esophageal cancer (16). However, tissue expression of SPINK1 in esophageal cancer has not been observed (75).

Serum SPINK1 concentrations, although higher in cancer patients than in controls (76), have not been found to be diagnostically useful in head and neck cancer (77). This was also the case in squamous cell carcinoma of the cervix (78) and lung cancer (79).

**Clinical Use of SPINK1 Determinations**
Development of RIAs with rabbit antisera facilitated measurement of SPINK1 in serum and urine (4, 13). Later, enzyme immunoassays (ELISAs) (80) and a time-resolved immunofluorometric assay (TR-IFMA) (81) were developed. Comparison of a commercial RIA, in-house ELISA, and TR-IFMA methods showed that these provide similar results for serum SPINK1 in clinical samples (80). The reference interval for SPINK1 in serum and plasma is 5–15 μg/L (80), whereas in urine it is 7–50 μg/g creatinine (14). Several biochemical companies
market ELISA methods for assaying SPINK1, and they report reference values identical to those determined by methods developed in our laboratory. Determination of SPINK1 in serum and urine by TR-IFMA is clinically used in Finland for diagnosis of pancreatitis and for ovarian, bladder, and kidney cancer. In particular, increased serum SPINK1 is a very sensitive marker for mucinous ovarian cancer, and unlike CA125, its concentrations correlate with tumor grade (82). Measuring serum concentration of SPINK1 is useful for monitoring treatment response and also as an indicator of recurrence.

SPINK1 in serum and urine can also be determined by LC-MS in combination with immunocapture with magnetic beads (83). With this method it is possible to simultaneously measure both wild-type and N34S-SPINK1. The N34S-SPINK1 mutation has been linked to increased susceptibility to chronic pancreatitis, but this mutation by itself is insufficient to cause pancreatitis (1). The role of N34S-SPINK1 in cancer has not been investigated. The LC-MS method can be used for identification of SPINK1 variants in archival serum and plasma samples when DNA is not available, and thus could be used to screen for this mutation in cancer patient samples. The ability to measure other SPINK1 variants is presently explored.

Potential Use of SPINK1 as a Therapeutic Target in Cancer

SPINK1 is expressed in variety of cancers and in the majority of them this indicates poor prognosis. Functionally SPINK1 has been shown to exert pleiotropic effects in cancer. Therefore SPINK1 would seem to be a candidate for molecularly targeted cancer therapy, and studies exploring this have been conducted. Knockdown of SPINK1 in 22RW1 prostate cancer cells by RNA interference or inhibition by a monoclonal SPINK1 antibody reduced cell proliferation, cell invasion, and tumor growth in xenograft assays, but it did not have any effect on DU145 and PC3 cells that do not express SPINK1. Both SPINK1 antibody and the EGFR antibody cetuximab attenuated tumor growth, and the combined effect of these was stronger than that of either one alone (31). Monoclonal antibodies to SPINK1 have also been used in xenograft models of colon and breast cancer to attenuate tumor growth (50, 51).

It has been suggested that humanized antibodies could be used for targeted therapy of cancers expressing SPINK1. However, systemic inhibition of SPINK1 may be problematic, as it protects the pancreas against premature activation of trypsinogen, and interference of this mechanism is likely to increase the risk of pancreatitis. Therefore either local delivery or cancer-specific targeting may be required for SPINK1 therapy. Several approaches are currently being developed for cancer-specific targeting, and some of them, such as nanoparticles containing human transferrin protein on the surface, have been tested in clinical trials (84). Thus, SPINK1–targeted therapy might become feasible in the future.

Another option for SPINK1-positive cancers would be targeting downstream effectors of SPINK1. A majority of the oncogenic functions of SPINK1 seem to be mediated by the MAPK pathway (27, 36, 59, 66). Current US Food and Drug Administration–approved targeted therapies include the RAF inhibitor sorafenib for treatment of kidney, liver, and thyroid cancer; the MEK inhibitor trametinib for treatment of melanoma; and the EGFR, ERK1/2, and Akt inhibitor lapatinib for treatment of breast cancer. Further, several inhibitors targeting MAPK effectors are currently in clinical trials. Therefore, factoring in the expression of SPINK1 as part of patient stratification in clinical trials might be warranted.

Conclusions

SPINK1 has many important roles in various physiological and pathological processes. In addition to its role of protecting the pancreas against premature activation of trypsinogen, it also acts as an acute-phase reactant, growth factor, and inhibitor of apoptosis, thereby contributing to cancer progression. These functions appear to be mediated by quite different mechanisms, as inhibition of trypsin protects against pancreatitis and tumor invasion, whereas binding to EGFR and capability to inhibit granzyme A-, serine protease-, and caspase-mediated apoptosis promotes development of cancer. Clinically, determination of SPINK1 in plasma, serum, or urine is useful for evaluation of prognosis of several cancers. However, when using SPINK1 as a tumor marker, it is important to consider the effects of impaired renal function and acute-phase reactions on the concentrations of SPINK1 in serum. The clinical utility of SPINK1 tissue expression in cancer remains to be determined, and its use in targeted anticancer therapy needs to be explored. Taken together, SPINK1 plays pleiotropic roles in cancer and can serve as a biomarker and possibly in the future as a therapeutic target.

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