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RESEARCH HIGHLIGHT

Role of the tumor-associated trypsin inhibitor SPINK1 in cancer development

Ulf-Håkan Stenman

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In most industrialized countries, prostate cancer is the most common non-skin cancer of men. When detected early, most cases are curable, but for tumors that have metastasized, there is no curative therapy. Pharmaceutical androgen ablation or castration induces remission in most patients with metastatic disease, but if the patient lives long enough, the tumor becomes androgen-independent. In some patients, chemotherapy induces remission, which mostly is of short duration. Therefore, new treatment modalities are needed for androgen-independent prostate cancer.

Increasing knowledge of the biology of prostate cancer promises to facilitate development of therapies that target specific disease mechanisms. The recent finding of overexpression of serine protease inhibitor Kazal type 1 (SPINK1) in prostate cancer may represent a new mechanism amenable to targeted therapy in selected patients. SPINK1 was initially found in pancreatic tissue[1] and pancreatic fluid and was therefore called pancreatic secretory trypsin inhibitor.[2] When later identified in urine from patients with ovarian cancer, it was called tumor associated trypsin inhibitor.[3,4] This name has been used in most studies on its role in cancer.[5] SPINK1 occurs at high concentrations in the pancreas, where it functions as a first line of defense against premature activation of trypsinogen. This role of SPINK1 is demonstrated by the finding that a mutation in the *SPINK1* gene is associated with increased risk of pancreatitis.[6]

High expression of SPINK1 in malignant diseases was first observed in ovarian cancer, but later this has been found in many other cancers.[4,5,7] In most tumor types, this is associated with adverse prognosis. The Expression of SPINK1 is invariably associated with expression of tumor-associated trypsin, which activates several matrix metalloproteinases. Because these have been shown to mediate cancer invasion, the association between SPINK1 expression and adverse prognosis in cancer has been ascribed to the expression of trypsin by the tumors.[5] This notion was supported by the finding of high tissue expression of trypsinogen-2 and adverse prognosis in ovarian cancer.[8] In other tumors, low expression of SPINK1 is associated with adverse prognosis and aggressive disease, i.e., ventricular and bladder cancer.[9,10] In these tumors, SPINK1 may inhibit tumor invasion by inhibiting protease cascades involving trypsin. Recent studies have revealed another function of SPINK1, i.e., it can promote development and growth of prostate cancer by stimulating the epidermal growth factor receptor (EGFR).[11]

The structure of SPINK1 resembles that of EGF; they have 50% sequence homology, a molecular size of approximately 6 kDa and three intrachain disulfide bridges. This prompted studies on the ability of SPINK1 to act as a growth factor. Early studies showed that SPINK1 binds to surface receptors and stimulates DNA synthesis of fibroblasts,[12] but EGF and several other growth factors do not compete for binding of SPINK1.[12,13] More recently, overexpression of SPINK1 in hepatocellular cancer has been shown to be strongly associated with high-stage, early tumor recurrence and short 5-year survival.[14] SPINK1 has been identified in the medium of the colon cancer cell line HT29 5M21 by proteomic techniques and shown to be the major proinvasive factor produced by these cells.[15] At physiological concentrations, recombinant SPINK1 increases invasion in collagen gel of several cell lines, but a SPINK1 mutant, SPINK1 K18Y, which does not inhibit trypsin, has no effect on invasion. Furthermore, an antibody to SPINK1 inhibits the invasiveness of HT29 5M21 cells.[15] More recently, increased SPINK1 expression in tumor tissue and SPINK1 concentrations in serum have been shown to be independent prognostic factors for liver metastasis and adverse prognosis in colorectal cancer.[16,17]

SPINK1 is also expressed in the prostate and its expression increases with high tumor grade.[18] Strongly increased SPINK1 expression is found in about 10% of all prostate cancers and this is associated with adverse prognosis.[19,20] In surgically resected patients, increased SPINK1 expression is inversely related to gene fusions involving erythroblastosis virus E26 transformation–specific (ETS) family of transcriptions factors.[19] However, in endocrine treated patients, this is not associated with adverse prognosis.[20]

New information on the mechanisms by which SPINK1 may be associated with adverse prognosis was provided by the recent study of Ateeq *et al.*[11] Using several independent techniques, they showed that SPINK1 contributes to the aggressive phenotype of the prostate cancer cell line 22RV1. Forced expression of SPINK1 increases cell proliferation and invasiveness, while knockdown of the expression reduces these effects. Furthermore, the effect of SPINK1 on cell proliferation, invasion and growth of SPINK1-expressing tumor xenografts in mice was strongly reduced by a monoclonal antibody. Administration of antibodies to either SPINK1 or EGFR to mice bearing 22RV1 xenografts attenuated tumor growth by about 60% and 40%, respectively, while the effect of those combined was 75%.[11] The neoplastic effects of SPINK1 were shown to be mediated by binding to EGFR, but other mechanisms

Deparment of Clinical Chmistry, Helsinki University and Helsinki University Central Hospital, FIN-00014 Helsinki University, PB 63, Finland
Correspondence: Dr U-H Stenman (ulf-hakan.stenman@helsinki.fi)

may also be involved. Thus SPINK1 can be classified as an autocrine growth factor, which is potentially druggable for treatment of an aggressive form of prostate cancer. These results and those on colon cancer provide a rationale for development of humanized monoclonal antibodies to SPINK1 and the use of those in combination with other tools for EGFR inhibition for treatment of selected cancer patients.[11,15]

Increased expression of EGFR and its ligands occurs in many cancers and the monoclonal EGFR antibody cetuximab is successfully used to treat breast cancer.[21] Attempts to use EGFR inhibitors to treat prostate cancer have shown a response in less than 10% of the cases.[22,23] It remains to be shown whether the response is associated with SPINK1 expression and whether combined use of EGFR inhibitors and SPINK1 antibodies improves the response.

Increased expression of EGFR and its ligands occurs in many cancers and mutations can increase EGFR activation. There are four EGFR receptors, which are activated by several growth factors that have been classified on the basis of which receptors they activate.[21] SPINK1 can now be classified as an EGFR ligand, but so far it is not known which receptor or receptors that it activates and which are expressed in the prostate. Studies on 3T3 Swiss albino cells show that EGF does not compete with SPINK1 for binding to its cell surface receptor.[13] This suggests that they bind to different EGFRs.

An interesting question is the cause of the increased SPINK1 expression in cancer. The *SPINK1* gene contains an IL-6 responsive element and in hepatoma cells, expression of SPINK1 is induced by IL-6 and IL-1.[24] Thus, it is not surprising that SPINK1 also behaves as an acute phase reactant in patients with severe injury and infections.[25,26] There is a strong association between infections and cancer.[27] It is therefore tempting to speculate that infections causing elevation of inflammatory cytokines cause increased SPINK1 expression leading to increased cancer risk.

While SPINK1 is primarily thought to act as an autocrine growth factor, the addition of SPINK to culture medium increased invasiveness also of cells that did not express it.[11] Therefore, conditions with increased SPINK1 concentrations may give information on its role in cancer development. Patients on hemodialysis have highly increased plasma concentrations of SPINK1[25] and they also have increased risk of cancers of the kidney, bladder and endocrine organs but not of prostate cancer.[28] The lack of effect on prostate cancer incidence may be explained by the slow development of this disease. The mechanisms behind the increased risk are not known and they may also be explained by increased levels of other growth factors in dialysis patients.

Taken together, the results of several studies show that SPINK1 acts as an autocrine growth factor in several cancers. Thus, it exerts dual functions as a growth factor and a protease inhibitor. In some cancers, it promotes tumor growth and invasiveness, while in other ones its loss is associated with aggressive disease. The promising results on the use of monoclonal antibodies to treat prostate cancer xenografts in mice will prompt development of antibodies that can be used for treatment of human tumors expressing SPINK1. Patients suitable for such treatment need to be selected on the basis of SPINK1 expression. Nearly half of the patients with advanced prostate cancer have clearly elevated serum concentrations of SPINK1.[18] Thus, measurement of SPINK1 in serum may also be useful for identification of suitable patients and for monitoring of response to treatment.

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