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Sphingosine kinase-1 activity and expression in human prostate cancer resection specimens

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ABSTRACT

Purpose: Sphingosine kinase-1 (SphK1) was shown in preclinical models and non-genito-urinary cancers to be instrumental in cancer progression, adaptation to hypoxia and in tumour angiogenesis. No data were available in human prostate cancer. The present study was designed to assess SphK1 expression and activity in radical prostatectomy specimens and to research correlations with clinical features.

Materials and methods: Transverse section of fresh tissue was obtained from 30 consecutive patients undergoing laparoscopic prostatectomy. SphK1 enzymatic activities of tumour and normal counterpart were determined. Relationships with PSA, Gleason sum, pathological stage, resection margin status and treatment failure were researched. SphK1 pattern of expression was then assessed on tissue microarray.

Results: A significant 2-fold increase in SphK1 enzymatic activity(11.1 ± 8.4 versus 5.9 ± 3.2 ($P < 0.04$)) was observed in cancer. The upper quartile of SphK1 activity was associated with higher PSA (16.7 versus 6.4 ng/ml, $P = 0.04$), higher tumor volumes (20.7 versus 9.8, $P = 0.002$), higher rates of positive margins (85.7% versus 28.6%, $P = 0.01$) and surgical failure (71.4% versus 9.5%, $P = 0.003$) than the lower three quartiles. Odds ratios (OR) for treatment failure showed a strong relationship with SphK1 activity (OR: 23.7, $P = 0.001$), positive resection margins (OR: 15.0, $P = 0.007$) and Gleason sum ($\geqslant 4 + 3$, OR: 8.0, $P = 0.003$). Tissue microarrays showed discrete epithelial expression that varied with Gleason sum with significant relationship between SphK1 expression and higher Gleason sum.

Conclusion: In complement to preclinical literature, the demonstrated relationships between SphK1-increased activity in cancer and relevant clinical features confirm a central role for SphK1 in prostate cancer that herald promising avenues in risk-assessment and treatment.

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1. Introduction

Sphingosine 1-phosphate (S1P) has emerged as a critical lipid mediator that promotes tumour cell proliferation, survival, migration and angiogenesis (reviewed in 1,2). It has been suggested that the balance between the intracellular levels of S1P and its metabolic precursors ceramide and sphingosine provides a rheostat mechanism that decides whether a cell undergoes apoptosis (via ceramide and sphingosine) or proliferates and survives by S1P.[3] A decisive regulator of this sphingolipid switch is the sphingosine kinase-1 (SphK1), the enzyme whose role is to convert the death-promoting sphingolipid sphingosine into the growth-promoting S1P. While SphK1 activity can be stimulated by a wide array of growth factors (reviewed in 4), anticancer treatments cause its down-regulation,[5–10] and small molecule inhibitors of SphK1 can reduce tumor volume in animal models.[11–15]

Further supporting a role for SphK1 in promoting cancer, SphK1 has been found to act as an oncogene,[16] and SphK1 mRNA levels have been found significantly higher in various tumour tissues such as those of breast, colon, lung, ovary, stomach, uterus, kidney, rectum and small intestine, than in normal tissues.[11,17] Bone marrow cells isolated from acute leukaemia and myelodysplastic syndrome patients also displayed a noticeable increase of SphK1 mRNA as compared to normal population.[18] Interestingly, increased SphK1 mRNA is correlated to increasing clinical grade in non-Hodgkin lymphomas.[19] More importantly, a correlation was found between the mRNA content of SphK1 with short survival in grade IV human astrocytoma,[20] and oestrogen-receptor positive breast cancer patients.[21] In non-small-cell lung carcinomas or pancreatic adenocarcinoma, strong immunopositive staining for SphK1 in cancerous lesions as compared with normal adjacent tissue[17,22] suggested that increased mRNA levels were generally reflected in increased protein expression. A significant correlation was noted between SphK1 expression and histopathological staging in astrocytomas, gastric and colon cancer, supporting the notion that SphK1 plays a role in progression of these diseases.[23–25] In addition, a remarkable correlation was found between shorter overall survival times of patients and high SphK1 expression for astrocytomas and gastric cancer, suggesting that SphK1 could also be a prognostic marker.[24,25]

Following lung cancer, prostate cancer has become the second leading cause of death by cancer in the United States. The American Cancer Society estimates are 192,000 new cases and 28,000 deaths in 2009.[26] So far, the relevance of the sphingolipid rheostat governed by SphK1 with regard to prostate cancer progression/resistance has only been suggested in cell[7–9,27,28] or pre-clinical animal models.[9,15]

Herein, we report for the first time the location and variability of SphK1 expression in prostate tumours. We further relate the quantification of the SphK1 enzymatic activity in freshly retrieved specimens of individual tumours and corresponding normal tissues to clinical features.

2. Materials and methods

2.1. Patients and specimens handling

Tissue samples of 30 prostate cancer cases were obtained after IRB approval (Clinical Trial 0305302 from the Hôpitaux de Toulouse) and informed consent from consecutive patients undergoing laparoscopic radical prostectomies performed for clinical T1c–T2c with at least two positive biopsies from 12/2003 to 7/2004. The clinical stage was assigned by the referring urologist according to the 2002 TNM classification.[29] No patients received neoadjuvant treatment by radiation or hormonal manipulation. All prostatectomy specimens were inked over their entire surface and processed according to the Stanford protocol.[30] Specimens were hardened for one hour at 4 °C before obtaining a 3 mm thick transverse specimen at the level of the *veru montanum* (Fig. 1A). Slices were kept at –20 °C pending analysis of whole-mount sections of the upper and lower edges of the section. Tumor location in the specimen was inferred from H&E analysis of the two adjacent whole-mount sections. For two patients, the mid-specimen section did not comprise tumours. Tumour sample and symmetrical control taken in the same zone according to the classical zonal anatomy[31] were then harvested and snap-frozen before processing.

Histological tumour grading was performed according to the Gleason grading system.[32] A positive surgical margin was defined as cancer cells in contact with the inked specimen surface. The pathological stage was defined according to the 2002 AJCC staging classification.[29] Tumour volume expressed in percent of the total gland volume was obtained by streamlined three-dimensional estimation method.[33] Prostate specific antigen serum levels (Abbott AxSYM PSA assay, Rungis, France), extracapsular extension, pathological stage, surgical margin status, radical prostatectomy specimen, primary and secondary Gleason scores, follow-up time and biochemical recurrence as defined by the European Association of Urology guidelines by two PSA readings >0.2 ng/ml[34] were available for all patients. Failure of surgical treatment was defined by biochemical recurrence or by adjuvant radiation or hormonal deprivation therapy.

2.2. Sphingosine kinase-1 activity

SphK1 activity was measured as previously published.[6] Briefly, tissue samples were homogenised in a buffer containing 20 mM Tris (pH 7.4), 20% glycerol, 1 mM β-mercaptoethanol, 1 mM EDTA, 1 mM sodium orthovanadate, 40 mM β-glycerophosphate, 15 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor and 0.5 mM 4-deoxypyridoxine. After 10 s sonication, samples were ultra-centrifuged for 90 min (105,000*g* at 4 °C) The SphK1 activity was determined in the cytosolic fractions in the presence of 50 μM sphingosine, 0.25% Triton X-100 and [γ32P]ATP (10 μCi, 1 mM) containing 10 mM $MgCl_2$. The labelled S1P was separated by thin layer chromatography on silica gel 60 with 1-butanol/ethanol/acetic acid/water (80:20:10:10, v/v) and