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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

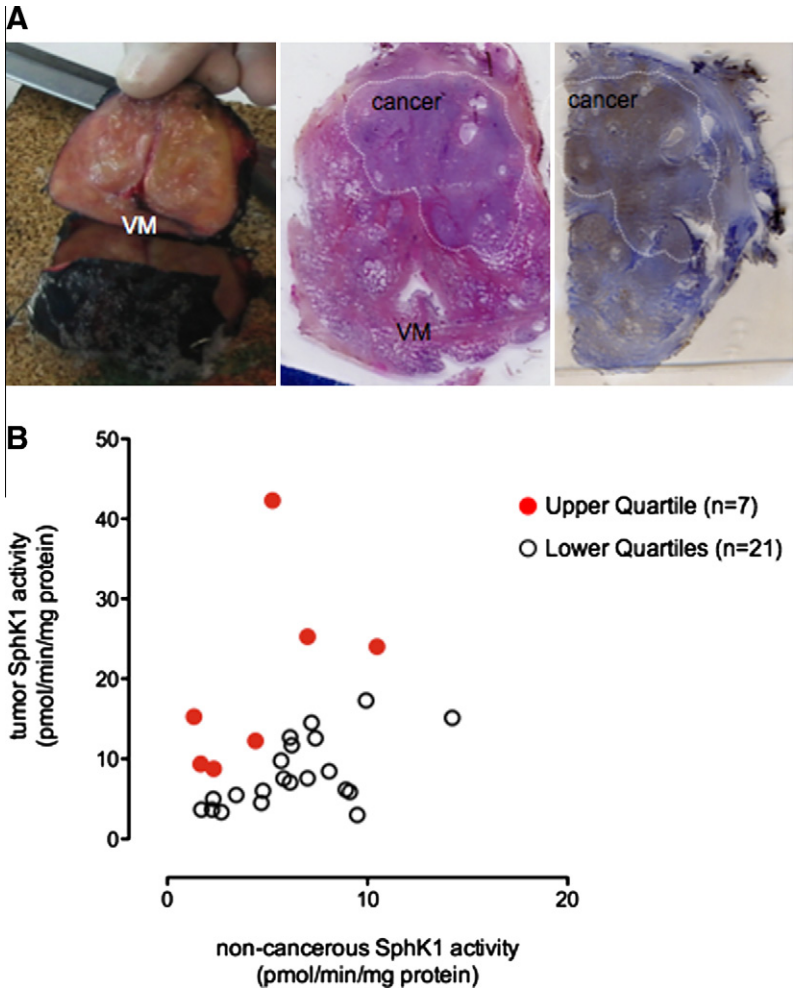

Fig. 1 – Sample harvesting and SphK1 activities in 28 individual pairs of cancer and non-cancerous lesions. (A) Left panel, one 3-mm thick specimen was taken at the level of *veru montanum* (VM) and kept at –20 °C pending enzymatic activity determination. Mid panel, two whole-mount sections were obtained above and under the specimen to verify the presence of cancer and outline its location. Right panel, low-power view of a representative anti-SphK1 immunohistochemistry (patient: THO.R). (B) Representation of SphK1 activities in non-cancerous (*x* axis) and cancer (*y* axis) showing the lack of significant correlation. Upper quartile cases are highlighted in red.

quantified by autoradiography. The SphK1-specific activity was expressed as picomoles of S1P formed/min/mg of protein.

2.3. *Immunohistochemistry*

Tissue microarray sections representative of prostate cancer Gleason scores ($n = 88$, Gleason scores 6, 7 and 8) were from our institution (C.M.) and used to assert the pattern of expression of SphK1 according to differentiation. All sections were deparaffinised, hydrated, boiled with 10 mmol/L of citrate buffer (pH 6) for 30 min, treated with 0.3% H_2O_2 for 5 min, pre-incubated in blocking solution (1% BSA in PBS) for 10 min at room temperature and incubated with the primary antibody (polyclonal anti-SphK1 Ab diluted 1:100,[35]) for 4 h at 4 °C. The sections were then washed with PBS and processed with the two-step EnVision+ HRP DAKO system (DAKO, Carpinteria, CA USA). Tumour Gleason sum was recorded for each individual microarray and as proposed by Rubin to report immunohistochemical results in prostate cancer,[36] the staining intensity was scored as negative (1), faint/equivocal (2), moderate(3) or strong (4).

2.4. *Statistical analysis*

The Spearman's rank test was used to assess a correlation between non-Gaussian quantitative variables. The two-sided Fisher's exact *t*-test was used to compare the distribution of qualitative variables and the Student's *t*-test for comparison of means between groups. For immunohistochemistry studies, the relationship between Gleason sum and staining intensities (negative and faint/equivocal versus moderate or strong) was analysed by the two-sided Fisher's exact *t*-test. Statistical significance was set at a p value < 0.05, all reported values are two-sided.

Table 1 – Patients' characteristics in a cohort of 28 males with prostate cancer treated by radical prostatectomy.

Patient	Age (years)	PSA (ng/ml)	pT	Resection margins	Gleason sum	% cancer	SphK1 non-cancer	SphK1 tumor	Ratio T/C	Follow-up (months)	Tx failure[a]
1	53.5	6.5	T3a	pos.	6	30	1.3	15.3	11.8	15	1
2	62.8	12.0	T3b	pos.	7	30	5.2	42.3	8.1	1	1
3	67.2	35.0	T3a	pos.	7	30	1.7	9.4	5.5	2	1
4	60.0	7.5	T3b	–	7	13	2.3	8.8	3.8	56	0
5	70.9	38.0	T2c	pos.	7	30	7.0	25.3	3.6	16	1
6	68.7	6.5	T3b	pos.	7	8	4.4	12.2	2.8	2	1
7	62.7	11.1	T2c	–	5	4	10.5	24.0	2.3	19	0
8	72.8	3.6	T2b	–	6	3	2.3	5.0	2.2	56	0
9	54.2	9.9	T3a	–	7	12	1.7	3.7	2.2	29	0
10	58.2	3.8	T2c	–	5	5	6.1	12.7	2.1	20	0
11	65.0	5.2	T2c	–	5	12	7.2	14.5	2.0	19	0
12	50.7	2.5	T2c	pos.	7	10	6.2	11.7	1.9	40	0
13	64.8	15.6	T3a	pos.	7	15	9.9	17.3	1.7	1	1
14	64.5	4.2	T2c	–	7	8	5.7	9.8	1.7	19	0
15	54.9	3.9	T2c	–	7	10	7.4	12.6	1.7	30	0
16	60.8	11.0	T2b	–	7	8	2.2	3.7	1.7	55	0
17	65.1	1.2	T2b	–	9	6	3.4	5.5	1.6	1	1
18	55.7	4.1	T2c	pos.	7	15	5.8	7.6	1.3	51	0
19	70.3	6.7	T3a	–	5	25	4.8	6.0	1.2	42	0
20	63.5	5.7	T2c	pos.	7	10	2.7	3.4	1.2	21	0
21	63.2	7.3	T2c	–	7	14	6.1	7.1	1.2	51	0
22	61.5	5.8	T3a	–	6	10	7.0	7.6	1.1	54	0
23	72.5	4.4	T2b	–	5	3	14.2	15.1	1.1	53	0
24	69.8	13.6	T3a	–	7	12	8.1	8.5	1.0	6	0
25	70.9	11.0	T3a	pos.	6	3	4.7	4.5	1.0	29	0
26	71.5	7.6	T2c	–	6	3	8.9	6.2	0.7	49	0
27	68.9	5.0	T3a	–	7	10	9.1	5.9	0.6	49	0
28	65.4	2.7	T3a	pos.	7	13	9.5	3.0	0.3	56	0

pT, pathological stage (TNM 2002); SphK1, sphingosine kinase-1 enzymatic activity; ratio T/C, ratio tumor/non cancer.
[a] Treatment failure was defined as detectable PSA after surgery or immediate adjuvant treatment after surgery. No PSA recurrence was observed during follow-up.

3. Results

3.1. *Sphingosine kinase-1 activity*

Thirty consecutive patients were enrolled. For two patients, no tumour was found in the mid-specimen section. Patients' characteristics are presented in Table 1. Median age was 63.9 years [95% confidence interval (CI): 61.5–66.3], median preoperative serum total PSA level was 9.0 ng/ml (95% CI: 5.7–12.3). Median follow-up before biochemical relapse or adjuvant treatment or no evidence of recurrence was 30 months (range 1–56.2 months). As a whole, median sphingosine kinase-1 (SphK1) activity was 11.0 pmol/min/mg (95% CI: 7.8–14.3) in tumour samples and 5.9 (95% CI: 4.7–7.1) in non-cancerous tissue accounting for a statistically significant ($P = 0.0039$) 2-fold increase in cancer tissue as compared to the individual normal counterpart (ratio SphK1 tumor/non-cancer: 2.4, 95% CI: 1.5–3.4). No correlations were found between SphK1 activity of tumor samples and their individual non-cancer counterparts (spearman's rank test $r = 0.318$, $P = 0.098$; Fig. 1B).

To take into account the individual variability of enzymatic activity, the ratio of tumour activity to non-cancerous counterpart was used for further analysis. The ratio distribution was split into quartiles and tumour characteristics were compared between the three lower quartiles (ratio < 2.3, $n = 21$) and the upper quartile (ratio $\geqslant$ 2.3, $n = 7$). As shown in Table 2, the upper quartile was associated with higher PSA (16.7 ± 13.7 versus 6.4 ± 3.8 ng/ml, $P = 0.039$), higher cancer volume (20.7 ± 11.8 versus 9.8 ± 5.2%, $P = 0.0023$), and higher incidence of surgical failure (71.4 versus 9.5%, $P = 0.0038$) than the lower three quartiles. No statistical relations were found between the SphK1 ratio and pT stage (pT2 versus pT3, ns), Gleason sum (GS $\leqslant$ 3 + 4 versus GS $\geqslant$ 3 + 4, ns) or the presence of positive resection margins (71.4 versus 28.5%, $P = 0.076$).

As anticipated, classical predictors of poor response to surgery such as PSA, tumour volume, resection status were also associated with treatment failure (Table 3).

The odds ratios (OR) for treatment failure were then obtained for: (i) SphK1 upper quartile (OR: 23.7, 95% CI: 2.64–212.9, $P = 0.001$), (ii) positive resection margins (OR: 15.0, 95% CI: 1.47–152.5, $P = 0.007$), (iii) Gleason Sum $\geqslant$4 + 3 (OR: 8.0, 95% CI: 1.2–55.3, $P = 0.003$), (iv) PSA ($\geqslant$10 ng/ml, OR: 5.7, 95% CI: 0.89–36.1, n.s. ($P = 0.06$)) and (v) pT stage (pT3, OR: 4.1, 95% CI, 0.63–26.1, n.s. ($P = 0.12$)).

3.2. *Tissue microarrays*

Immunohistochemistry evidenced faint SphK1 expression in the cytoplasm of normal prostate epithelium but no reactivity in the stroma (Fig. 2A, benign hyperplasia tissue) consistent with the reports of low mRNA levels of SphK1 in all epithelia tested to date[11,17]. Representative patterns of expression are

Table 2 – Patient's characteristics according to quartile distribution of ratios of SphK1 activity in cancer to non-cancerous counterpart.

	Three lower quartiles SphK1 ratio < 2.3 (n = 21)	Higher quartile SphK1 ratio ⩾ 2.3 (n = 7)	P^*
Age (years)	64.0 ± 6.4	63.7 ± 5.8	ns (0.90)
PSA (ng/ml)	6.4 ± 3.8	16.7 ± 13.7	0.0039
Prostate Weight (g)	47.0 ± 19.6	54.9 ± 13.5	ns (0.17)
PSA density (ng/ml/g)	0.156 ± 0.11	0.341 ± 0.35	0.039
Cancer volume (%)	9.8 ± 5.2	20.7 ± 11.8	0.0023
Pathological stage			ns (0.48)
pT2b	4	0	
pT2c	9	2	
pT3a	8	2	
pT3b	0	3	
Resections margins			ns (0.076)
Positive	6	5	
Negative	15	2	
Primary Gleason grade			ns (0.40)
2	1	0	
3	16	4	
4	4	3	
Secondary Gleason grade			ns (0.78)
2	3	1	
3	8	4	
4	9	2	
5	1	0	
Gleason sum			ns (0.88)
5	4	1	
6	4	1	
7	12	5	
9	1	0	
Treatment failure			0.0038
No	19	2	
Yes	2 (9.5%)	5 (71.4%)	

* Statistical significance between lower three and upper fourth quartiles of SphK1 ratios was sought by analysis of variance for quantitative variables and by Fisher's exact t-test for qualitative variables.

Table 3 – Univariate analysis of patients' characteristics in relation to treatment failure as defined by adjuvant radiation or hormonal deprivation therapy or biochemical recurrence.

	No treatment failure (n = 21)	Treatment failure (n = 7)	P
PSA (ng/ml)[a]	6.5 ± 3.1	16.4 ± 14.4	0.0055
pT stage (pT3)	8/21 (38.0%)	5/7 (71.4%)	ns (0.06)
Tumor volume (%)	9.5 ± 5.3	21.3 ± 11.2	0.0009
Positive margins	5/21 (23.8%)	6/7 (85.7%)	0.0069
Higher quartile SphK1 ratio	2/21 (9.5%)	5/7 (71.4%)	0.0038

[a] Mean and standard deviation.

presented in Fig. 2 (Fig. 2B, mixed Gleason grades 2 and 3; 2C, Gleason grade 3; and 2D, Gleason grade 4). In semi-quantitative analysis, SphK1 expression showed a distinct cytoplasmic pattern that increased in intensity with tumour grade ($P < 0.008$) (Fig. 3).

4. Discussion

Sphingolipids signalling has emerged as a key integrated system controlling a wide array of molecular functions involved in crucial steps of cancer natural history from promotion and progression to response to treatments (reviewed in 2). Across the complexities of sphingolipid biology, sphingosine kinase type 1 (SphK1) has the pivotal role of phosphorylating anti-apoptotic ceramide/sphingosine into sphingosine 1-phosphate (S1P) that can act intracellularly or through dedicated extracellular receptors to promote cell motility, proliferation and survival, as befits its reported oncogenic nature.[37] Of note, a second isoform – sphingosine kinase type 2 – was shown to exhibit pro-apoptotic function in relation to structural homology with a pro-apoptotic subgroup of the Bcl-2 family[38] but independent of the activation of sphingosine-1-receptors.[39] SphK2 role in cancer has yet to be fully investigated but preliminary studies failed to correlate SphK2 expression and clinical features both in solid cancers[20,21] and blood malignancies.[18,19]

Regarding SpK1, the first validation in human samples was conducted by French and co-workers who tested SphK1 mRNA expression in solid tumours such as breast, colon, lung, ovary, stomach, uterus and kidney cancers showing a 2–3-fold increase in expression, as compared to normal tissue, of note, prostate cancer was not investigated.[11]

Few clinical studies researched correlations between SphK1 and the natural history of cancer. In a series of 48 malignant astrocytomas, SphK1 mRNA expression correlated with patient's survival with a 3-fold increase in median survival in low as compared to high-expression.[20] Prognostic value was then confirmed in breast cancer where the upper quartile of mRNA SphK1 expression correlated with poor prognosis, irrespective of the oestrogen receptor status.[21] Assessing tumour SphK1 activity was also suggested in ovarian cancer to have diagnostic capabilities, as shown by a significant increase of the product of its activity (sphingosine 1-phosphate), in ascites.[40]

Intriguingly no reports addressed the expression of this oncogene in prostate cancer, which might be related to the difficulties encountered for mRNA studies in harvesting fresh prostate tissue. In the present series all specimens were from routine laparoscopic prostatectomy where after resection the prostate was retained 30–45 min at body temperature in the abdominal cavity for urethro-vesical anastomosis. This delay compromised the recovery of mRNA and prompted us to use the demanding technique of enzymatic assessment. It represents the first human study to date of SphK1 enzymatic activity in cancer.

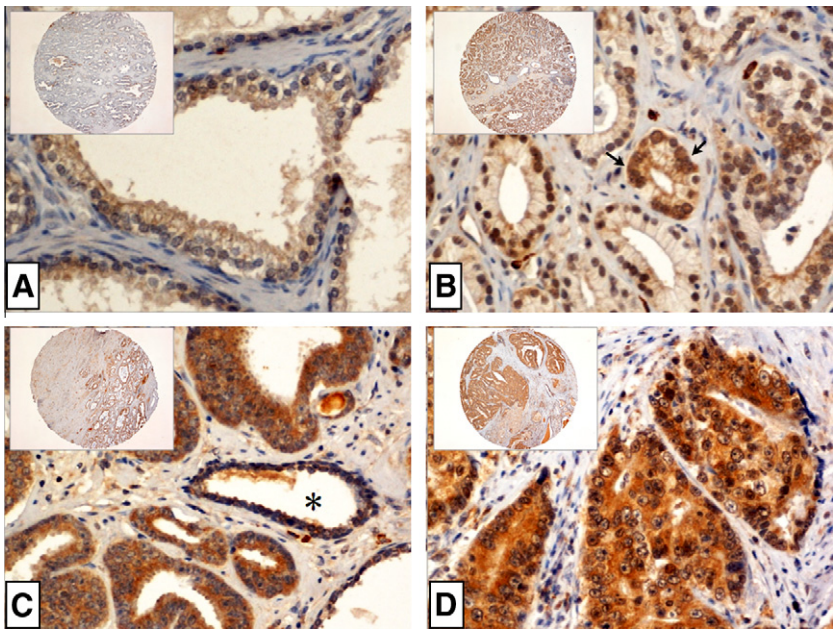

Fig. 2 – Representative patterns of SphK1 expression in benign and cancer glands. (A) TMA and high power magnification of benign glands showing faint reactivity in the cytoplasm of basal and differentiated luminal cells. (B) TMA and high power magnifications of well-differentiated Gleason score 5 prostate cancer. Note the heterogeneity of staining in well-differentiated cancer glands as some cells stain positive (arrow) while adjacent ones only exhibit faint reactivity. (C) Gleason score 6 cancer. Note the lack of staining in atrophic glands (*) and the homogeneous and intense cytoplasmic reactivity of cancer cells. (D) Poorly-differentiated Gleason score 8 cancer showing intense and diffuse staining in the cancer cells cytoplasm.

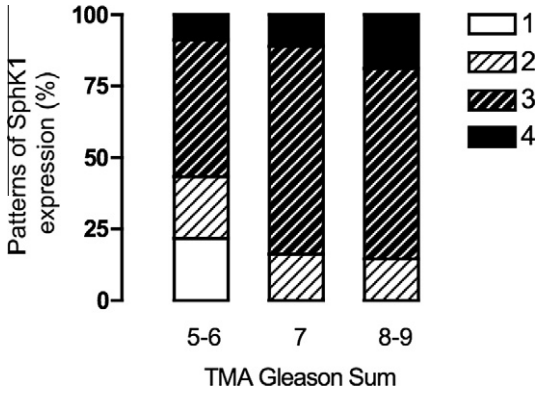

Fig. 3 – Semiquantitative analysis of SphK1expression. Representative set of 8 tissue microarrays obtained from 88 radical prostatectomy specimens (Gleason sum 5 and 6: 23 spots, GS 7: 44 spots, GS 8 and 9: 21 spots). Staining intensity was scored as negative (1), faint/equivocal (2), moderate (3) or strong (4).

Harvesting functional samples in specimens with small tumour volumes [mean tumour volume 12.6 ± 8.6%, range (3–30%)] was critical to the study and led to the development of an original method where the location of relevant samples in a slice of fresh tissue taken at the level of the *veru-montanum* was inferred from the analysis of two adjacent whole-mount sections. Before whole-mount sections could be made available for analysis, the slices had to be kept at –20 °C for 2 d. Preliminary studies of transurethral resection chips stored at –20 °C confirmed the stability of prostate SphK1 activity for up to 2 weeks (data not shown).

A 2-fold increase in prostate cancer enzymatic activity (ratio SphK1Tu/Normal: 2.4, 95% CI: 1.5–3.4) was observed and correlated to adverse clinical features. Interestingly, SpHK1 demonstrated higher odds ratio for treatment failure than the most robust clinical predictors. This report therefore gives evidence that SphK1 expression and oncogene activity impacts on the natural history of human prostate cancer.

Nava and Cuvillier first showed that SphK1 activity was associated to radioresistance of prostate cancer cell line[8] demonstrating the link between resistance to treatment and increased SphK1 activity. This concept was further extended by our group and others to other cell lines and treatments including chemotherapy,[15,28] targeted therapy[6] and androgen suppression.[7] One step further, we recently reported two proof-of-concept studies where direct inhibition of SphK1 could revert chemoresistance in prostate cancer[15] and imatinib-resistance in chronic myeloid leukaemia.[5]

Finally, a strong link between SphK1 and hypoxia was recently demonstrated.[27] Hypoxia contributes to the development of aggressive phenotype, resistance to radiation therapy and chemotherapy. It is in general predictive of a poor clinical outcome[41] as recently confirmed in the prostate by Turaka and coworkers who observed that reduced oxygen concentrations were strongly associated to tumour grade and PSA levels and independent predictors of tumour recurrence after radiation.[42] In that line, the recent report in

a prostate cancer model that SphK1 was upstream of Hypoxia-Inducible Factor-1 alpha in the molecular cascade triggered by hypoxic conditions[27,43] brought further evidence that sphingosine kinase-I bears the hallmarks of a target of choice in prostate cancer.

It could also carry potentials in the risk stratification of prostate cancer. Because of potential discrepancies between mRNA and protein expression in cancer[44] establishing strong correlations between protein expression (immunohistochemistry) and function (enzymatic activity) and clinical features was required to extend to the prostate cancer the mRNA preliminary data obtained in other tumour sites. The present 2-fold increase in SphK1 activity is in keeping with SphK1 mRNA increases observed in other tumour sites.[11] It confirms that, when technically feasible, mRNA expression might be a valid proxy for SphK1 metabolic activity measurement. Of note, an increase of the same magnitude was shown in breast cancer to correlate to adverse factors such as tumour grade and oestrogen receptor status.[21] Albeit shown here to be feasible, measuring enzymatic activity in freshly retrieved human tissue and comparing cancer and non-cancer controls is technically demanding and hardly amenable to routine use as a prognostic factor. On the contrary, immunochemistry of readily available paraffin-embedded tissues – which in the present work confirmed that similar to enzymatic activity, protein expression was related to aggressive features – could be used to research SphK1 dysregulation in archived human specimens.

As a whole, the correlation of activity and expression with adverse clinical features is in line with preclinical reports on the central role of sphingosine kinase-1 pathway in prostate cancer response adaptation to hypoxia[27] and resistance to treatment.[7–9,15,27] Addressing the sphingolipid rheostat may thus open new avenues in prostate cancer risk-assessment and treatment.

5. Conclusion

The reported relationship between activity and prognostic factors such as PSA, tumour volume and positive resection margin supports a central role for SphK1 in the early stages of prostate cancer, complementing preclinical studies where it was shown to be a key regulator of response to local treatment and chemotherapy. We believe that SphK1 fully meets the main criteria required for the development of targeted therapy[45] and supports promising perspectives in prostate cancer risk-assessment and treatment.

Conflict of interest statement

None declared.

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