Expression profile of sphingosine kinase 1 isoforms in human cancer tissues and cells: importance and clinical relevance of the neglected 1b-isoform

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Abstract

Introduction: Overexpression of sphingosine kinase 1 (SphK1) is casually associated with many types of cancer and inhibitors of SphK1 sensitize tumors to chemotherapy. SphK1 is expressed as two major isoforms, SphK1a and SphK1b. To date, no information has been reported on the SphK1 isoform expression profile and its clinical relevance. The purpose of this study was to examine the expression profile of the SphK1a and SPhK1b isoforms in human cancer and noncancer tissues and cell lines and to explore its clinical relevance.

Methods: We used PCR to qualitatively examine the expression profile of these two isoforms in breast, liver, and prostate cancer tissues plus paired adjacent tissues, and in 11 cancer and normal cell lines (breast, cervical, bone, prostate, colon, brain, and mesothelioma tumors and benign and human kidney cells).

Results: We found that SphK1a was ubiquitously expressed in all cancer cells and tissues tested; in contrast, SphK1b was only expressed in selective cell types in breast, prostate, and lung cancer.

Conclusion: Our data suggest that SphK1a is important for generic SphK1/S1P functions and SphK1b mediates specialized and / or unique pathways in a specific type of tissues and could be a biomarker of cancer. This discovery is important for future SphK1-related cancer research and may have clinical implications in drug development associated with SphK1-directed cancer treatment.

1. Introduction

The Sphingosine kinase 1 (SphK1) isoforms are bioactive lipid enzymes involved in the phosphorylation of sphingosine to produce the active form sphingosine-1-phosphate (S1P), thereby regulating the balance between S1P, sphingosine and ceramide [1–3]. Maintaining the balance of SphK/S1P signalling is important in normal cellular and physiological processes including cell proliferation, survival, cell death, adhesion, angiogenesis, migration, and inflammation and is key in the prevention and progression of cancer [4–12]. Here, we examined the expression profile of endogenous SpK-1a and – 1b in cancer tissue samples and matching noncancer tissues from the 3 most common types of cancer patients (lung, breast, and prostate cancer) and complemented these studies by examining SphK-1a and 1b expression in a variety of human cancer and non-cancerous cell lines. It is expected that the study will advance our understanding of the functional significance of relative SphK1a and SphK1b in normal and cancer cells, and the clinical relevance of these isoforms. Our data support that SphK1b has a significant impact on cancer progression and/or treatment outcome, an effect that is more likely to be cancer-type specific. Conceivably, the cell specificity of SphK1b expression may play a significant role in modulating or enhancing SphK1 signaling and physiological functions.

Overexpression of SphK1 is linked to many cancer types [8,13,14] and has been identified as oncogenic due to ‘gain of function’ in preference to any identifiable mutations. In other words, cancer cells have become

reliant on SphK1 expression for survival, a phenomenon termed 'non-oncogenic' addiction [15]. There are two major human SphK1 isoforms, SphK1a (SphK1a-isoform 3, SphK1-43kDa - GenBank accession: NM_001142601; UniProt ID: Q9NYA1-1) and SphK1b (SK1b-isoform 2, SphK1-51kDa - GenBank accession: NM_182965; UniProt ID: Q9NYA-2) [16]. Both isoforms share common amino acid sequences, with the exception that the SphK1b isoform has a unique extra 86 amino acid sequence at the N-terminus [16]. This unique N-terminal domain alters the conformation of the SphK1b isoform, allowing in common and additional distinct interaction patterns of SphK1a and SphK1b [17]. Our knowledge of alternative splicing of SphK1 isozymes on cell function is very limited, and many aspects of the functions of the 2 isoforms have not been explored. The first SphK1 isoform described was SphK1a (43kDa), or isoform 3, and most of the *in vitro* human SphK1 functional studies have focused on this isoform [8]. SphK1a and SphK1b share the same catalytic domain [18] and no significant differences in S1P activity have been observed [17]. Moreover, there are no overt phenotypic differences in cell morphology or cell proliferation when either SphK1a or SphK1b are stably overexpressed, however, there is little doubt that the expression of one or both isoforms has the capacity to alter downstream cell signaling events [17]. We have previously shown that the expression of the SphK1a and SphK1b isoform in a target cell determines the nature and magnitude of the response to SphK-S1P signalling and can be initiated similar and distinct downstream signalling pathways that alter cellular events [17].

There is some evidence to suggest that changes in SphK1a/SphK1b ratios affect drug treatment regimens and altered vulnerability to cancer treatment. In breast cancer cells, specific protein interactions with either SphK1b or SphK1a isoform alters SphK1 signaling events [17]. For example, SphK1b preferentially interacts with dipeptidyl peptidase 2 (DPP2), a protein involved in the regulation of glucose metabolism [19]. Treatment of hormone-dependent breast cancer cells with a DPP2 inhibitor increases SphK1b expression, but does not have an effect on SphK1a expression [17]. In prostate cancer cells, alteration in SphK1a and SphK1b expression is associated with chemotherapy resistance [20]. Changes in SphK1a and SphK1b levels result in specific changes in ceramide and S1P levels leading to induced apoptosis of androgen sensitive, but not androgen-independent, LNCaP prostate cancer cells [20]. These studies indicate that the imbalance of SphK1a and SphK1b may be causally associated with cancer progression and resistance to chemotherapy [20,21].

Most previous *in vivo* studies so far have explored overall SphK1 expression and activity, not individual isoforms. Historically, most *in vitro* studies use overexpression of the SphK1a isoform to explore SphK1 function, and SphK1b is relatively neglected. Differential isoform expression is proving to be important in cancer definition in general [22], and we and others have shown the subtleties of differential functional effects of SphK1 isoform expression in cell studies *in vitro*. Although it is important to examine the endogenous expression status of SphK1a and SphK1b in different cell and tissue types, no specific study thus far has been conducted to explore the expression profile of SphK1a and SphK1b in different cancer cells and tissues.

2. Materials And Methods

The materials and methods are described in brief. Detailed description can be found in the thesis by Nahal Haddadi, 2019 [23].

2.1 Cell culture

Cancer cells were routinely cultured in either Dulbecco's modified eagle medium (DMEM) or Roswell Park Memorial Institute medium (RPMI1640) with 10% foetal bovine serum (FBS) depending on the cell type. Apha-minimum essential medium (α -MEM) supplemented with 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, 20% FCS, 10 ng / ml recombinant basic fibroblastic growth factor was used for primary cell lines. The Lonza MycoAlert™ Plus mycoplasma detection kit was routinely used to check mycoplasma negatively.

2.2 MCF-7SphK1 isoform expressing control cell lines.

MCF-7 breast cancer cells (ATCC:HTB-22™) were used to stably overexpress SphK1a (43kDa) and SphK1b (51kDa) containing a C-terminal FLAG-TAG previously described [17,24]. The SphK1a and SphK1b isoforms were derived from human umbilical vein endothelial cells (HUVEC) [17,24]. SphK1a and SphK1b cDNA clones expressed the SphK1 isoform 3, variant 3 (NM_001142601), and SphK1 isoform 2, variant 2 (NM_182965)[16].

2.3 Cell lines.

Details of cell lines used for the detection of SphK1a and Sphk1b isoforms are listed in Table 1. Mesothelioma cell lines were kindly donated by Dr Glen Reid (Asbestos Disease Research Institute – ADRI, Concord, Sydney) and Drs Rayleen Bowman, Walter Berger and Walter Klepetko, as indicated. The prostate cancer cell lines were kindly donated by the late Robert Sutherland (Garvan Institute of Medical Research, Sydney).

Table 1
Summary of SphK1 isoform expression in different cell line types (in vitro)

Cell type	Cell origin	SphK1a*	SphK1b**
Breast	epithelial	2/2	1/2
Cervical	epithelial	1/1	0/1
Bone	epithelial	1/1	0/1
Prostate	epithelial	4/4	0/4
Colon	epithelial	2/2	0/2
Brain	epithelial	1/1	0/1
Mesothelioma	epithelioid	6/6	5/6
Mesothelioma	biphasic	3/3	0/3
Mesothelioma	benign	3/3	2/3
Human embryonic kidney (HEK)	epithelial	1/1	0/1
Total		24/24	8/24
* SphK1a primers, F3-R4; ** SphK1b primers, F1-R2			

2.4 Collection of clinical cancer tissue samples.

Human breast and prostate cancer tissues and adjacent matching tissues were collected from the Third Affiliated Hospital of Sun Yet-sen University. Human liver cancer, hepatocellular carcinoma (HCC) tissues, as well as adjacent tissues from the cancer site, were from the First Affiliated Hospital of Sun Yet-sen University. The use of human tissues for this project was approved by an institutional approval, Human Ethics GZSCHE 2016 – 00122.

2.5 Western blots.

Cells were lysed in cell lysis buffer for the isolation of whole cell proteins and protein levels were estimated using the BioRad BCA assay (Biorad Lab Inc. CA) as previously described [25,26]. Equal amounts of protein were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. The overexpression of the isoforms in MCF-7SphK1a and MCF-7SphK1b was verified by Western blot analysis using Anti-Flag m2 mouse F1804-1MG from Sigma-Aldrich.

2.6 RNA extraction from cell lines.

Total RNA was isolated from cultured cells using either 1) TRIzol (Life Technologies) extraction and RNeasy Plus Mini system (Qiagen), or 2) the Maxwell ESC simply RNA cell system (Promega). Cultured cells were harvested and processed according to the manufacturer's instructions. In all cases, the quality

and quantity of RNA were determined by agarose gel electrophoresis and nanodrop absorbance measurements.

2.7 RNA extraction from clinical tissue samples.

Fresh samples of breast, prostate, and HCC cancer tissues and adjacent non-cancer tissues were stored in RNA*later*. RNA extraction was performed using TRIzol TM according to the manufacturer's instructions. Total RNA purity and concentrations were measured using a spectrophotometer at wavelength 280 nm.

2.8 Reverse transcription polymerase chain reaction (PCR).

Reverse transcription (RT) was performed using the SuperScript III First Strand Synthesis SuperMix (Life Technologies) with oligo (dT) priming, according to the manufacturer's protocol. RT-PCR reactions were optimized and conducted using the Eppendorf master cycler gradient instrument.

2.9 Optimisation of the SphK1-PCR primers.

SphK1-specific primers were designed to pair in different combinations (Suppl. Figure 1) with locations as shown on the SphK1 sequence (accession number NM_182965.2) (Suppl Fig. 1A). The expected sizes of the SphK1-PCR amplified products, alongside the SphK1 sequence locations are listed in Suppl. Figure 1B. Each of the 6 primer sets were tested to determine the optimal PCR conditions for each primer set using cDNA derived from the MCF-7-SphK1b and SphK1a stably transfected cells.

3. Results

3.1. Differential expression of SphK1a and SphK1b isoform is cell line dependent

In the absence of antibodies to clearly define the true nature of endogenous SphK1a (43kDa) and SphK1b (51kDa) protein expression by Western blot, we chose to develop a simple, economic, easy to use, differential SphK1a and SphK1b RT-PCR technique. Using this SphK1-RT-PCR diagnostic test, we can determine unique SphK1b expression in any cell and tissue type. We designed and evaluated a number of specific SphK1 PCR primers, forward (F) and reverse (R) (Suppl. Figure 1 and Fig. 1A). The N' terminal region of the SphK1b-a gene is a high G-C rich area and contains a number of secondary hairpin loop structures, making the upstream 'SphK1b-a' region difficult to amplify. After careful testing, we chose a unique 164bp (base pair) PCR primer set (F1, R2), which overlaps the SphK1a-1b N' terminal region thus identifying cells containing only SphK1b, and the 289bp PCR primer set (F3, R4), within the SphK1a region picking up both SphK1 isoforms. These two PCR primer sets (F1-R2) and (F3-R4) proved to be the most robust and consistent for routine testing of SphK1 isoform products in both cell lines and patient tissue samples. Cells stably overexpressing SphK1a and SphK1b manufactured previously [17], were used as controls to test the effectiveness of PCR primers and the efficiency of amplification of SphK1 PCR products. Overexpression of MCF-7 (SphK1a-43kDa; isoform 3) and MCF-7 (SphK1b-51kDa; isoform 2) was confirmed by Western blot (Fig. 1b). MCF-7 cells have very low but detectable levels of endogenous

SphK1a and 1b. We tested 8 different cancer cell types for SphK1a and 1b expression, namely, breast, prostate, colon, brain, bone, ovarian, and mesothelioma (epithelioid and biphasic), as well as benign mesothelioma and human embryonic kidney (HEK) (24 cell lines), as listed in Suppl. Table 1. All cell lines, irrespective of cell type, showed the 289 bp PCR corresponding to a region within the SphK1a domain (Fig. 1b). In contrast, the 164 bp PCR product, which is unique for the SphK1b isoform, was cell line specific. SphK1b was only detected in selective breast and mesothelioma cell lines. The unique SphK1b primers (F1-R2) did not detect a product in prostate cancer (androgen dependence and independence), colon, ovarian, brain, and bone cell lines. Of the 2 breast cancer cell lines tested, only MCF-7 cells detected SphK1b expression. Interestingly, the majority of the mesothelioma epithelioid cell lines expressed detectable SphK1b (5/6) as well as 2/3 benign mesothelioma cells. The 3 biphasic mesothelioma cell lines tested did not express detectable SphK1b products. These results are summarized in Table 1.

Table 2
Summary of SphK1 isoform expression in liver, prostate and breast cancer and non-tumour tissue.

Tissue type	SphK1a* (cancer)	SphK1a* (adjacent)	Total	SphK1b** (cancer)	SphK1b** (adjacent)	Total
Liver	6/6	6/6	12/12	0/6	0/6	0/12
Prostate	7/7	7/7	14/14	5/7	4/7	9/14
Breast	14/15	10/15	24/30	9/15	8/15	17/30
Total	27/28	23/28	50/56	14/28	12/28	26/56

* SphK1a primers, F1-R2,** SphK1b primers, F3-R4.

3.2. Expression of SphK1a is ubiquitous in different human tissue types, SphK1b isoform is tissue-type dependent

Resections from 6 patients diagnosed with HCC, 7 patients diagnosed with prostate cancer, and 15 patients with breast cancer, along with matching adjacent tissues were analysed for expression of SphK1a and SphK1b isoforms using RT-PCR. Patient details, diagnosis information, age of diagnosis, subtype, gender, and hormonal status, where appropriate, alongside SphK1 isoform expression analysis are listed in Suppl. Table 2. Visual representations of the SphK1a and SphK1b PCR amplified products are shown in Fig. 2.

The SphK1a isoform was expressed in all liver cancer tissues and corresponding adjacent tissues, in contrast, the SphK1b isoform was not detected in any of the liver cancer tissues or corresponding adjacent tissues (Table 2, Fig. 2A).

All human prostate samples, both cancer and adjacent tissue, expressed the SphK1a isoform (Table 2, Fig. 2B). The SphK1b isoform was detected in 71% (5/7) prostate cancer tissues and 57% (4/7) corresponding adjacent tissues; there was no demarcation in SphK1 isoform expression to distinguish

tumour stage (Table 2, Fig. 2B). In contrast, no endogenous expression of SphK1b was observed in any of the prostate cancer cell lines tested *in vitro*, as shown in Table 1 and Fig. 1.

There appeared to be some discrepancies in the detection of SphK1a and SphK1b in breast tissue. In breast tissue, 93% (14/15) breast cancer samples and 66% (10/15) corresponding adjacent tissue expressed detectable levels of SphK1a (Fig. 2C, Table 2). Detection of the unique SphK1b isoform was 60% (9/15) and adjacent 53% (8/15) breast cancer patient samples. When SphK1 isoform expression status was considered by breast cancer grade (Table 3) and by hormonal status (ER + or ER-) (Table 4), we found that most of the Grade 1, 2, IDC and ILC tissue resections had undetectable levels of SphK1b isoform, whereas all Grade 3 breast tissues proved positive for SphK1b expression (Table 4). Hormone receptor positive breast cancers were more likely to express both SphK1 isoforms.

Table 3
Analysis of SphK1 isoform expression in breast cancer patients by Grade

Grade	Total no.	Cancer		Adjacent	
		SphK1a	SphK1b	SphK1a	SphK1b
Grade 1 + 2	8/15	7/8	2/8	4/8	2/8
Grade 3	6/15	6/6	6/6	6/6	6/6
ILC	1/15	1/1	0/1	0/1	0/1
ILC = Invasive lobular carcinoma.					

Table 4
Analysis of SphK1 isoforms in breast cancer patients by hormonal status.

Type	Total no.	Cancer		Adjacent	
		SphK1a	SphK1b	SphK1a	SphK1b
Breast overall	15	14/15	9/15	10/15	8/15
ER+ (8/15)	8	8/8	6/8	8/8	5/8
ER- (7/15)	7	6/7	3/7	3/7	2/7

In summary, profiling SphK1 isoform expression of patient tissue samples show that SphK1a is detected in liver, prostate, and breast, both in cancer and adjacent tissues, however, there are some discrepancies in individual breast samples where SphK1 is not detected. These results are consistent with the findings that all cells tested *in vitro*, independent of cell type, detected a product within the common SphK1a. Expression of SphK1b was found to be cell type specific, it was not detected in the liver, and was not expressed in all prostate and breast tissues tested (Table 2 and Figs. 2A, 2B, and 2C). Again, this is consistent with cell line profiling, where SphK1b is not universally expressed in all cell lines and cell types.

In summary, all human tissues tested were positive for SphK1 independent of tissue type, and although this RT-PCR assay is not quantitative, there was no clear difference in SphK1 expression between cancer and adjacent tissues. The SphK1b isoform was not detected in any sample of human liver patients. SphK1b was detected in over 64% of human prostate and 57% of breast tissues tested. suggesting a functional role for SphK1b in reproductive tissues. Given that breast cancer cells and prostate cancer cells have shown different drug responses depending on SphK1a or SphK1b expression, this may have some relevance in potential drug therapy [17,21,27,28].

3.3 Comparative stability of SphK1a and SphK1b RNA structure

Given that in all experiments SphK1b was less abundantly expressed than SphK1a, we compared the folding of mRNA and the secondary structures formed by the 2 isoforms to look at the stability of the two RNA structures. Using computational modelling predictions [29], the b-isoform RNA was predicted to be more unstable than the shorter SphK1a isoform (Fig. 3), consistent with our previous findings, where the SphK1a protein isoform was found to be more stably expressed [17]. This prediction is based on the concept that mRNAs associated with stress have higher free energy, longer loop length, and more single strands that enable them to undergo conformational changes in response to their environment [30]. Moreover, some mRNA isoforms transcribed from a single gene can have different half-lives depending on their environmental conditions [31,32]. Investigations by Geisberg and his group, examining stabilizing and destabilizing elements in mRNAs and isoform half-lives, suggested that double-stranded structures at the 3'region' are crucial in mRNA stability [33]. Taking all these predictions into account, SphK1b with the higher free energy and the longer loop length is predicted to be less stable than the SphK1a isoform.

4. Discussion

The SphK- 1a and − 1b isoforms have distinct as well as compensatory functions in the cell, however we know little about their definitive expression in human normal and cancer tissues. The little we do know is mainly derived from the few *in vitro* studies where the individual isoform is overexpressed and may have significance in altering SphK1 function, both in normal physiology and in cancer pathophysiology. The main aim of this study was to determine if both endogenous SphK1a expression and SphK1b were expressed in human tissue using a simple PCR test to, and if the loss or gain of one or both isoforms could be linked to cancer. We found that SphK1a was ubiquitously and dominantly expressed in all cell lines and human tissue types tested, implying that SphK1a is important for generic SphK1/S1P functions and may be the principal isoform involved in mediating the known pro-survival and cell maintenance functions of SphK1 in cell [8]. Conversely, the selective cell and tissue expression of SphK1b in breast, prostate, and lung, suggests this isoform mediates specialised and/or unique pathways, whereby the 1b-isoform tissue specificity is important in regulating or modulating signalling pathways for specific cell functions. Although one of our objectives was to determine if there was a demarcation between SphK1a

and SphK1b expression, comparing cancer tissues and adjacent tissues, in this study we did not find any clear distinction between the isoform between cancer tissues and adjacent tissues.

There is little, if any, information available on the endogenous expression of SphK isoforms in human tissues, and most of the *in vitro* human SphK1 functional studies have either focused on the SphK1a shorter isoform or not specified which isoform is being studied, reviewed in [8,16]. However, knowledge of the expression of SphK1a and 1b in patient tumors may be significant in the selection of treatment given *in vitro* experiments showing the importance of 1b-isoform in drug sensitivity [17,20,21]. *In vitro* studies focusing on the expression of the two main SphK1 isoforms (1a and 1b) suggest that an imbalance or aberrant expression of these isoforms plays a role in subverting the signaling pathways involved in resistance to treatment [8,16]. In particular, discrepancies in SphK1 isoform expression have been observed in tumors of the reproductive glands, including prostate [20,21,28] and breast [17]. Proteomic studies by our group demonstrated common interacting partners for both isoforms, while the unique SphK1b-86kDa upstream region provided subtle and not so subtle differences in protein interactions and downstream signalling pathways in MCF-7 breast cancer cells [17]. Experiments showing the importance of the 1b-isoform in drug sensitivity have been conducted in cell lines *in vitro*, however, how this translates to clinical importance is unknown. The discovery in this study that the 1b-isoform is expressed in breast, prostate, and lung, supports further experiments into exploring how alterations in SphK isoforms may affect drug responsiveness and drug resistance in both prostate and breast cancer [17,20,21].

It is observed that both in cell lines (*in vitro*) and in human tissues (*in vivo*), when expressed, the SphK1b isoform was consistently less abundant compared to the SphK1a isoform, given the limitation that this assay was qualitative and not quantitative. When we compared the mRNA folding and secondary structures formed by the 2 isoforms, using computational modelling predictions [29], the b-isoform was predicted to be more unstable than the shorter SphK1a isoform (Fig. 3). These predictions, although implied, may provide one reason as to why the SphK1b isoform is seemingly of lesser abundance compared to its shorter 1a isoform, *in vitro* (cell lines) and *in vivo* (patient tissue samples), i.e. SphK1b RNA may be more unstable and/or susceptible to degradation within the cell milieu.

In our study, at the protein level, the unique C-terminal 86 amino acids of SphK1b allow conformational changes to facilitate preferential isoform interactions with proteasomal proteins and ubiquitin-protein ligases [17]. Therefore, at both the mRNA and protein level, the longer SphK1b isoform may preferentially be more susceptible to degradation control.

We demonstrate that SphK1a is expressed in all cancer and tissue types tested, and expression of the 1b-isoform is cell- and tissue-specific; resected human liver samples did not express the 1b-isoform, whereas the human prostate and breast expressed both SphK1 isoforms. The selective tissue expression of SphK1b suggests that the 1b isoform is important for specialized cell functions. Speculatively, a possible function for SphK1b in reproductive tissues (prostate and breast) may be hormone related. As mentioned in the limited published *in vitro* information available, the expression of SphK1b may negatively affect treatment outcome in some breast and prostate cancers. A previous study, demonstrating the important

differences of SphK1a and SphK1b as drivers of distinct and common signaling pathways of the two major SphK1 isoforms in a hormone-responsive breast cancer cell model [17], provides some insight into the importance of the two isoforms in directing cell signaling pathways and function. The findings that both isoforms are expressed in reproductive tissue in breast and prostate cancer samples from patients and adjacent tissues support the diverse signalling pathways of both isoforms and are important in the normal physiological and abnormal pathophysiological function of the cell.

At this stage, we have no direct evidence to suggest SphK1 isoform association with chemo-resistance or hormone resistance. Nonetheless, aberrant SphK1 isoform expression has been causally associated with prostate cancer therapy resistance in preclinical laboratory experiments. Although SphK1 inhibitors have been successful in increasing chemo-sensitivity [34–36], there are examples demonstrating discriminatory chemo-sensitivity depending on the expression of the two major SphK1 isoforms in hormone-dependent and independent prostate cancer cell lines [37,38]. Similarly, differences in SphK1a and SphK1b expression had different functional responses in breast cancer cells [17]. One interesting finding was the expression of SphK1a and SphK1b in all grade 3 breast cancers and adjacent tissues, whereas the 1b-isoform was not detected in the majority of grade 1 and 2 breast cancers. Potentially, from a cancer prognostic viewpoint, this information will aid our understanding of SphK1 isoform expression and its relevance to treatment outcomes in patients with hormone-responsive cancers.

The limitations of our study included that the assessment of SphK1 isoform expression was done based only on data from qualitative PCR, rather than quantitative PCR. Furthermore, the sample size of the patients is relatively small, limiting the generalization of the findings. Therefore, interpretation of the findings should be done with caution.

Conclusions

This is the first report specifically examining the expression profile of SphK1a and SphK1b in 3 common human cancer tissues (lung, breast, and liver) and 11 human cell types. Our results provide the first insight into the ubiquitous nature of SphK1a expression and the selectively of SphK1b expression in different types of cancer and adjacent tissues, supporting SphK1b has a significant impact on cancer progression and/or treatment outcome, an effect that is more likely to be cancer-type specific. Conceivably, the cell specificity of SphK1b expression may play a significant role in modulating or enhancing SphK1 signaling and physiological functions. Alternatively, SphK1b expression may function as a biomarker for some cancer types.

Declarations

Data Availability

The data used to support the findings of this study are included within the article.

Author contributions:

Conception: EM, YL; interpretation or analysis of data: HC, NH, DH, NTN, SC, YL, EM; preparation of the manuscript: EM, YL, HC, NH; revision for important intellectual content: HC, NH, DH, NTN, SC, YL, EM; supervision: EM, YL, SC.

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Figures

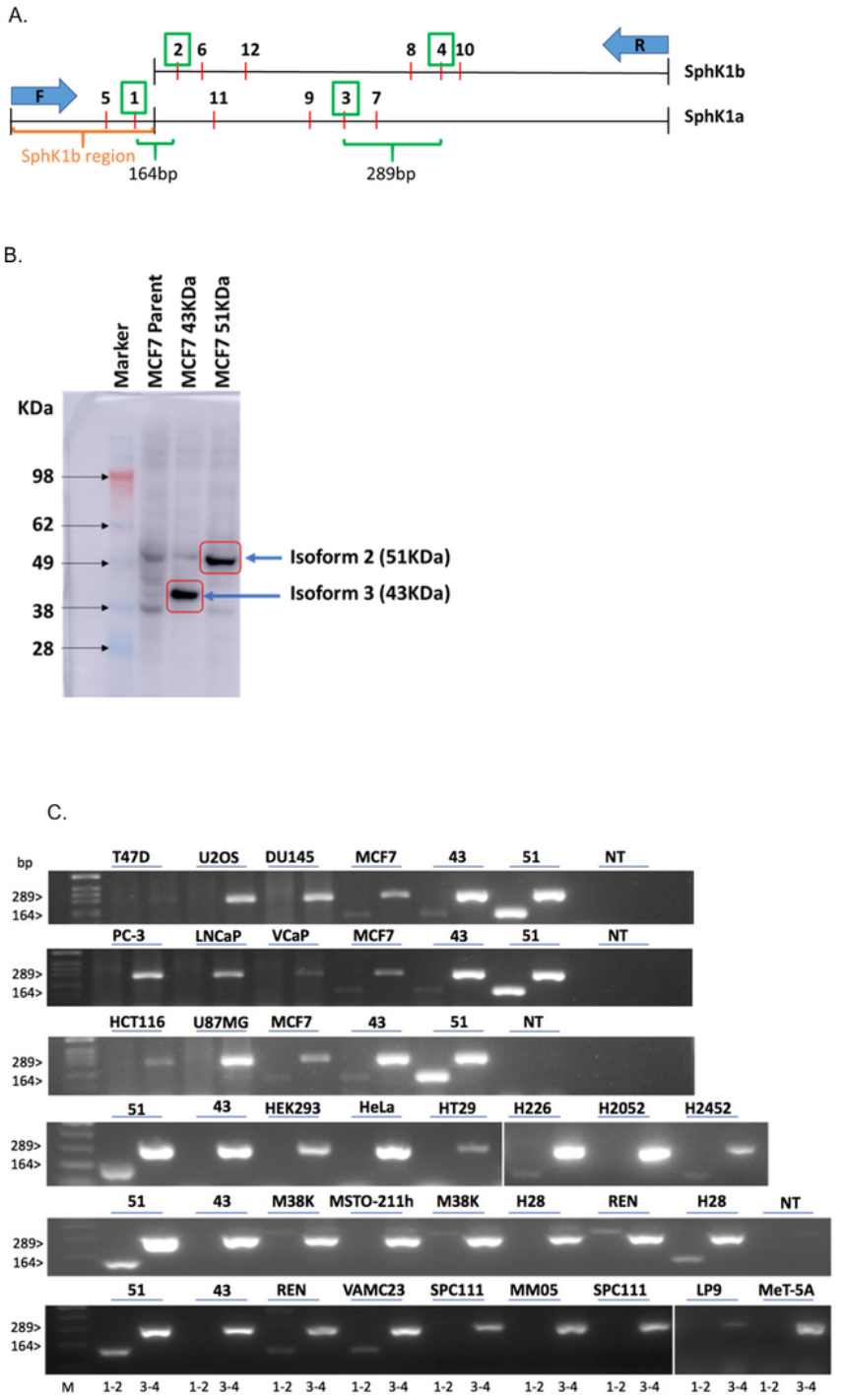

Figure 1

Differential expression of SphK1a and 1b isoforms in cancer cells *in vitro*. A. Schematic of the SphK1a and 1b primers (F=forward, R=reverse) locations (reference Suppl Fig. 1, primer sequences and SphK1 sequence locations). B. Western blot visulalistion of SphK1a (isoform 3, 43kDa) and SphK1b (isoform 2, 51kDa) in stably transfected MCF7 cells detected using a Flag-tag antibody [17]. C. Representative gels of RT-PCR amplification products of SphK1 isoforms from cancer and non-cancer cell lines (described in

Tables 1 and 2). RT-PCR was performed using SphK1 primers F1-R2 (1-2) and F3-R4 (3-4). Primers F1-R2 amplified a product of 164 bp in length (overlapping the SphK1a-b N' terminal region) and primers F3-R4 amplified a product of 289 bp (within the SphK1a region). MCF-7SphK1b (51) and MCF-7SphK1a (43) and no DNA template (NT) were used as controls for every set of RT-PCR reactions. These panels are representative of repeat experiments.

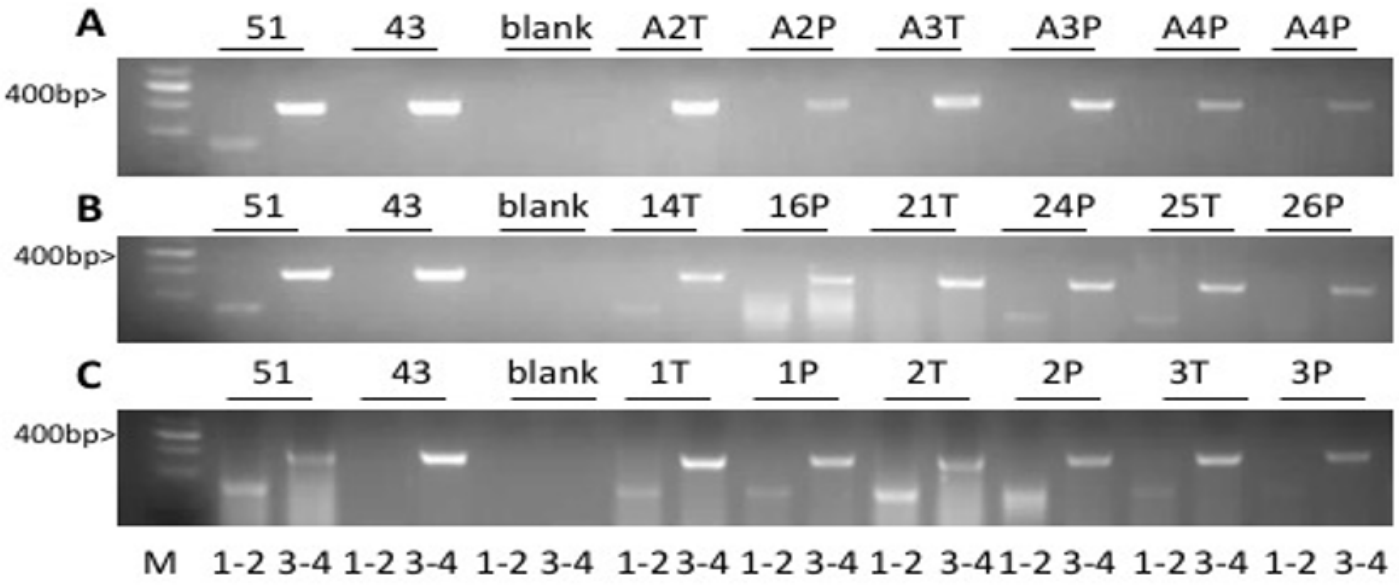

Figure 2

Comparative analysis of SphK1a and SphK1b isoform expression in human cancer and adjacent tissues. Representative gels of RT-PCR amplification products of SphK1 isoforms from human cancer and adjacent tissue samples. RT-PCR was performed using SphK1 primers F1-R2 and F3-R4. Primers F1-R2 amplified a product of 164 bp unique to the SphK1b isoform and primers F3-R4 amplified a product of 289 bp within the SphK1a region. A) Liver, B) Prostate and C) Breast cancer and adjacent tissues. MCF-7SphK1b (51) and MCF-7SphK1a (43) and no DNA template (NT) were used as controls; T = tumour, P= adjacent tissue. Each sample was amplified 2x with similar results.

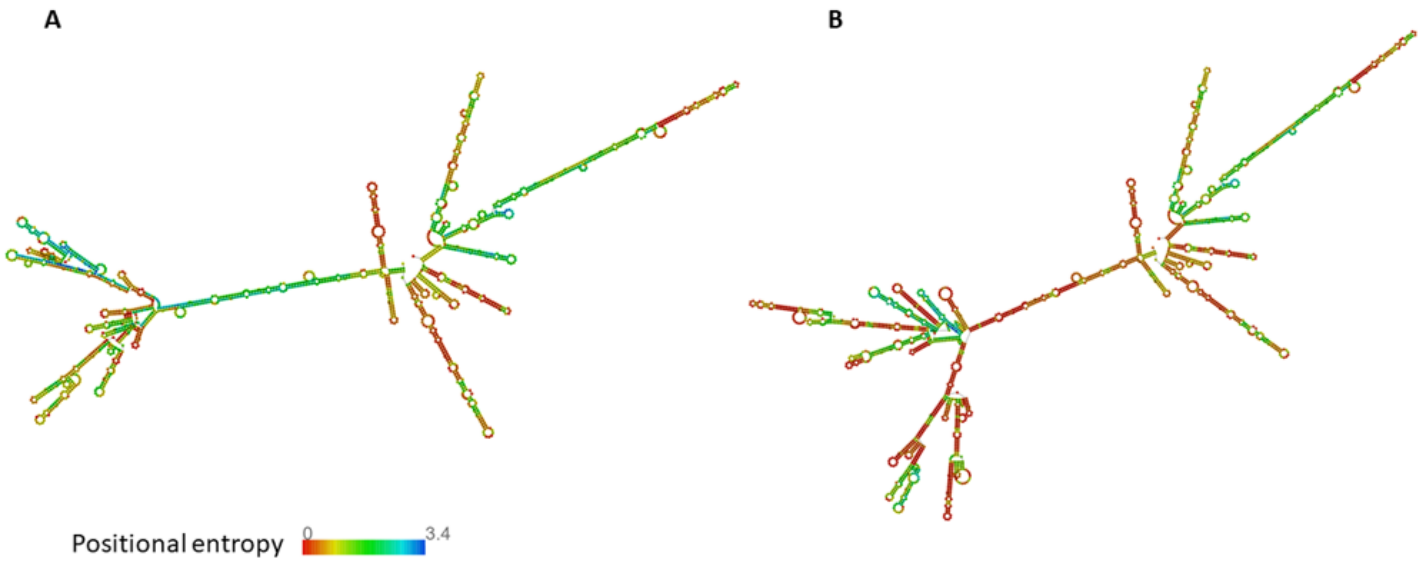

Figure 3

RNA secondary structure prediction by minimum free energy that may have higher fidelity of the predicted structures for SphK1a (A) and SphK1b (B) isoforms are shown. The positional entropy with low entropy are predicted with high confidence. The mRNA secondary structure fold predictions were performed based on highly probable base pairs and the lowest free energy structure for each sequence as determined by RNAfold WebServer on The ViennaRNA Web Services (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi).

Supplementary Files

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- SupplementaryFigureTables.pdf