Does Sphingosine Kinase 1 (SPHK1) Play a Role in Endometriosis?

Spielt Sphingosinkinase 1 (SPHK1) eine potenzielle Rolle bei Endometriose?

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Abstract

Sphingolipids are important constituents of cell membranes, which play key roles as regulatory molecules for various cellular functions. Sphingosine-1-phosphate produced by sphingosine kinase 1 (SPHK1) promotes cell proliferation, regulates angiogenesis, and influences invasion as well as the attachment of cells. Since these processes are believed to be involved in the development of endometriosis, we analyzed the expression of SPHK1 in human eutopic and ectopic endometrium using immunohistochemistry and microarray analysis. Epithelial cells of both eutopic and ectopic endometrium showed highly variable immunostaining with polyclonal antibody directed against SPHK1. However, strong expression of SPHK1 was largely restricted to the epithelial endometrial cells of peritoneal endometriotic lesions (n = 8/23, 34.8%). Only n = 1/15 (6.7%) of the adenomyosis samples and n = 3/41 (7.3%) of the eutopic endometrium samples displayed strong antigen expression (p = 0.008, χ^2 test). No association between SPHK1 and Ki-67 expression was detectable. Still further research is needed in order to clarify the role of sphingolipids in the development of endometriosis, and particularly in invasive growth.

Zusammenfassung

Sphingolipide sind wichtige Bestandteile der Zellmembran, die eine Schlüsselrolle in der Regulation verschiedener Zellfunktionen haben. Sphingosine-1-Phosphat, das durch Sphingosinkinnase 1 (SPHK1) entsteht, fördert die Zellproliferation, reguliert die Angiogenese und beeinflusst sowohl die Invasivität als auch die Adhäsion von Zellen. Da diese Prozesse in der Entwicklung der Endometriose bekanntermaßen eine wichtige Rolle haben, wurde die Expression von SPHK1 im eutopen und ektopen humanen Endometrium durch Immunhistochemie und Mikroarray-Analysen untersucht. Die Epithelzellen von Endometrioseherden des Peritoneums zeigen häufiger eine starke Expression von SPHK1 verglichen mit denen des eutopen Endometriums oder der Adenomyosis uteri (p = 0,008, χ^2-Test). Eine Assoziation zwischen SPHK1 und Ki67-Epxression konnte nicht gefunden werden. Diese ersten Ergebnisse sollten Anlass für weitere Untersuchungen zur Rolle der Sphingolipide in der Entwicklung der Endometriose, insbesondere des invasiven Wachstumspotenzials, sein.

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Introduction

Together with cholesterol and glycerophospholipids, sphingolipids are important constituents of cell membranes. However, sphingolipids – particularly ceramide, sphingosine, and sphingosine-1-phosphate (S1P) – also play key roles as regulatory molecules for various cellular functions [1,2]. S1P promotes cell proliferation and survival as well as cell differentiation, regulates angiogenesis, and influences cell invasion/migration and the attachment of tumor cells in vitro [3]. In addition, evidence has recently been found that the S1P/SPHK1 pathway contributes to carcinogenesis in colon cancer [4]. Several cell-culture and animal models have indicated that the SPHK1/S1P pathway has carcinogenic potential. In mammals sphingosine is phosphorylated to S1P by two different sphingosine kinases, SPHK1 and SPHK2. SPHK1 is induced by numerous growth factors and cytokines and appears to be a critical regulator of sphingolipid functions [5–7].

Endometriosis is one of the most common gynecological diseases. Although endometriosis has

Table 1 Patients characteristics.

	Sample group Ectopic endometrium A Peritoneal endometriosis	B Adenomyosis	Eutopic endometrium C Endometriosis but no evidence of adenomyosis in hysterectomy specimen	D No evidence of endometriosis or adenomyosis
Total number of samples	n = 23	n = 21	n = 2	n = 19
Age	33.7 ± 5.7	48.9 ± 6.4	47, 49	45.9 ± 4.6
Diagnosis	AFS I/II, n = 20	adenomyosis and AFS I, n = 1		Uterus myomatosis, n = 15
	AFS III/IV, n = 3	adenomyosis and AFS III/IV, n = 2	AFS III/IV, n = 2	CIN, n = 4
		adenomyosis, n = 18		
Menstrual phase				
▸ proliferative phase	n = 9	n = 7		n = 9
▸ secretory phase	n = 5	n = 6	n = 2	n = 6
▸ hormone therapy	n = 4	n = 1		n = 4
▸ atrophy		n = 5		
▸ unknown	n = 5	n = 2		

been the subject of numerous scientific investigations, the central elements involved in the pathogenesis of endometriosis are as yet unexplained [8, 9]. Irrespective of different proposed models for the pathogenesis of endometriosis, it is common belief that both cell differentiation and cell survival play a fundamental role. Therefore the present study investigated the expression of SPHK1 in human eutopic and ectopic endometrium, using immunohistochemical staining.

Methods

Materials

Endometrial biopsies were taken from consecutive patients undergoing hysterectomy, dilation and curettage, and other procedures for benign gynaecological diseases with IRB approval and informed consent as previously described [10]. All peritoneal biopsy specimens were taken from the lateral abdominal wall. Characteristics of the patients are given in **Table 1**. The polyclonal antibody directed against SPHK1 was obtained from Imgenex (IMG-72025, San Diego, CA). Ki-67 staining was done by using a MIB-1 antibody (Dako, Danmark, M7240). Secondary goat anti-mouse antibody (FAST-RED) was purchased from Dianova, Hamburg (Germany). The study was approved by the local ethics committee.

Immunohistochemistry

Immunohistochemical analyses were performed as previously described [7, 11, 12]. In brief, paraffin sections (2 µm) were mounted on Superfrost Plus slides, dewaxed in xylene and rehydrated through graduated ethanol to water. Antigens were retrieved by microwaving sections in 10 mM citrate buffer (pH 6.0) for 20 min at 800 W. Blocking was performed using antibody dilution buffer (DCS-Diagnostics, Hamburg, Germany) at room temperature for 15 min. Subsequently, antibodies were diluted 1 : 100 individually in this buffer. Sections were incubated with antibodies 1 h at room temperature. For negative controls, the primary antibodies were replaced with PBS. For secondary antibody incubations and detection the Dako REAL Detection System Alkaline Phosphatase/RED (Dako, Danmark) was used following the protocol of the supplier and sections were counterstained with Mayer's hematoxylin.

Statistical analyses

All reported p values are two-sided. P values of less than 0.05 were considered to indicate a significant result. The non-parametric Mann-Whitney test and Kruskal-Wallis test were used to compare expression values between different tissues. All analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, IL).

Results

Immunohistochemical analysis of SPHK1 expression in eutopic and ectopic endometrium

We analyzed the expression of SPHK1 in n = 79 endometrium samples from n = 65 patients (**Table 1**). In almost all cases epithelial cells of both eutopic and ectopic endometrium showed immunostaining with polyclonal antibody directed against SPHK1. In stromal cells none or only a weak staining was detected with no differences between eutopic and ectopic endometrium. By contrast, the epithelial endometrial cells of peritoneal endometriotic lesions showed increased expression of SPHK1 (**Fig. 1 a**) in comparison with the epithelial cells of eutopic endometrium (**Fig. 1 b**) and of adenomyosis (not shown). As presented in **Table 2** strong staining was observed in the ectopic glandular endometrial cells for n = 8/23 (34.8%) of the peritoneal endometriosis samples. In contrast only n = 1/15 (6.7%) of the adenomyosis samples and n = 3/41 (7.3%) of the eutopic endometrium samples displayed strong antigen expression ($p = 0.008$, χ^2 test).

Since S1P produced by SPHK1 is known to regulate cell growth we analyzed a potential association between Ki-67 and SPHK1 expression in epithelial cells of eutopic and ectopic endometrium. We determined the percentage of Ki-67 positive cells for n = 54 of the samples which have been analyzed for SPHK1 expression. However, as shown in **Fig. 2** we observed no signifi-

cant correlation of SPHK1 expression and Ki-67 in neither the ectopic nor the eutopic endometrium.

For 42 samples information on SPHK1 expression in epithelial cells and data on the menstrual cycle phase were available (14 samples from ectopic endometrium and 28 from eutopic endometrium). However, no significant differences in SPHK1 expression were found among ectopic endometrium between proliferative phase (n = 7/9) and secretory phase (n = 5/5) or among eutopic endometrium (n = 7/15 and n = 10/13, respectively). These results are in line with gene expression data from microarray analyses of Burney et al. [13]. As shown in **Fig. 3** no significant difference of SPHK1 expression detected by microarray were obtained when comparing eutopic endometrium from different phases of the menstrual cycle. We did also not find a correlation of the immunostaining with the antibody directed against SPHK1 and hormone intake or histopathological endometrial findings in any of the tissues examined.

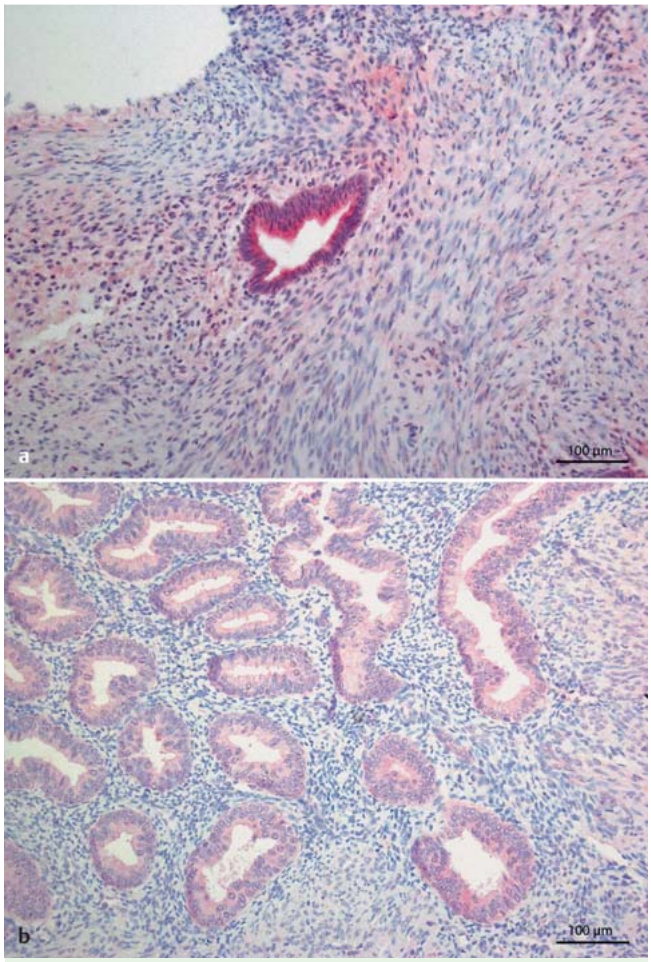

Fig. 1 Immunostaining of sphingosine kinase 1 in ectopic and eutopic endometrium. Representative examples of the results from immunohistochemistry using an SPHK1 antibody (red staining) are shown for samples from ectopic (A) and eutopic (B) endometrium (magnification 20×).

Discussion

Better understanding of sphingolipid function has been hindered by the considerable complexities involved in the networks of the sphingolipid metabolism and their compartmentalization, coupled with inherent experimental difficulties in studying lipid metabolism and function. However, it is known that S1P, produced by SPHK1, promotes cell growth and survival as well as cell differentiation, regulates angiogenesis, influences cell invasion/migration and the attachment of tumor cells in vitro, and inhibits apoptosis [3–5]. In epithelial ovarian cancer (EOC), for example, S1P affects EOC invasiveness in vitro in a dose-dependent manner by regulating extracellular matrix (ECM) proteolysis, matrix metalloproteinase 2 (MMP2), urokinase-type plasminogen activator (uPA), and N-cadherin [5]. Interestingly, it has long been known that these pathways also play an important role in the pathogenesis of endometriosis. From this point of view, the increased expression of SPHK1 we had observed in peritoneal endometriosis lesions provides a basis for considering the extent to which sphingolipids may also be involved in the development of endometriosis. In EOC, a small amount of S1P leads to increased invasiveness in vitro, while a high concentration of S1P leads to inhibition [5]. This does not contradict the hypothesis that increased expression in peritoneal endometriosis lesions, in contrast to eutopic endometrium, plays a role in the invasive growth of endometriosis in vivo and in vitro that has been recognized for many years. No threshold values are known, either for eutopic or ectopic endometrium, for concentrations at which effects on the pathways mentioned above might be reached. Such thresholds could only be assessed using in vitro studies. On the other hand, the present study also identified a high level of SPHK1 expression in mesothelial cells (data not shown). On the basis of the current data it is not clear whether the observed expression of SPHK1 either leads to inhibition or activation of adhesion, proliferation, and invasive mechanisms in the ectopic endometrial cells. The question also arises which might represent the

Table 2 Analysis of SPHK1 expression in glandular cells from peritoneal endometriosis, adenomyosis, and eutopic endometrium in patients with adenomyosis and control individuals.

	Ectopic endometrium		Eutopic endometrium		
Staining pattern	Peritoneal endometriosis	Adenomyosis*	Control	Patients with endometriosis but without adenomyosis	Patients with adenomyosis*
Total	n = 23	n = 15	n = 19	n = 2	n = 20
Negative	0	0	0	0	1
Weak	2	9	8	0	9
Moderate	12	5	8	2	10
Strong	8	1	3	0	0

* In the tissue sections examined from patients with adenomyosis uteri, no endometrium was found in one case and no focus of adenomyosis was found in six cases. As significant results were not seen, no further immunohistochemical examination of additional step sections was carried out.

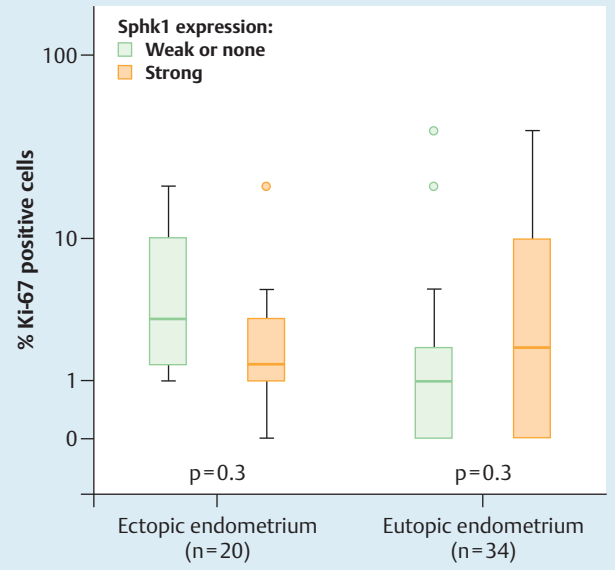

Fig. 2 Correlation of Ki-67 and SPHK1 expression in epithelial cells of ectopic and eutopic endometrium. Box plots of the percentage of Ki-67 positive epithelial cell are shown for the subgroups of ectopic and eutopic endometrium with strong and weak/none SPHK1 expression, respectively. Ki-67 staining results were obtained for n = 54 samples. P values according to Mann-Whitney U-test are given revealing no significant differences.

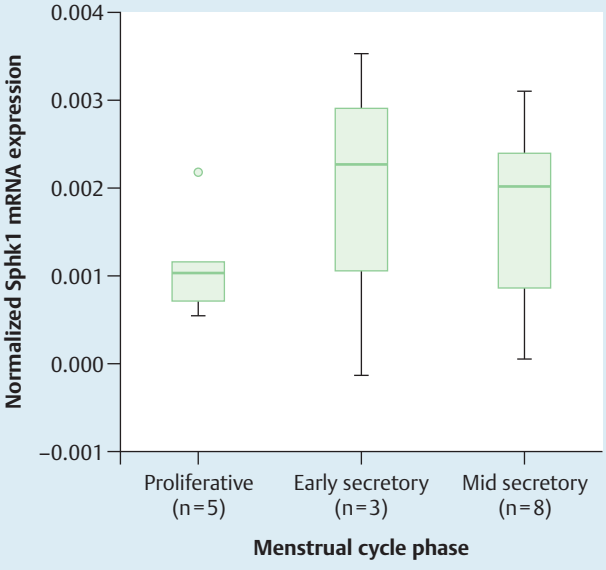

Fig. 3 Analysis of SPHK1 mRNA expression detected by microarray in eutopic endometrium from different phases of the menstrual cycle. Box plots of SPHK1 mRNA expression in eutopic endometrium are given for samples from proliferative, early secretory and mid secretory phase, respectively. Data are based on a microarray study of Burney et al. [13]. No significant difference was obtained (p = 0.49, Kruskal-Wallis test).

appropriate reference tissue in this context – eutopic endometrium, peritoneum, or another type?

Pain is one of the principal symptoms of endometriosis. Numerous hypotheses have been proposed for which factors are decisive in the origination of pain. Simple explanations are altered anatomy whereby adhesions, stenoses, etc., can cause the pain. However, frequently even women with minimal endometriosis suffer from pain. In these cases local production of prostaglandin is thought to play a role. It has been shown in colon carcinoma that SPHK1/S1P is involved in the carcinogenetic process through an influence on the COX-2/prostaglandin E_2 pathway [4]. With regard to endometriosis COX-2, in addition to its role in the origination of pain, may also be involved in the development of the endometriosis lesions themselves. This has been a topic of discussion for several years [14]. In vitro, inhibition by COX-2 inhibitors leads to reduced proliferation and induction of apoptosis in endometrial epithelial cells [15]. In experimental animal models, regression of endometriosis can be achieved by administering COX-2 inhibitors [16, 17]. The above considerations demonstrate that there is a connection between sphingolipids and numerous pathways for which a role in the development of endometriosis has long been known. Further research is needed in order to clarify the role of sphingolipids in the development of endometriosis, and particularly in invasive growth. Thus at the moment the question whether SPHK1 plays a role in endometriosis cannot be answered definitely based on the current available data.

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Competing interests: None.

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