Sphingosine 1-phosphate acts as proliferative and fibrotic cue in leiomyoma cells

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Objective: To determine whether the bioactive sphingolipid sphingosine 1-phosphate (S1P) modulates cellular proliferation and synthesis of fibrotic proteins in leiomyoma differently than myometrial cells.
Design: A basic science study using human leiomyoma and myometrial cells.
Subjects: Not applicable. This is an in vitro study performed on cellular models.
Exposure: Leiomyoma and myometrial cells were treated with S1P, as well as with selective antagonists for S1P-specific G protein–coupled receptors and secondarily with inhibitors of extracellular signal-regulated kinase 1/2 (ERK1/2) and ezrin.
Main Outcome Measures: The main outcome measures included cellular proliferation and fibrogenesis. Bromodeoxyuridine Cell Proliferation Assay was employed to measure deoxyribonucleic acid synthesis and proliferation, whereas western blot analysis was used to assess the expression of the fibrotic markers N-cadherin, α-smooth muscle actin, transgelin, and collagen type I alpha 1.
Results: Sphingosine 1-phosphate stimulates cellular proliferation of leiomyoma but not myometrial cells. The mitogenic effect elicited by S1P relies on the engagement of its specific receptor $S1P_2$ and is mediated by ERK1/2 and ezrin activation. Furthermore, S1P exerts a profibrotic effect in a S1P-specific G protein–coupled receptor–dependent manner in leiomyoma but not myometrial cells.
Conclusions: These results, besides extending the knowledge on the molecular mechanism underlying uterine leiomyoma development and fibrosis, demonstrate the pathogenetic role of S1P in leiomyoma and support the rationale for targeting S1P signaling pathway as innovative potential treatment. (F S Sci® 2025;6:99–106. ©2024 by American Society for Reproductive Medicine.)

Key Words: Uterine fibroids, proliferation, fibrosis, sphingosine 1-phosphate, sphingosine 1-phosphate receptors

F&S SCIENCE CLINICAL QUICK TAKE

What clinical problem is addressed by this study?

- Uterine fibroids, or leiomyomas, are the most common benign gynecologic tumors causing significant morbidity and affecting reproductive function with an impact on fertility and pregnancy outcome. Despite that sex steroid hormones play a critical role in the formation and growth of fibroids, the complex pathogenesis of the disease is still under investigation.

What are the key findings?

- The bioactive lipid sphingosine 1-phosphate has a critical role in leiomyoma pathogenesis acting both as proliferative and fibrotic cue in leiomyoma cells.

How do these findings apply to human fertility or the reproductive process?

- Sphingosine 1-phosphate signaling could represent an innovative target to counteract leiomyoma growth and fibrosis, thus increasing women fertility and ameliorating pregnancy outcome.

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Uterine fibroids, or leiomyomas, represent the most common benign pelvic tumors in women of reproductive age (1). Although in some cases they are asymptomatic, uterine fibroids are associated with a variety of symptoms, including abnormal uterine bleeding or heavy menstrual bleeding and pelvic pain, and may affect patient fertility (2). The complex pathogenesis of the disease is under investigation: sex steroid hormones (estrogens and progesterone) play a critical role in the formation and growth of fibroids, and several growth factors and cytokines regulate inflammation, fibrosis, proliferation, and angiogenesis (2, 3). In addition to cell proliferation, fibrosis is one of the main features of leiomyomas (4), causing excessive production of extracellular matrix leading to the rigid structure of these tumors and abnormal bleeding and pelvic pain (5–8).

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid crucially involved in the modulation of several cellular processes such as proliferation, survival, migration, inflammation, and fibrosis (9, 10). It is physiologically present in plasma at submicromolar concentration and exerts its biologic actions mainly by binding to a family of 5 G protein–coupled receptors (S1PR, $S1P_1$–$S1P_5$) (11) and activating downstream signaling pathways such as the extracellular signal-regulated kinases 1 and 2 (ERK1/2) (12, 13) and the cytoskeleton-related proteins ezrin, radixin, and moesin (ERM) (14, 15).

In recent years, a dysregulation of the S1P signaling pathway has been found in diverse gynecologic disorders such as endometriosis, adenomyosis, and uterine fibroids (15–21).

Recently, we demonstrated that S1P signaling and metabolism are deeply dysregulated in leiomyoma. In particular, the expression of both isoforms of the enzyme responsible for S1P synthesis, sphingosine kinase (SK), SK1 and SK2, and the receptors $S1P_2$, $S1P_3$, and $S1P_5$ resulted to be increased in leiomyoma compared with that in adjacent myometrium (22).

In the present study, we investigated in leiomyoma vs. myometrial cells: the role of S1P on cellular proliferation, characterizing the receptor isoform and the downstream signaling pathway involved, and the role of S1P in fibrogenesis.

MATERIALS AND METHODS

Cell culture and treatments

The myometrial (A009) and leiomyoma (A010) cell lines, immortalized with human papilloma virus type 16, were provided by Dr. William H. Catherino, M.D., Ph.D. (Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, Maryland) (23). The cells were cultured in a 1:1 mixture of Dulbecco's Modified Eagle Medium and Nutrient Mixture F-12 Ham (Merck Life Science, Burlington, MA) supplemented with 10% fetal bovine serum (Merck Life Science), 1% penicillin-streptomycin (Merck Life Science), 1% amphotericin B (Fungizone; Euroclone, Milan, Italy), and 1% glutamine (Merck Life Science) at 37 °C in 95% air and 5% CO_2. The cells were starved overnight before every treatment in the starvation medium (Dulbecco's Modified Eagle Medium and Nutrient Mixture F-12 Ham supplemented with 10% charcoal stripped fetal bovine serum [Merck Life Science], 1% penicillin-streptomycin, 1% amphotericin B, and 1% glutamine). When required, cells were preincubated with pharmacologic inhibitors or antagonists (ezrin inhibitor, NSC668394; ERK1/2 inhibitor, U0126; $S1P_1$/$S1P_3$ antagonist, VPC23019; and $S1P_2$ antagonist, JTE013; Merck Life Science) 1 hour before the treatment with S1P (Merck Life Science). The institutional review board protocol number was 13742.

Bromodeoxyuridine Cell Proliferation Assay

Myometrial and leiomyoma cells were seeded in 96-well plates. Cell proliferation was evaluated using the Bromodeoxyuridine (BrdU) Cell Proliferation Assay (Merck Life Science) according to the manufacturer's instructions. Briefly, the cells were incubated with BrdU for the last 24 hours of treatment, fixed, and incubated with the anti-BrdU antibody and with the secondary horseradish peroxidase-conjugated antibody. Finally, substrate was added, and the absorbance was read using a spectrophotometric plate reader (Tecan Trading AG, Männedorf, Switzerland).

Western blot analysis

Cells were collected using a scraper and lysed for 30 minutes at 4 °C in 50 mM Tris, pH 7.5, 120 mM NaCl, 6 mM ethylene glycol tetraacetic acid, 1 mM ethylenediaminetetraacetic acid, 20 mM NaF, 15 mM $Na_4P_2O_7$, and 1% Nonidet, with the addition of protease inhibitor cocktail and phosphatase inhibitor cocktail (Merck Life Science). Protein lysates were then centrifuged at 10,000 × *g* for 15 minutes at 4 °C, and the supernatant was collected. Finally, proteins (10 μg) from total cell lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequently to Western blot (WB) analysis using polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA). The membranes were incubated overnight at 4 °C with primary antibodies against the targets of interest (anti-phospho-ERM and anti-phospho-ERK1/2 [Cell Signaling Technology, Danvers, MA]; anti-N-cadherin, anti-vinculin, and anti–collagen type I alpha 1 [COL1A1; Santa Cruz Biotechnology, Santa Cruz, CA]; anti-α-smooth muscle actin [αSMA; Merck Life Science]; anti-transgelin [Everest Biotech Ltd, Upper Heyford, United Kingdom]; and anti–glyceraldehyde-3-phosphate dehydrogenase [Santa Cruz Biotechnology]). Membranes were then incubated with secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) for 1 hour at room temperature, and bound antibodies were revealed by chemiluminescence with the use of enhanced chemiluminescence reagents (Bio-Rad) employing Amersham Imager 600 (GE HealthCare Europe GmbH, Freiburg, Germany).

Densitometric analysis was performed by the ImageJ software (National Institutes of Health, Bethesda, MD). Band intensity of target proteins was reported as fold increase relative to the respective control, set as 1.

Statistical analysis

Data were analyzed using the Student *t*-test and 1-way or 2-way analysis of variance followed by the Bonferroni post hoc test. A P value of $<.05$ was considered statistically significant. Graphical representation of the data and statistical analysis were performed with the use of the GraphPad Prism 8 software.

RESULTS

S1P stimulates leiomyoma but not myometrial proliferation via $S1P_2$ engagement

The effect of S1P on the induction of proliferation was evaluated in myometrial and leiomyoma cells using the BrdU incorporation assay. Obtained results (Fig. 1) show that 48-hour stimulation with 1 μM S1P significantly increased the proliferation of leiomyoma cells but not that of myometrial cells. To characterize the S1PR implicated in S1P-induced proliferation, leiomyoma cells were pretreated with selective $S1P_1$ antagonist W146 (10 μM), $S1P_2$ antagonist JTE013 (1 μM), $S1P_1$/$S1P_3$ antagonist VPC23019 (10 μM), or $S1P_4$ antagonist CYM50358 (1 μM). Data reported in Figure 2 clearly showed that the mitogenic effect elicited by S1P was abolished in the presence of JTE013, demonstrating that the sphingolipid stimulates cellular proliferation via $S1P_2$ signaling in leiomyoma cells. In contrast, the pharmacologic blockade of the other receptor isoforms did not affect the mitogenic effect elicited by S1P in leiomyoma cells (Fig. 2).

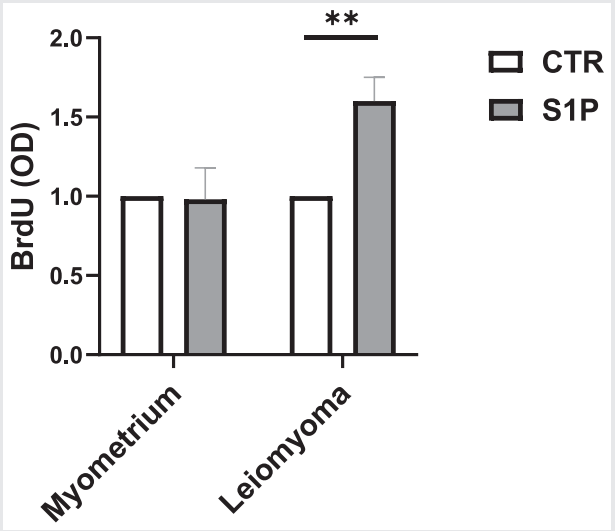
S1P-induced leiomyoma cell proliferation requires ERK1/2 and ERM activation

The possible involvement of ERK1/2 and ERM activation in the S1P mitogenic effect was then investigated. WB analysis of phosphorylated ERM (p-ERM) and ERK1/2 (p-ERK1/2) performed in leiomyoma cells incubated with 1 μM S1P for different time intervals (1, 5, 10, 15, and 30 minutes) revealed that the sphingolipid significantly activated both ERM and ERK1/2. In particular, ERM phosphorylation was maximal between 1 and 10 minutes and still high at 30 minutes, whereas ERK1/2 were maximally activated between 5 and 10 minutes (Fig. 3A). Next, to evaluate the involvement of ERK1/2 and ERM activation in the S1P-induced leiomyoma proliferation, specific pharmacologic inhibitors for ERK1/2 (U0126, 5 μM) or ezrin (NSC668394, 1 μM) were used to pretreat the cells before stimulation with 1 μM S1P for 48 hours. As shown in Figure 3B, the inhibition of ERK1/2 and ezrin abolished the proliferative effect of S1P, demonstrating a crucial role of both signaling pathways in mediating the mitogenic effect of the sphingolipid.

S1P induces fibrosis in leiomyoma but not myometrial cells

WB analysis of fibrotic markers (N-cadherin, αSMA, and transgelin) was performed in both leiomyoma and

FIGURE 1



Sphingosine 1-phosphate (S1P) stimulates proliferation in leiomyoma but not myometrial cells. Human myometrial or leiomyoma cells were treated for 48 hours with 1 μM S1P, and cell proliferation was evaluated with the bromodeoxyuridine (BrdU) incorporation assay. Data are shown as the means ± standard deviations of 3 independent experiments. The BrdU incorporation of the treated cells is reported as relative to the control, set as 1. Differences were statistically significant according to the Student *t*-test (**$P < .01$).

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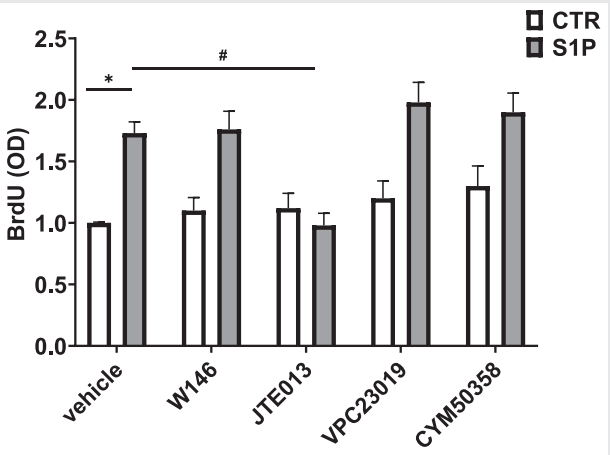
FIGURE 2



Sphingosine 1-phosphate (S1P)–stimulated proliferation in leiomyoma cells is dependent on $S1P_2$ engagement. Human leiomyoma cells were pretreated for 1 hour with the specific $S1P_1$ antagonist W146 (10 μM), $S1P_2$ antagonist JTE013 (1 μM), $S1P_1$/$S1P_3$ antagonist VPC23019 (10 μM), or $S1P_4$ antagonist CYM50358 (1 μM), before being challenged for 48 hours with 1 μM S1P. Cell proliferation was evaluated with the bromodeoxyuridine (BrdU) incorporation assay. Data are shown as the means ± standard deviations of 3 independent experiments. The BrdU incorporation of the cells is reported as relative to the untreated control, set as 1. The effect of $S1P_2$ blockade in S1P-induced proliferation (*$P < .05$) was statistically significant by 2-way analysis of variance followed by the Bonferroni post hoc test (#$P < .05$).

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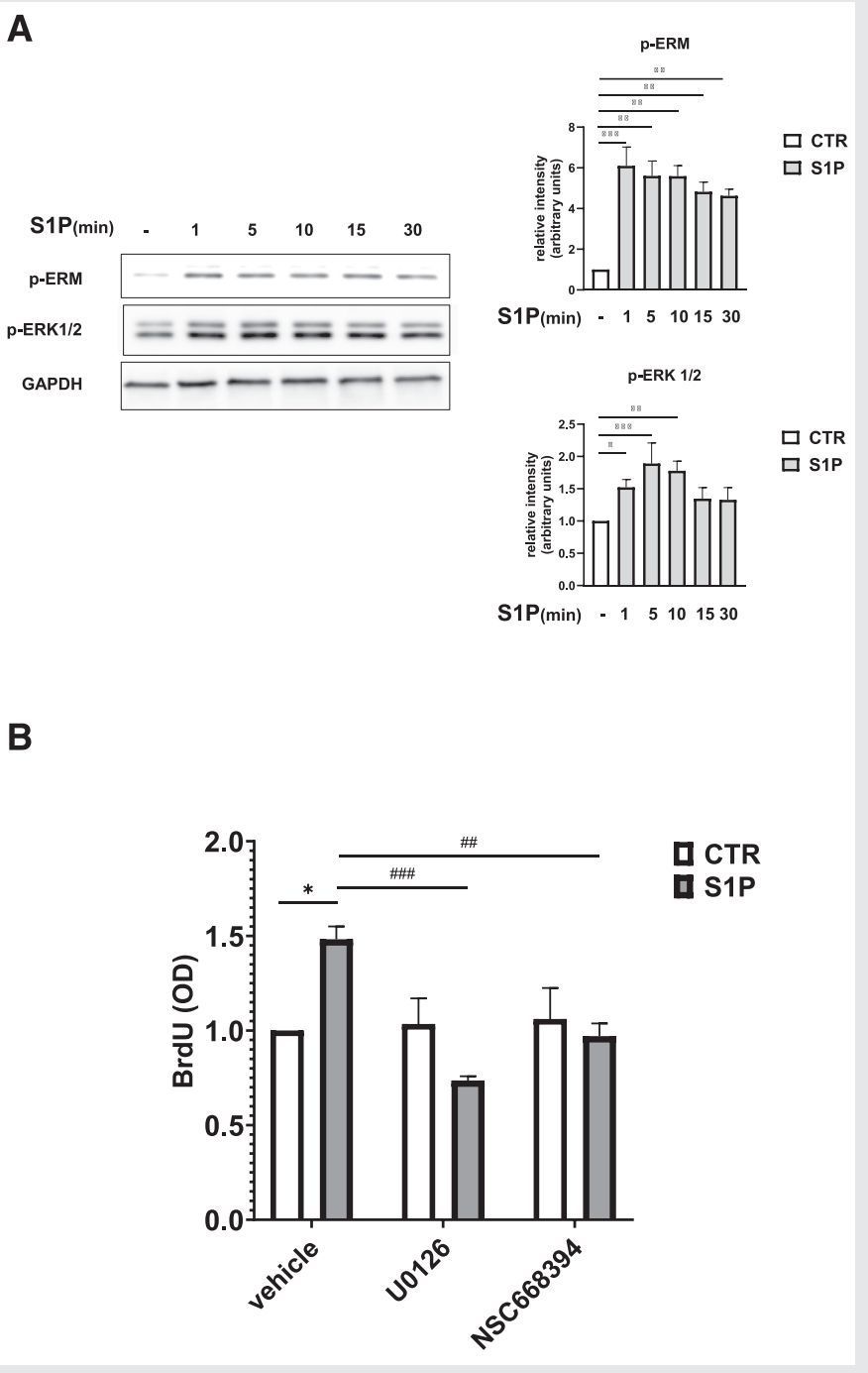
FIGURE 3



Sphingosine 1-phosphate (S1P) stimulates leiomyoma cell proliferation via extracellular signal-regulated kinase 1/2 (ERK1/2) and ezrin. (**A**) Western blot analysis was performed in leiomyoma cells after treatment with 1 μM S1P for the indicated time points, using specific antibodies against p-ERM and p-ERK1/2. A blot representative of 3 independent experiments with analogous results is shown. The histograms represent the means ± standard deviations of the densitometric analysis of 3 independent experiments. Data are reported as protein expression normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fold change over control, set as 1. Differences were statistically significant according to 1-way analysis of variance followed by the Bonferroni post hoc test (*$P < .05$, **$P < .01$, ***$P < .001$). (**B**) Leiomyoma cells were pretreated for 1 hour with the specific ERK1/2 inhibitor U0126 (5 μM) or the ezrin inhibitor NSC668394 (1 μM) before being challenged for 48 hours with 1 μM S1P. Cell proliferation was evaluated with the bromodeoxyuridine (BrdU) incorporation assay. Data are shown as the means ± standard deviations of 3 independent experiments. The BrdU incorporation of the cells is reported as relative to the untreated control, set as 1. The effect of ERK1/2 or ezrin inhibition in S1P-induced proliferation (*$P < .05$) was statistically significant by 2-way analysis of variance followed by the Bonferroni post hoc test (##$P < .01$; ###$P < .001$).

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myometrial cells stimulated with 1 μM S1P for 24 and 48 hours. Data shown in Figure 4A demonstrated that S1P exerted a profibrotic action in leiomyoma cells, significantly increasing the expression of the evaluated fibrotic markers at both time points of treatment. In contrast, S1P did not affect the levels of the same fibrotic markers in myometrial cells (Fig. 4A). The S1P profibrotic effect in leiomyoma cells was mediated by S1PR. Indeed, the increase in the expression of N-cadherin, αSMA, transgelin, and COL1A1 elicited by the bioactive sphingolipid was abolished by the pretreatment with the pharmacologic antagonists of $S1P_1$/$S1P_3$ (VPC23019, 10 μM) and $S1P_2$ (JTE013, 1 μM) (Fig. 4B).

DISCUSSION

Despite the high prevalence of uterine leiomyomas, the molecular mechanisms underlying the pathogenesis and development of the disease are yet to be fully clarified (1, 2, 24–28).

In this study, the biologic action of the bioactive sphingolipid S1P in leiomyoma cells was deeply investigated. The obtained results clearly demonstrate that S1P exerts a crucial role in leiomyoma, promoting fundamental processes for its pathogenesis, such as proliferation and fibrosis.

Sphingosine 1-phosphate signaling pathway has been recently shown to be deeply dysregulated in uterine fibroids: the expression levels of the enzymes SK1 and SK2 and of the receptors $S1P_2$, $S1P_3$, and $S1P_5$ significantly increase in leiomyoma tissue compared with those in adjacent myometrium (22).

Here, S1P has been shown to potently stimulate BrdU incorporation into deoxyribonucleic acid in leiomyoma cells but not in myometrial cells. Accordingly, the role of S1P in regulating cell proliferation is well established both in physiological (13, 29) and pathological conditions, being implicated in the progression of different types of cancer (30–33). To investigate the receptor isoform specifically involved in the S1P-induced mitogenic effect, S1PR antagonists were employed. Interestingly, $S1P_2$ was found to be implicated in the transmission of S1P-induced cell proliferation, similarly to what previously demonstrated in mesoangioblasts (34) and satellite cells (13). Considering that increased proliferation is one of the first pathogenetic mechanism of uterine fibroids (2, 28), $S1P_2$ could be proposed as a potential target for their treatment.

In this study, the molecular mechanisms by which S1P stimulates leiomyoma cell proliferation were also investigated. Sphingosine 1-phosphate rapidly and potently increased the activation of ERK1/2 and ERM in leiomyoma cells. Both ERM and ERK1/2 signaling pathways were found to be necessary for the mitogenic response to S1P because the selective pharmacologic inhibition of ERK1/2 and ezrin abrogated the biologic action of the sphingolipid. The activation of ERK1/2 has long been associated with mitogenic signaling being also aberrantly activated in different types of cancer (35–37). Notably, ERK1/2 signaling transduces the proliferative effect of 17β-estradiol (38) and leptin (39) in leiomyoma cells. Data reported here are in agreement with a number of other studies that established a crucial role for ERK1/2 activation in the mitogenic effect of S1P (34, 40–42).

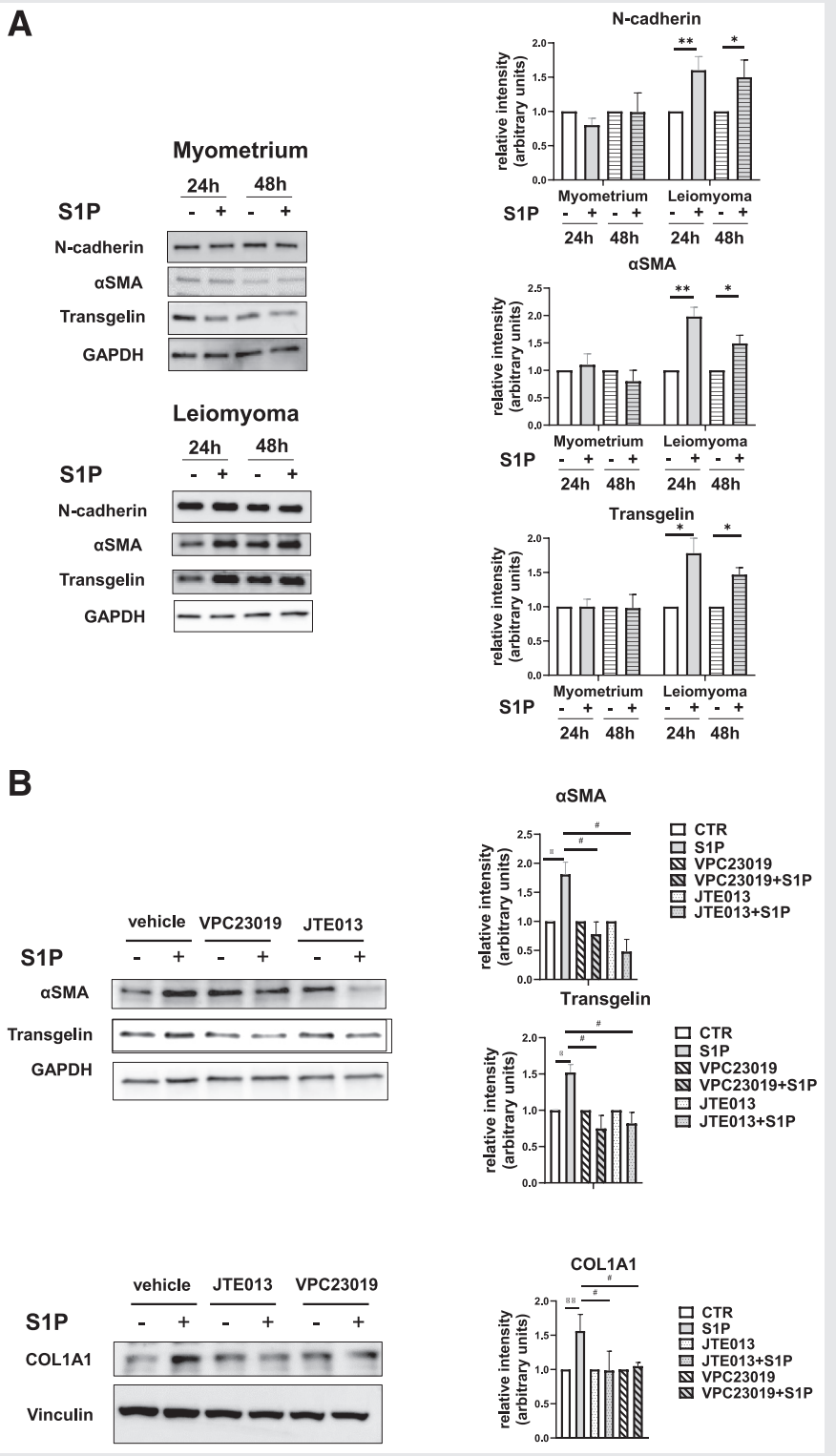
The S1P-dependent activation of the ERM complex described in the present study is in agreement with previous findings on epithelial otic vesicle progenitors (14), breast cancer (43), vascular smooth muscle (44), and epithelial endometriotic (15) cells. Besides being implicated in cellular migration and invasion (45), in accordance with our findings, it has been reported that ERM proteins modulate cellular proliferation in rheumatoid fibroblast-like synoviocytes (46) and ovarian cancer cells (47).

Beyond identifying S1P as key regulator of cell proliferation, data reported here demonstrate a crucial role of the sphingolipid, via ligation to its receptors, in the promotion of fibrosis in leiomyoma cells. Indeed, S1P significantly increased the levels of the fibrotic markers N-cadherin, αSMA, transgelin, and COL1A1 in leiomyoma cells, whereas it did not alter their levels in myometrial cells. These data corroborate our previous findings obtained at the messenger RNA level (22). The S1P signaling pathway has been crucially linked to the development of fibrosis in different tissues, such as the skeletal muscle, lung, kidney, and heart (10). Of note, we have recently demonstrated that SK/S1P signaling axis, through the specific engagement of $S1P_2$/$S1P_3$, mediates the profibrotic action of activin A in leiomyoma cells (22).

In agreement to the pivotal role of S1P signaling in leiomyoma, the overexpression of SK1 in rat leiomyoma cells resulted in increased proliferation and augmented levels of the cell cycle regulator cyclin D1 (48). Moreover, in the same cellular model, Raymond et al. (19) demonstrated that the down-regulation or pharmacologic inhibition of SK1 significantly reduced the antiapoptotic effect of endothelin. The exogenous administration of S1P was also shown to inhibit apoptosis besides increasing the expression of cyclooxygenase 2 in rat leiomyoma cells (19).

Our data highlight S1P signaling as a crucial driver of leiomyoma progression, stimulating cell proliferation and fibrosis in leiomyoma but not in myometrial cells. It has been previously shown that the content of S1P and other sphingolipids in human fibroids and pair-matched healthy uterus tissue remains constant (49). However, it should be taken into consideration that not the total variations but rather very localized alterations of S1P levels, restricted at a specific district of the membrane, are crucial to activate a specific receptor isoform and evoke a biologic outcome. Moreover, similar to our previous study on myoblasts (50), our recent findings on the increased expression of $S1P_2$, $S1P_3$, and $S1P_5$ in leiomyoma tissue compared with that in the adjacent myometrium (22), support the hypothesis that the remodeling of S1PR expression in leiomyoma may be responsible for the switch of the final biologic response evoked by S1P. However, our attempt to show any differences in S1PR protein levels by WB analysis in leiomyoma vs. myometrial cells was unsuccessful (data not shown). Because the present work has been performed in immortalized cellular models, additional studies employing primary cell cultures will be required to strongly support the involvement of the sphingolipid and dissect the molecular mechanisms implicated in the promotion of growth and fibrosis of leiomyoma.

FIGURE 4



Sphingosine 1-phosphate (S1P) induces fibrosis in leiomyoma but not myometrial cells. (**A**) Western blot analysis was performed in myometrial or leiomyoma cells after treatment with 1 μM S1P for 24 or 48 hours, using specific antibodies against N-cadherin, α-smooth muscle actin (αSMA), and transgelin. Representative blots of 3 independent experiments with analogous results are shown. The histograms represent the means $\pm$ standard deviations of the densitometric analysis of 3 independent experiments. Data are reported as protein expression normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fold change over control, set as 1. Differences were statistically significant according to 1-way analysis of variance followed by the Bonferroni post hoc test (*$P < .05$, **$P < .01$). (**B**) Leiomyoma cells were pretreated for 1 hour with the $S1P_1/S1P_3$ antagonist VPC23019 (10 μM) or the $S1P_2$ antagonist JTE013 (1 μM) before being challenged with 1 μM S1P for 24 hours.

FIGURE 4 Continued

Western blot analysis was performed using specific antibodies against αSMA, transgelin, and COL1A1. A blot representative of 3 independent experiments with analogous results is shown. The histograms represent the means ± standard deviations of the densitometric analysis of 3 independent experiments. Data are reported as protein expression normalized to GAPDH or vinculin, fold change over control, set as 1. The effect of $S1P_1$/$S1P_3$ or $S1P_2$ blockade in S1P-induced fibrosis (*$P < .05$, **$P < .01$) was statistically significant by 2-way analysis of variance followed by the Bonferroni post hoc test (#$P < .05$).

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CONCLUSION

In conclusion, the present data obtained in leiomyoma cells show that S1P increases proliferation through $S1P_2$, acting via ERK1/2 and ERM pathways, and exerts a profibrotic role through S1PR. These results extend the knowledge of the molecular mechanism underlying uterine leiomyoma development and fibrosis, reinforcing the pathogenetic role of S1P and supporting the rationale for a potential treatment targeting the S1P signaling pathway.

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CRediT Authorship Contribution Statement

Margherita Rossi: Investigation, Data curation, Writing – original draft. Isabelle Seidita: Investigation, Data curation, Writing – original draft. Matteo Prisinzano: Investigation, Data curation. Maryam Raeispour: Investigation, Data curation. Lucia Romeo: Investigation, Data curation. Flavia Sorbi: Data curation. Massimiliano Fambrini: Data curation. Pasquapina Ciarmela: Writing – review & editing. Felice Petraglia: Conceptualization, Writing – review & editing, Funding acquisition. Caterina Bernacchioni: Conceptualization, Formal analysis, Writing – review & editing, Funding acquisition. Chiara Donati: conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Interests

M. Ro. has nothing to disclose. I.S. has nothing to disclose. M.P. has nothing to disclose. M. Ra. has nothing to disclose. L.R. has nothing to disclose. F.S. has nothing to disclose. M.F. has nothing to disclose. P.C. has nothing to disclose. F.P. reports funding from Fondi di Ateneo (ex 60%) and Fondazione Careggi, Project on Woman's Health, for the submitted work. C.B. reports funding from Fondi di Ateneo (ex 60%) for the submitted work. C.D. reports funding from Fondi di Ateneo (ex 60%) for the submitted work.

REFERENCES

1. Yang Q, Ciebiera M, Bariani MV, Ali M, Elkafas H, Boyer TG, et al. Comprehensive review of uterine fibroids: developmental origin, pathogenesis, and treatment. Endocr Rev 2022;43:678–719.
2. Islam MS, Protic O, Stortoni P, Grechi G, Lamanna P, Petraglia F, et al. Complex networks of multiple factors in the pathogenesis of uterine leiomyoma. Fertil Steril 2013;100:178–93.
3. Islam MS, Greco S, Janjusevic M, Ciavattini A, Giannubilo SR, D'Adderio A, et al. Growth factors and pathogenesis. Best Pract Res Clin Obstet Gynaecol 2016;34:25–36.
4. Malik M, Norian J, McCarthy-Keith D, Britten J, Catherino W. Why leiomyomas are called fibroids: the central role of extracellular matrix in symptomatic women. Semin Reprod Med 2010;28:169–79.
5. Islam MS, Ciavattini A, Petraglia F, Castellucci M, Ciarmela P. Extracellular matrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics. Hum Reprod Update 2018;24:59–85.
6. Leppert PC, Catherino WH, Segars JH. A new hypothesis about the origin of uterine fibroids based on gene expression profiling with microarrays. Am J Obstet Gynecol 2006;195:415–20.
7. Leppert PC, Jayes FL, Segars JH. The extracellular matrix contributes to mechanotransduction in uterine fibroids. Obstet Gynecol Int 2014;2014:1–12.
8. Bulun SE. Uterine fibroids. N Engl J Med 2013;369:1344–55.
9. Cartier A, Hla T. Sphingosine 1-phosphate: lipid signaling in pathology and therapy. Science 2019;366:eaar5551.
10. Donati C, Cencetti F, Bernacchioni C, Vannuzzi V, Bruni P. Role of sphingosine 1-phosphate signalling in tissue fibrosis. Cell Signal 2021;78:109861.
11. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 2012;22:50–60.
12. Nincheri P, Bernacchioni C, Cencetti F, Donati C, Bruni P. Sphingosine kinase-1/S1P1 signalling axis negatively regulates mitogenic response elicited by PDGF in mouse myoblasts. Cell Signal 2010;22:1688–99.
13. Calise S, Blescia S, Cencetti F, Bernacchioni C, Donati C, Bruni P. Sphingosine 1-phosphate stimulates proliferation and migration of satellite cells: role of S1P receptors. Biochim Biophys Acta 2012;1823:439–50.
14. Cencetti F, Bernacchioni C, Bruno M, Squecco R, Idrizaj E, Berbeglia M, et al. Sphingosine 1-phosphate-mediated activation of ezrin-radixin-moesin proteins contributes to cytoskeletal remodeling and changes of membrane properties in epithelial otic vesicle progenitors. Biochim Biophys Acta 2019;1866:554–65.
15. Bernacchioni C, Rossi M, Vannuzzi V, Prisinzano M, Seidita I, Raeispour M, et al. Sphingosine-1-phosphate receptor 3 is a non-hormonal target to counteract endometriosis-associated fibrosis. Fertil Steril 2024;121:631–41.
16. Bernacchioni C, Capezzuoli T, Vannuzzi V, Malentacchi F, Castiglione F, Cencetti F, et al. Sphingosine 1-phosphate receptors are dysregulated in endometriosis: possible implication in transforming growth factor β-induced fibrosis. Fertil Steril 2021;115:501–11.
17. Di Paolo A, Vignini A, Alia S, Membrino V, Delli Carpini G, Giannella L, et al. Pathogenic role of the sphingosine 1-phosphate (S1P) pathway in common gynecologic disorders (GDs): a possible novel therapeutic target. Int J Mol Sci 2022;23:13538.
18. Prisinzano M, Bernacchioni C, Seidita I, Rossi M, Raeispour M, Cencetti F, et al. Sphingosine 1-phosphate signaling axis mediates neuropeptide S-induced invasive phenotype of endometriotic cells. FEBS J 2024;291:1744–58.
19. Raymond MN, Bole-Feysot C, Banno Y, Tanfin Z, Robin P. Endothelin-1 inhibits apoptosis through a sphingosine kinase 1-dependent mechanism in uterine leiomyoma ELT3 cells. Endocrinology 2006;147:5873–82.

20. Seidita I, Tusa I, Prisinzano M, Menconi A, Cencetti F, Vannuccini S, et al. Sphingosine 1-phosphate elicits a ROS-mediated proinflammatory response in human endometrial stromal cells via ERK5 activation. FASEB J 2023;37:e23061.
21. Vannuzzi V, Bernacchioni C, Muccilli A, Castiglione F, Nozzoli F, Vannuccini S, et al. Sphingosine 1-phosphate pathway is dysregulated in adenomyosis. Reprod Biomed Online 2022;45:15–8.
22. Bernacchioni C, Ciarmela P, Vannuzzi V, Greco S, Vannuccini S, Malentacchi F, et al. Sphingosine 1-phosphate signaling in uterine fibroids: implication in activin A pro-fibrotic effect. Fertil Steril 2021;115:1576–85.
23. Malik M, Webb J, Catherino WH. Retinoic acid treatment of human leiomyoma cells transformed the cell phenotype to one strongly resembling myometrial cells. Clin Endocrinol (Oxf) 2008;69:462–70.
24. Walker CL, Stewart EA. Uterine fibroids: the elephant in the room. Science 2005;308:1589–92.
25. Ramaiyer MS, Saad E, Kurt I, Borahay MA. Genetic mechanisms driving uterine leiomyoma pathobiology, epidemiology, and treatment. Genes 2024;15:558.
26. Ali M, Ciebiera M, Vafaei S, Alkhrait S, Chen HY, Chiang YF, et al. Progesterone signaling and uterine fibroid pathogenesis; molecular mechanisms and potential therapeutics. Cells 2023;12:1117.
27. Stewart EA, Laughlin-Tommaso SK, Catherino WH, Lalitkumar S, Gupta D, Vollenhoven B. Uterine fibroids. Nat Rev Dis Primer 2016;2:16043.
28. Ono M, Yin P, Navarro A, Moravek MB, Coon 5th JS, Druschitz SA, et al. Paracrine activation of WNT/β-catenin pathway in uterine leiomyoma stem cells promotes tumor growth. Proc Natl Acad Sci U S A 2013;110:17053–8.
29. Olivera A, Spiegel S. Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature 1993;365:557–60.
30. Hirose Y, Nagahashi M, Katsuta E, Yuza K, Miura K, Sakata J, et al. Generation of sphingosine-1-phosphate is enhanced in biliary tract cancer patients and is associated with lymphatic metastasis. Sci Rep 2018;8:10814.
31. Miura K, Nagahashi M, Prasoon P, Hirose Y, Kobayashi T, Sakata J, et al. Dysregulation of sphingolipid metabolic enzymes leads to high levels of sphingosine-1-phosphate and ceramide in human hepatocellular carcinoma. Hepatol Res 2021;51:614–26.
32. Nagahashi M, Miyoshi Y. Targeting sphingosine-1-phosphate signaling in breast cancer. Int J Mol Sci 2024;25:3354.
33. Nojima H, Shimizu H, Murakami T, Shuto K, Koda K. Critical roles of the sphingolipid metabolic pathway in liver regeneration, hepatocellular carcinoma progression and therapy. Cancers (Basel) 2024;16:850.
34. Donati C, Cencetti F, Nincheri P, Bernacchioni C, Brunelli S, Clementi E, et al. Sphingosine 1-phosphate mediates proliferation and survival of mesoangioblasts. Stem Cells 2007;25:1713–9.
35. Chen J, Jiao Z, Wang A, Zhong W. Lidocaine inhibits melanoma cell proliferation by regulating ERK phosphorylation. J Cell Biochem 2019;120:6402–8.
36. Niu Y, Ji H. Current developments in extracellular-regulated protein kinase (ERK1/2) inhibitors. Drug Discov Today 2022;27:1464–73.
37. Omokehinde T, Jotte A, Johnson RW. gp130 cytokines activate novel signaling pathways and alter bone dissemination in ER+ breast cancer cells. J Bone Miner Res 2022;37:185–201.
38. Nierth-Simpson EN, Martin MM, Chiang TC, Melnik LI, Rhodes LV, Muir SE, et al. Human uterine smooth muscle and leiomyoma cells differ in their rapid 17beta-estradiol signaling: implications for proliferation. Endocrinology 2009;150:2436–45.
39. Reschke L, Afrin S, El Sabah M, Charewycz N, Miyashita-Ishiwata M, Borahay MA. Leptin induces leiomyoma cell proliferation and extracellular matrix deposition via JAK2/STAT3 and MAPK/ERK pathways. F S Sci 2022;3:383–91.
40. Kimura T, Watanabe T, Sato K, Kon J, Tomura H, Tamama K, et al. Sphingosine 1-phosphate stimulates proliferation and migration of human endothelial cells possibly through the lipid receptors, Edg-1 and Edg-3. Biochem J 2000;348:71–6.
41. Van Brocklyn J, Letterle C, Snyder P, Prior T. Sphingosine-1-phosphate stimulates human glioma cell proliferation through Gi-coupled receptors: role of ERK MAP kinase and phosphatidylinositol 3-kinase beta. Cancer Lett 2002;181:195–204.
42. Pébay A, Toutant M, Prémont J, Calvo CF, Venance L, Cordier J, et al. Sphingosine-1-phosphate induces proliferation of astrocytes: regulation by intracellular signalling cascades. Eur J Neurosci 2001;13:2067–76.
43. Canals D, Jenkins RW, Roddy P, Hernández-Corbacho MJ, Obeid LM, Hannun YA. Differential effects of ceramide and sphingosine 1-phosphate on ERM phosphorylation: probing sphingolipid signaling at the outer plasma membrane. J Biol Chem 2010;285:32476–85.
44. Morris TG, Borland SJ, Clarke CJ, Wilson C, Hannun YA, Ohanian V, et al. Sphingosine 1-phosphate activation of ERM contributes to vascular calcification. J Lipid Res 2018;59:69–78.
45. Song Y, Ma X, Zhang M, Wang M, Wang G, Ye Y, et al. Ezrin mediates invasion and metastasis in tumorigenesis: a review. Front Cell Dev Biol 2020;8:588801.
46. Huang H, Xiao Y, Lin H, Fu D, Zhan Z, Liang L, et al. Increased phosphorylation of ezrin/radixin/moesin proteins contributes to proliferation of rheumatoid fibroblast-like synoviocytes. Rheumatology 2011;50:1045–53.
47. Li MJ, Xiong D, Huang H, Wen ZY. Ezrin promotes the proliferation, migration, and invasion of ovarian cancer cells. Biomed Environ Sci 2021;34:139–51.
48. Jeng YJ, Suarez VR, Izban MG, Wang HQ, Soloff MS. Progesterone-induced sphingosine kinase-1 expression in the rat uterus during pregnancy and signaling consequences. Am J Physiol Endocrinol Metab 2007;292:E1110–21.
49. Paweł K, Chabowski A, Górski J. Sphingolipid content in the human uterus and pair-matched uterine leiomyomas remains constant. Lipids 2013;48:245–50.
50. Cencetti F, Bernacchioni C, Nincheri P, Donati C, Bruni P. Transforming growth factor-beta1 induces transdifferentiation of myoblasts into myofibroblasts via up-regulation of sphingosine kinase-1/S1P$_3$ axis. Mol Biol Cell 2010;21:1111–24.