



**HAL**  
open science

## The role of sphingosine 1-phosphate metabolism in bone and joint pathologies and ectopic calcification

Alaeddine El Jamal, Carole Bougault, Saida Mebarek, David Magne, Olivier Cuvillier, Leyre Brizuela

### ► To cite this version:

Alaeddine El Jamal, Carole Bougault, Saida Mebarek, David Magne, Olivier Cuvillier, et al.. The role of sphingosine 1-phosphate metabolism in bone and joint pathologies and ectopic calcification. *BONE*, 2020, 130, pp.115087 -. 10.1016/j.bone.2019.115087 . hal-03489320

**HAL Id: hal-03489320**

**<https://hal.science/hal-03489320v1>**

Submitted on 20 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

# **The role of sphingosine 1-phosphate metabolism in bone and joint pathologies and ectopic calcification**

Alaeddine El Jamal<sup>1</sup>, Carole Bougault<sup>1</sup>, Saida Mebarek<sup>1</sup>, David Magne<sup>1</sup>, Olivier Cuvillier<sup>2</sup>,  
Leyre Brizuela<sup>1</sup>

<sup>1</sup> Univ Lyon, Université Claude Bernard Lyon 1, CNRS UMR 5246, ICBMS, F-69622, Lyon, France

<sup>2</sup> Institut de Pharmacologie et de Biologie Structurale, IPBS, CNRS UMR 5089, F-31077, Toulouse, France

To whom requests should be addressed:

Dr Leyre Brizuela Madrid, CNRS UMR 5246 ICBMS, F-69622, Lyon, France

E-mail: [leyre.brizuela-madrid@univ-lyon1.fr](mailto:leyre.brizuela-madrid@univ-lyon1.fr)

Running title:

Sphingosine 1-phosphate in bone and joint pathologies

Conflict of interest statement:

no disclosures

Highlights:

- S1P contributes to the development of several rheumatic disorders.
- S1P could also be involved in vascular or cancer-derived ectopic calcifications.
- Targeting S1P pathway might both reduce inflammation and restore bone homeostasis.
- Fingolimod may be a promising drug candidate in this regard.

## **ABSTRACT**

Sphingolipids display important functions in various pathologies such as cancer, obesity, diabetes, cardiovascular or neurodegenerative diseases. Sphingosine, sphingosine 1-phosphate (S1P), and ceramide are the central molecules of sphingolipid metabolism. Sphingosine kinases 1 and 2 (SK1 and SK2) catalyze the conversion of the sphingolipid metabolite sphingosine into S1P. The balance between the levels of S1P and its metabolic precursors ceramide and sphingosine has been considered as a switch that could determine whether a cell proliferates or dies. This balance, also called « sphingolipid rheostat », is mainly under the control of SKs.

Several studies have recently pointed out the contribution of SK/S1P metabolic pathway in skeletal development, mineralization and bone homeostasis. Indeed, SK/S1P metabolism participates in different diseases including rheumatoid arthritis, spondyloarthritis, osteoarthritis, osteoporosis, cancer-derived bone metastasis or calcification disorders as vascular calcification. In this review, we will summarize the most important data regarding the implication of SK/S1P axis in bone and joint diseases and ectopic calcification, and discuss the therapeutic potential of targeting SK/S1P metabolism for the treatment of these pathologies.

Keywords:

sphingosine 1-phosphate, sphingosine kinases, arthritis, spondyloarthritis, osteoporosis.

## **SUMMARY**

### 1. Insight on sphingolipids and sphingosine 1-phosphate signaling

#### 1.1 Sphingolipid metabolism

#### 1.2 Sphingosine 1-phosphate and sphingosine kinases

#### 1.3 Intracellular and extracellular sphingosine 1-phosphate signaling

### 2. Role of sphingosine kinases/sphingosine 1-phosphate metabolic pathway in bone and joint pathologies and ectopic calcification

#### 2.1 Rheumatoid arthritis

#### 2.2 Spondyloarthritis

#### 2.3 Osteoarthritis

#### 2.4 Osteoporosis

#### 2.5 Cancer-derived bone metastasis

#### 2.6 Vascular calcification

### 3. Potential of targeting sphingosine kinases/sphingosine 1-phosphate metabolic pathway for the treatment of bone and joint pathologies and ectopic calcification

### 4. Conclusions

# 1. INSIGHT ON SPHINGOLIPIDS AND SPHINGOSINE 1-PHOSPHATE SIGNALING

Sphingolipids have been classically considered as structural molecules, mainly membrane components. Nowadays sphingolipids are widely recognized as bioactive lipids that control pivotal biological functions such as proliferation, survival, differentiation, migration, inflammatory response, cellular cycle or apoptosis <sup>(1-3)</sup>. Importantly, sphingolipids play key roles in skeletal development, mineralization, regulation of bone mass or osteoimmunology <sup>(4-6)</sup>. Moreover, this class of lipids has been involved in multiple pathologies including cancer, obesity, diabetes, cardiovascular diseases, and more recently associated with bone and joint pathologies <sup>(1-5,7)</sup>.

Ceramide, sphingosine and sphingosine 1-phosphate (S1P) are the central molecules of sphingolipid metabolism with often opposite roles in the cell. Ceramide and sphingosine are generally pro-apoptotic and anti-proliferative while S1P stimulates proliferation, migration and cell survival *in vitro*, *in vivo*, and *ex vivo* <sup>(8-10)</sup>.

## 1.1 Sphingolipid metabolism

Several enzymes control the synthesis or the degradation of these metabolites. Ceramide is the key molecule of this network, at the crossroads between multiple signaling pathways (**Figure 1**). It can be synthesized through *de novo* synthesis, with the condensation of serine with palmitoyl-CoA and subsequent actions of dihydroceramide synthases and dihydroceramide desaturase. Of note, other components for the first reaction of *de novo* pathway have been recently suggested such as alanine or glycine with stearate or myristate <sup>(2)</sup>. Ceramide can also be directly produced from sphingomyelin (SM), the most abundant sphingolipid in mammalian cells, after stimulation of sphingomyelinases (SMases). Three categories of

SMases exist based on their optimal pH of action: acidic, alkaline and neutral SMases <sup>(11,12)</sup>. Moreover, several sphingomyelin synthases (SMS) can catalyze the transfer of a phosphocholine head from phosphatidylcholine (PC) to ceramide, producing diacylglycerol (DAG) and SM <sup>(11)</sup> (**Figure 1**). Two isoforms of SMS exist with SMS1 localized in the trans-Golgi apparatus and SMS2 mainly expressed in plasma membrane <sup>(13)</sup>. Ceramide can then be hydrolyzed by ceramidases to yield sphingosine while ceramide synthases control the opposite reaction. Finally, sphingosine kinases 1 and 2 (SK1, SK2) catalyze the conversion of sphingosine into S1P.

## 1.2 Sphingosine 1-phosphate and sphingosine kinases

S1P is a pleiotropic phospholipid that regulates various biological activities such as proliferation, migration, inflammation, or angiogenesis <sup>(1)</sup>. S1P is implicated in a number of pathophysiological conditions and diseases that affect almost every organ of the body including cancer, diabetes, atherosclerosis, neurodegenerative diseases, inflammatory disorders (asthma, rheumatoid arthritis, inflammatory bowel diseases, autoimmune diseases...) <sup>(1-3)</sup>.

S1P content in resting cells is low and controlled through a finely regulated equilibrium between its synthesis and its degradation. The balance between the intracellular levels of S1P and its metabolic precursors, ceramide and sphingosine, has been regarded as a switch determining whether a cell lives or dies <sup>(8)</sup>.

S1P is produced from sphingosine in a reaction catalyzed by two sphingosine kinase isoforms, SK1 and SK2 (**Figure 1**). Although differing in size, SK1 and SK2 do share a high degree of sequence similarity <sup>(14)</sup>. They have different developmental expression, tissue distribution and subcellular localization profiles, suggesting that the two enzymes might have distinct

physiological functions <sup>(15)</sup>. During mouse embryonic development for example, *Sphk1* is highly expressed at day 7, while *Sphk2* form is detected later, at day 11 <sup>(16)</sup>. Regarding their tissue localization, *SPHK1* is highly expressed in lung and spleen, and is also expressed in brain, heart, thymus, and kidney <sup>(17)</sup>; whereas *SPHK2* is highly expressed in liver and kidney <sup>(14)</sup>. Both enzymes have multiple spliced variants of unknown functional significance and most studies are conducted on the shortest isoforms, which might not necessarily be the most physiologically relevant ones in a given cell model studied <sup>(18)</sup>. If SK1 almost universally promotes proliferation and cell survival, the functions of SK2 appear to be much more complex <sup>(19)</sup>. SK1 is activated by various stimuli, among which growth and survival factors are prominent, thus primarily promoting cell survival and proliferation <sup>(20,21)</sup>. On the contrary, the role of SK2 appears by far more complex, generally displaying a pro-apoptotic role but anti-apoptotic effects have also been reported <sup>(21)</sup>. The subcellular localization of both SK1 and SK2, by impacting compartmentalization of generated S1P, is likely critical in dictating the biological effects of S1P <sup>(19,21)</sup>. Activation of SK1 is mediated by its phosphorylation on Ser225 by ERK1/2, which markedly enhances its catalytic activity <sup>(22)</sup>. This phosphorylation is crucial for SK1 to translocate from the cytosol to the inner leaflet of the plasma membrane <sup>(22)</sup>. This translocation is essential for its enzymatic activity <sup>(23)</sup>. Alike SK1, SK2 phosphorylation on Ser351 and/or Thr578 by ERK1/2 leads to its activation <sup>(24)</sup>. SK2 distribution is more complex than SK1 because it exhibits nuclear localization and export signals in its sequence. Thus, it can be found in the cytosol and in the nucleus. Adding to that, SK2 can also be localized in the endoplasmic reticulum (ER) or in the mitochondria <sup>(25)</sup>.

S1P content is also controlled by its degradation by S1P phosphatases (SPP1 or SPP2) <sup>(26–28)</sup> that convert S1P into sphingosine, and S1P lyase (SPL) that irreversibly degrades S1P into phosphoethanolamine and hexadecenal <sup>(29,30)</sup> (**Figure 1**). Both SPPs are localized in the ER and catalyze reversible S1P dephosphorylation. However, they have different tissue



distribution: SPP1 is mainly expressed in placenta and kidney while SPP2 is present in brain, heart, colon, kidney, lung and small intestine <sup>(16,26)</sup>. Of note, S1P can also be dephosphorylated by non-specific phosphatases such as lipid phosphate phosphatases 1, 2 and 3 <sup>(31)</sup>. On the other hand, the irreversible degradation of S1P by SPL is localized in the ER and in the mitochondrial inner membrane. SPL is highly expressed in small intestine, colon, thymus, spleen and moderately expressed in liver, kidney, lung, stomach and testis <sup>(32)</sup>. S1P degradation products -phosphoethanolamine and hexadecenal - are substrates for the synthesis of fatty acids or phospholipids as phosphatidylethanolamine <sup>(32)</sup>.

Interestingly, SPL expression has been shown to be inversely correlated to SK1 expression in cancer <sup>(33)</sup> and Alzheimer's disease <sup>(34)</sup>, demonstrating its prominent role in regulating S1P content in cells.

### **1.3 Intracellular and extracellular sphingosine 1-phosphate signaling**

With regard to the mode of action of S1P, many lines of evidence indicate an intracellular role of S1P affecting a number of intracellular targets including notably histone deacetylases <sup>(35)</sup>, tumor necrosis factor (TNF- $\alpha$ ) signaling <sup>(36)</sup>, human telomerase reverse transcriptase <sup>(37)</sup> or peroxisome proliferator-activated receptor- $\gamma$  <sup>(38)</sup> (**Figure 2**). However, most of the well-known actions of S1P are mediated through five specific high-affinity G protein-coupled receptors (S1P<sub>1-5</sub>) (**Figure 2**), with specific (sometimes antagonistic) effects dictated by the expression profile of S1P receptor subtypes, expressed in a particular tissue <sup>(39)</sup>. As S1P is synthesized intracellularly, it has been proposed the model of inside-out signaling where S1P must be released to the extracellular environment to interact with its receptors in an autocrine or paracrine manner <sup>(40)</sup> (**Figure 2**). Finally, several transporters of S1P have been identified including ABC transporter family members and more recently Mfsd2b <sup>(41)</sup> or Spns2 (Spinster

Homologue 2). Spns2 is a member of the family of non-ATP-dependent organic ion transporters which has attracted a lot of attention as an S1P transporter<sup>(42,43)</sup> (**Figure 2**).

## **2. ROLE OF SPHINGOSINE KINASES/SPHINGOSINE 1-PHOSPHATE METABOLIC PATHWAY IN BONE AND JOINT PATHOLOGIES AND ECTOPIC CALCIFICATION**

In the past ten years, an increasing number of studies have highlighted the importance of SK/S1P metabolic pathway in numerous diseases such as rheumatoid arthritis, spondyloarthritis, osteoarthritis, osteoporosis, and cancer-derived bone metastasis or calcification disorders like vascular calcification. Here we provide a “state of the art” of the involvement of SK/S1P metabolic axis for each disease.

### **2.1 Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a common autoimmune disease that is associated with progressive pain and disability, systemic complications and early death. RA is characterized by persistent synovial inflammation (synovitis), autoantibody production (rheumatoid factor and anti-citrullinated protein antibody) and cartilage and bone destruction<sup>(44)</sup>. In the synovial membrane of the joint, adaptive and innate immune pathways integrate to promote tissue remodeling and damage. Leukocytes, fibroblast-like synoviocytes (FLS), chondrocytes and osteoclasts, interact together and with the molecular products of damage, to drive the chronic inflammation. Consequently, RA synovium experiments neoangiogenesis, local fibroblast activation and deep architectural reorganization<sup>(45)</sup>.

Interestingly, SK/S1P metabolic pathway has been implicated in a wide variety of physiological processes that are deregulated in the context of RA <sup>(2,3)</sup>. Studies on SKs and S1P in the context of RA can be divided in two groups focusing on immune cells or joint cells. This section will summarize the main studies involving S1P metabolism pathway in joint cells, i.e. FLS and osteoclasts.

The formal evidence for the involvement of SKs in RA pathophysiology was provided by Lai and colleagues in the mouse model of collagen-induced arthritis (CIA). Inhibition of SKs and particularly SK1, was shown to diminish disease severity <sup>(46)</sup>. First, the pharmacological inhibition of SKs by administration of DMS (*N,N*-dimethylsphingosine) significantly reduced articular inflammation and joint destruction. Second, the specific *Sphk1* knock-down by siRNA decreased the incidence and activity of the disease <sup>(46)</sup>. The role of SK1 isoform in RA-like disease was further confirmed in another *in vivo* model in which a TNF $\alpha$ -induced chronic inflammation causes the development of a spontaneous erosive arthritis <sup>(47)</sup>. In this model, a reduction of joint inflammation and pathology was obtained by genetic deletion of *Sphk1*. COX-2 (cyclooxygenase 2) protein expression was downregulated in joint protein extracts from *Sphk1*-deleted mice. In addition, joint RNA extraction and analysis by microarray revealed that *Socs3*, a natural regulator of interleukin (IL)-6 signaling, was increased in the absence of *Sphk1*, thus probably decreasing the signaling of IL-6 <sup>(47)</sup>. The lack of *Sphk1* globally decreased the pro-inflammatory signaling in the joint, even if these whole tissue analyses did not allow discriminating the relative contribution of skeletal cells versus immune cells present in the joint. Noteworthy, the loss of *Sphk1* led to diminished osteoclastogenesis. Multinucleated mature osteoclasts appeared less activated and bone resorbing was therefore decreased in transgenic hTNF $\alpha$  mice <sup>(47)</sup>. Therefore, two SK1/S1P dependent mechanisms may be simultaneously implicated in RA development: inflammation and bone catabolism. Lastly, a Fas-deficient *MRL/lpr* mouse model, which spontaneously

develops autoimmune arthritis, was used to investigate the crosstalk occurring between the Fas and S1P/S1P<sub>1</sub> signaling pathways *via* NF-κB<sup>(48)</sup>. In the mandibular condyles of these arthritic mice, immunohistochemical assays showed that protein levels of SK1 and S1P<sub>1</sub> were increased. Of note, S1P<sub>1</sub> was also overexpressed in the inflamed synovium of RA patients<sup>(49)</sup>. Moreover, the blockade of NF-κB signaling by a synthetic inhibitory peptide was sufficient to reduce osteoclastogenesis and joint bone loss<sup>(48)</sup>. Meanwhile, a marked decrease in the number of SK1- and S1P<sub>1</sub>-positive cells was observed, whereas the expression of S1P<sub>3</sub> remained unaffected<sup>(48)</sup>. This study confirms the implication of SK1/S1P pathway in the deregulation of osteoclast maturation in a context of autoimmune arthritis. Furthermore, previous data on the human RA synovial cell line MH7A suggested that FLS may be implicated in this S1P-mediated osteoclastogenesis<sup>(50)</sup>. MH7A cells express RANKL (receptor activator of NF-κB ligand), which is the master inducer of osteoclast maturation. The authors showed that S1P increased its expression, probably *via* Gi/Go-dependent S1P/S1P<sub>1</sub> signaling<sup>(50)</sup>. In fact, S1P metabolic pathway seems dysregulated in FLS from RA patients<sup>(51)</sup>. When compared to normal FLS, they have lower levels of intracellular S1P correlated with an increase in both S1P phosphatase 1 (*SGPPI*) and S1P lyase 1 (*SGPLI*) gene expression. Yet, S1P concentration is particularly elevated in synovial fluids of RA patients (17.5 μM), when compared to osteoarthritis patients (3.5 μM)<sup>(46)</sup>. RA synovium is characterized by local FLS activation, migration and invasion. Actually, S1P addition on RA patients-derived FLS enhances cell migration<sup>(52)</sup> and conversely, knockdown of *SPHK1* by siRNA significantly reduced spontaneous migration and invasion of RA patients-derived FLS<sup>(53)</sup>. Various intracellular signaling pathways have been implicated in these processes, including p42/44 MAPK, p38 MAPK, Rho kinase and PI3K/Akt<sup>(52,53)</sup>. In addition, production and secretion of the matrix metalloproteinases (MMP)-2 and MMP-9 were reduced by *SPHK1* knockdown<sup>(53)</sup>. Finally, S1P signaling has been shown to stimulate

migration and favor invasion of RA patients-derived FLS in the surrounding tissues by facilitating matrix degradation. On the other hand, it has been established that S1P mediate inflammation in FLS, which may contribute to the synovitis characteristic of RA. Indeed, S1P addition on human RA FLS triggered production of pro-inflammatory cytokines and chemokines, such as IL-6, IL-8, MCP-1 (monocyte chemotactic protein-1), RANTES (regulated on activation normal T cells expressed and secreted) and PGE<sub>2</sub> (prostaglandin E2)<sup>(49,52)</sup>. S1P-induced cytokine secretion was partly mediated by p42/44 MAPK, p38 MAPK, and Rho kinase particularly<sup>(52)</sup> and PGE<sub>2</sub> upregulation was due to enhanced COX-2 expression, probably *via* Gi/Go-dependent S1P/S1P<sub>1</sub> signaling<sup>(49)</sup>. Cell autocrine activation of S1P<sub>2</sub> and S1P<sub>3</sub> signaling also contributed to IL-8 and MCP-1 secretion<sup>(51)</sup>. In fact, all of the three receptors seem to be involved in the response of FLS to exogenous addition of S1P; S1P<sub>1</sub> and S1P<sub>3</sub> stimulating FLS migration, S1P<sub>2</sub> and S1P<sub>3</sub> inducing cytokine and chemokine secretion and S1P<sub>1</sub> protecting cells from apoptosis<sup>(52)</sup>. Overall, the deregulation of the S1P metabolic pathway in FLS may cause the normally thin synovium to become inflamed and thickened in RA. **Figure 3A** details the most important actions of SK/S1P metabolic pathway in RA context.

## 2.2 Spondyloarthritis

Spondyloarthritis (SpA) is a group of chronic rheumatic inflammatory diseases, which is the second most common type of inflammatory arthritis after RA<sup>(54)</sup>. This pathology is characterized by two clinical events primarily occurring in the enthesis: inflammation (enthesitis) and excessive bone formation (peripheral enthesophytes and/or vertebral syndesmophytes)<sup>(55)</sup>. Entheses, where ligaments and tendon transmit mechanical forces to bone through fibrocartilaginous connections, are thus the primary targets of SpA. It is

noteworthy that little is known about the involvement of SK/S1P metabolism in SpA pathophysiology.

We were the first group to report that S1P serum levels are significantly elevated in SpA patients compared to healthy donors ( $6.1 \pm 4.2$  versus  $1.6 \pm 0.9$   $\mu\text{M}$ )<sup>(56)</sup>. Since then, others have confirmed our findings<sup>(57)</sup>. Newly, we showed that cyclic stretch enhanced *Sphk1* gene expression in cultured osteoblasts and chondrocytes<sup>(58)</sup>. In chondrocytes, TNF- $\alpha$  or IL-17 treatment (two cytokines involved in enthesitis) further increased the stretch-induced *Sphk1* upregulation<sup>(58)</sup>. Thus, S1P production by chondrocytes could be stimulated in response to enthesal inflammation and/or mechanical stress, in SpA context. *In vitro*, differentiation into mineralizing cells was accompanied by increased gene expression and activity of both SK1 and SK2, together with augmented gene expression of the S1P-specific transporter *Spns2* and increased S1P secretion<sup>(56)</sup>. Adding to that, we demonstrated that *Slpr1* and *Slpr3* gene expression rose during osteoblasts maturation and chondrocytes hypertrophy<sup>(58)</sup> (**Figure 3B**). Moreover, pharmacological inhibition of SKs decreased matrix mineralization, alkaline phosphatase activity and the mRNA expression of Runx2 (a bone master transcription factor) and of its transcriptional target osteocalcin (an osteoblast specific hormone) in primary murine osteoblasts and hypertrophic chondrocytes<sup>(56)</sup>. Therefore, SK1 and SK2 enzymatic activity seem crucial in the cell mineralizing process. Despite the fact that the studies involving S1P in SpA are rather scarce, there is growing evidence that S1P metabolism is deregulated in SpA and that S1P could participate in enthesal pathological ossification. Consequently, the inhibition of S1P metabolism could be a novel therapeutic approach to treat abnormal ossification.

### 2.3 Osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease characterized by deterioration in the integrity of cartilage, reduced cartilage cellularity, subchondral sclerosis, and low-grade synovial inflammation. It is a leading cause of pain, disability, and shortening of adult working life around the world. Multiple factors are implicated in the etiology of OA. However, no approved treatment is available that can modify or arrest the disease progression<sup>(59,60)</sup>. Chondrocyte is the only cell type present in mature cartilage, which dominates the degenerative process of cartilage in OA<sup>(61)</sup>. Chondrocyte death by apoptosis is implicated in the initiation of articular cartilage degradation and appears to positively correlate with the severity of matrix depletion and destruction that are observed in cartilage of OA patients. Therefore, maintaining viability and proliferation, and inhibiting apoptosis of chondrocytes are essential for preserving the integrity of articular cartilage<sup>(62)</sup>. Interestingly, S1P is present in the synovial fluid of OA patients<sup>(46)</sup> and SK/S1P metabolic pathway is also implicated in different aspects of OA disease.

First, SK/S1P may be involved in the regulation of chondrocytes survival. In most cells, overexpression of the SK isoforms (SK1 and SK2) had opposing biological actions with regard to cellular proliferation and apoptosis. In contrast to pro-survival SK1, SK2 overexpression was reported to inhibit cell growth and enhance apoptosis in NIH3T3 fibroblasts and HEK 293 cells, for instance<sup>(25)</sup>. In chondrocytes specifically, DANCR (differentiation antagonizing non-protein coding RNA) acted as a competitive endogenous RNA to sponge miR-577, which targeted the mRNA of SK2 to regulate the survival of chondrocytes in OA<sup>(63)</sup>. Moreover, a selective SK2 inhibitor (e.g. ABC294640) was hypothesized to block the enhanced chondrocyte apoptosis in a rat model of MIA (monoiodoacetate)-induced OA. Treatment with ABC294640 during four weeks attenuated several parameters of knee joint histological damage and improved the weight bearing pain

response in OA rats <sup>(64)</sup>. Moreover, cell motility and apoptosis in chondrocytes were in part regulated by the crosstalk between the TGF- $\beta$ /Smad3 and S1P/S1P<sub>3</sub> signaling pathways as shown in temporomandibular joint OA <sup>(65)</sup>. Consequently, we can hypothesize that blocking SK2/S1P/S1P<sub>3</sub> pathway could improve OA manifestations by promoting chondrocyte survival.

Second, SK/S1P may modulate chondrocyte metabolism to favor degradation of the cartilage matrix. Importantly, S1P stimulated secretion of PGE<sub>2</sub> *via* COX-2 and MAPK and regulated VEGF expression in human articular chondrocytes <sup>(66,67)</sup>. Furthermore, S1P-produced PGE<sub>2</sub>, decreased proteoglycan aggrecan expression in human chondrocytes <sup>(68)</sup>. As explained before, S1P can regulate several cell functions intracellularly activating directly various signaling pathways, including NF- $\kappa$ B <sup>(7,69)</sup>. NF- $\kappa$ B is a central signaling pathway responsible for catabolic events in human articular chondrocytes during OA <sup>(70)</sup>. NF- $\kappa$ B also influenced the accumulation and remodeling of extracellular matrix proteins and had indirect positive effects on downstream regulators of terminal chondrocyte differentiation (including  $\beta$ -catenin and Runx2) <sup>(70)</sup>. Collectively, these data suggest that diminishing S1P secretion could lead to the downregulation of inflammatory and angiogenic factors, and NF- $\kappa$ B signaling, consequently reducing catabolic process in OA cartilage. However, this statement is somewhat in contradiction with two other studies. Indeed, on the other hand, the ankylosis protein ANK is a major regulator of SK1 activity. Interestingly, the ANK/SK1 interaction stimulated NF- $\kappa$ B activity and decreased expression of catabolic markers, including the aggrecanase ADAMTS-5 and the collagenase MMP-13, and the inflammatory markers COX-2, IL-6, iNOS in IL-1 $\beta$ -treated *ank/ank* human articular chondrocytes or femoral head explants <sup>(71)</sup>. In parallel, S1P can also act extracellularly, *via* its specific receptors. S1P<sub>2</sub> was identified as the most prevalent S1P receptor subtype in human OA cartilage <sup>(72)</sup>. In human chondrocytes, S1P inhibited IL-1 $\beta$ -induced regulation of ADAMTS-4, MMP-13 and iNOS in particular *via* the



p38 MAPK pathway, downstream S1P<sub>2</sub> receptor, but not S1P<sub>1</sub> and S1P<sub>3</sub> <sup>(72)</sup>. These two studies therefore suggest that the activation of SK1/S1P/S1P<sub>2</sub> pathway could impede the vicious circle of inflammation, and diminish cartilage degradation in the specific context of IL-1 $\beta$ -mediated process.

In conclusion, SK/S1P metabolic pathway involvement in the development of OA has been evidenced, but its role remains controversial (**Figure 3C**). Despite numerous studies on cultured chondrocytes, *in vivo* data are lacking to conclude about the possible beneficial effect of blocking S1P production in OA. S1P action seems dual because it can affect the survival of chondrocytes as well as their catabolic behavior. In addition, the SK1 and SK2 isoforms do not seem to have the same roles, and neither the receptors S1P<sub>2</sub> and S1P<sub>3</sub>. Therefore, it appears likely that defining therapeutic targets very specifically would be needed.

## 2.4 Osteoporosis

Osteoporosis is a skeletal disease that occurs when the equilibrium between bone resorbing (*via* osteoclasts) and bone formation (*via* osteoblasts) is disrupted, leading to bone loss. It is characterized by a diminution in bone mass and architectural modifications; in consequence, bone becomes porous. In general, the disease is accelerated in post-menopausal woman due to the drop of oestrogen production. This hormonal deficiency promotes bone destruction. In addition, the lack of vitamin D and calcium also contributes to the onset of osteoporosis <sup>(73)</sup>. Importantly, the sphingomyelin (SM) pathway (**Figure 1**) has been implicated in the development of osteoporosis. It has been recently shown that multiple variants of a particular SM synthase (*SGMS2*, gene coding for the isoform SMS2) exist in osteoporosis patients <sup>(74)</sup>. Subjects with nonsense mutations, leading to a catalytically inactive SMS2, presented childhood-onset osteoporosis with or without cranial sclerosis. Moreover, subjects with

missense mutations presented a more severe phenotype with neonatal fractures, severe short stature, and spondylometaphyseal dysplasia. In these cases, SMS2 was accumulated in ER and SM *de novo* synthesis was enhanced. Moreover, *SGMS2* did not affect serum S1P concentration <sup>(74)</sup>. It has been also reported that genetic deletion of one of the SMases (the neutral SMase 2, *Smpd3*<sup>-/-</sup>) induced dysproteostasis, skeletal growth inhibition, malformation, and chondrodysplasia in mice <sup>(75)</sup>. Moreover, these mice presented unimpaired mineralization in primary and secondary endochondral ossification centers <sup>(75)</sup>.

Further, the role of S1P in osteoporosis was demonstrated in several clinical studies <sup>(76-78)</sup>. One such study was conducted in postmenopausal woman, and showed that S1P plasma levels are high and correlate with the bone fracture risk <sup>(78)</sup>. S1P is a master regulator of osteoblast-osteoclast coupling, which is dysregulated in osteoporosis. On the one hand, the SK1/S1P/S1P<sub>1</sub> axis stimulates osteoblast differentiation by activating Wnt/BMP pathways <sup>(79)</sup>. This effect, which has been demonstrated in human mesenchymal stem cells, can be abolished by blocking S1P<sub>1</sub> with a specific antagonist <sup>(79)</sup>. S1P also stimulates osteoblast migration and survival <sup>(80)</sup>. Recently, Weske and colleagues showed that S1P<sub>2</sub> could also play an osteoanabolic role in the disease. S1P was able to stimulate osteoprotegerin secretion in MC3T3-E1 osteoblastic cells through activation of S1P<sub>2</sub> <sup>(81)</sup>. More recently, the same authors demonstrated that the activation of S1P<sub>2</sub> with a specific agonist (CYM5520) increased bone mass of long bone and vertebrae and the number of osteoblasts in ovariectomized mice <sup>(82)</sup>.

On the other hand, S1P controls osteoclasts precursors' maturation and migration <sup>(80,83,84)</sup>. In blood vessels, where S1P concentration is high, S1P<sub>1</sub> is downregulated and S1P<sub>2</sub> stimulates their migration towards the bone, where they attach and mature. Ishii and colleagues have shown in ovariectomized mice, a post-menopausal osteoporosis model, that a S1P<sub>1</sub> agonist reduced the number of osteoclasts attached to bone <sup>(83)</sup>. Moreover, the blockade of S1P<sub>2</sub> by the specific antagonist JTE013, limited osteoclastic resorption <sup>(84)</sup>. Activation of RANKL *via*

COX-2 and PGE<sub>2</sub> is also part of the mechanisms leading to the S1P-mediated stimulation of osteoclastogenesis<sup>(80)</sup>. Experiments using bone marrow-derived macrophages showed that the osteoclastogenic factor RANKL enhanced *Sphk1* mRNA and thereby S1P production and secretion<sup>(80)</sup>. Surprisingly, *Sphk1* gene expression silencing by siRNA increased osteoclastogenesis indicating that S1P produced by SK1 would exert a negative feedback regulating osteoclast functions<sup>(80)</sup>. One can assume that high concentrations of S1P in osteoporosis could downregulate osteoclasts precursors' S1P<sub>1</sub> expression and favor their migration to bone via activation of S1P<sub>2</sub> signaling.

In summary, these studies suggest that targeting S1P<sub>2</sub> (either by activation in osteoblasts or by blockade in osteoclasts) could be a potential therapeutic approach in the treatment of osteoporosis (**Figure 3D**).

## **2.5 Cancer-derived bone metastasis**

Bone is a preferential site for the establishment of cancer-derived metastasis; it represents the third most frequent site for metastasis, behind lung and liver. Bone metastasis (BM) is a common complication in multiple myeloma (70-95% of incidence), in prostate cancer (PCa) (65-90%) or in breast cancer (BCa) (65-75%). BM can appear in other cancers but to a lesser extent as in lung cancer (15-65%), melanoma (14-45%), renal cell carcinoma (25%) or colorectal cancer (10%). Globally, PCa and BCa-derived metastasis represent the large majority of the skeletal metastases (up to 70%)<sup>(85-87)</sup>. Patients with BM often experience skeletal complications called skeletal-related events (SREs), including severe pain, increased risk of fracture, hypercalcemia or spinal cord compression and injury among others. SREs strongly increase morbidity in patients. Moreover, when BCa or PCa become metastatic, the

5-year overall survival is reduced to 20%. Therefore therapeutic options need to be developed to reduce or slow BM <sup>(88)</sup>.

SK/S1P signaling has been extensively studied because of its implication in tumorigenesis <sup>(2,3)</sup>. It also drives complex physiological mechanisms like bone homeostasis <sup>(4)</sup>. Interestingly, bone remodeling is dysregulated during BM <sup>(5)</sup>, and few reports suggest a role of altered SK/S1P signaling as a cause for BM.

BM can be divided in three subtypes: osteolytic, osteoblastic or mixed, according to the imbalance in osteoblast-osteoclast coupling:

- Osteolytic BMs are characterized by destruction of normal bone, mainly caused by osteoclasts and they appear in multiple myeloma and particularly in BCa <sup>(89)</sup>. BCa-associated BMs are frequently osteolytic even if in 15-20% of the cases they can be osteoblastic or mixed. Noteworthy, SK1 expression was stimulated by TGF- $\beta$  and correlated to the metastatic potential of MDA-MB-231 cultured cells derived from BCa <sup>(90)</sup>. Moreover, in the MDA-MB-231 subline 1833, derived from a BM, S1P<sub>3</sub> receptor was strongly upregulated and correlated with increased S1P-induced intracellular calcium. Activation by S1P had pro-inflammatory and pro-metastatic potential by inducing COX-2 expression and PGE<sub>2</sub> signal transduction <sup>(91)</sup>.
- Osteoblastic BMs are characterized by an abnormal formation of new bone by osteoblasts. This new bone is synthesized rapidly and is considered as a bone of “poor quality”, which is woven and prone to fractures. Osteoblastic BMs are present in lung cancer patients but they are typically found in advanced PCa patients. Unfortunately, the molecular mechanisms of PCa-derived BM remain poorly understood. To our knowledge, there is only one study implicating SK/S1P metabolism in PCa-derived BM. This study demonstrated that murine or human osteoblastic cells showed high

SK1 activity and stimulated proliferation of PCa cell lines in a S1P-dependent manner. In addition, osteoblastic-derived S1P induced resistance of PCa cells to standard chemotherapy and radiotherapy. Moreover, pharmacological inhibition or knock-down of SK1 abolished the proliferative and survival effects of osteoblasts on PCa models <sup>(92)</sup>.

- Mixed BMs are characterized by areas of osteolysis and areas of bone formation. In addition to BCa, squamous cancers or gastrointestinal cancers can present this type of lesions. With regard to gastrointestinal cancers, several studies confirmed the participation of SK/S1P signaling to the pathobiology of colorectal or colitis-associated cancer <sup>(93)</sup>. Recently, SK1 was implicated in the promotion of metastasis in colorectal cancer <sup>(94)</sup>. Moreover, patients with aggressive colorectal cancer present an upregulated SK1 expression. In these patients, high SK1 was an independent predictor of distant metastasis <sup>(95)</sup>.

Collectively, these studies put forward the implication of the SK1/S1P signaling in the development of cancer-associated BM.

## **2.6 Vascular calcification**

Given the involvement of S1P metabolism in bone formation, the putative role of SK/S1P metabolic axis in vascular calcification (VC) deserves particular investigation. VC appears in three common age-related diseases: chronic kidney disease (CKD), type 2 diabetes mellitus (T2DM), where it develops in the vascular media; and atherosclerosis, where it occurs in the intima. VC is a major pathological problem as it significantly decreases life expectancy in the general population <sup>(96)</sup>, and more dramatically in patients with CKD, where it contributes to cardiovascular mortality <sup>(97–100)</sup>.

In all three diseases and despite intense efforts, it still remains difficult to precisely evaluate the precise involvement of cell-mediated mineralization as compared with cell-independent calcium-phosphate precipitation. It can however be summarized that if cell-independent precipitation probably contributes to VC in the three diseases, endochondral and/or endomembranous ossification, and thus chondrocyte and osteoblast differentiation are also involved. To begin with, endochondral ossification governs the ossification of atherosclerosis plaques in apolipoprotein-E deficient mice <sup>(101,102)</sup>. However, in the case of human atherosclerosis, the plaques show either ossifications or cell-independent calcifications, mainly depending of the arterial bed <sup>(103)</sup>. VC associated with T2DM also shows evidence of endochondral ossification. Studies of human medial calcification in T2DM and aging showed that the expression of chondrocyte differentiation markers such as type II collagen in foci of cartilaginous metaplasia <sup>(104,105)</sup>. Finally, VC in CKD also relies at least in part on endochondral ossification. Both calcified aortic sections from transplant donors with CKD, and calcified aortas from rats with chronic renal failure induced by adenine supplementation, showed immunohistological evidence of the chondrocyte markers Sox9, type II collagen, and Runx2 <sup>(106,107)</sup>. To summarize, VC in atherosclerosis, T2DM and CKD involved at least to some extent arterial differentiation of chondrocyte- and osteoblast-like cells. Since S1P modulates bone cell behavior, it may therefore also impact VC.

To our knowledge, only very few studies explored the effects of S1P metabolism on vascular cell trans-differentiation and calcification. In bovine vascular smooth muscle cells (VSMCs) allowed to transdifferentiate upon addition of  $\beta$ -glycerophosphate, mineralization was accompanied by increased SK1 and SK2 expression, SK activity and S1P release <sup>(108)</sup>. Pharmacological inhibition of SMases, which contribute to ceramide and sphingosine formation, reduced mineralization, whereas exogenously added S1P accelerated mineralization <sup>(108)</sup>. Mechanistically, S1P promoted the phosphorylation of ezrin-radixin-

moesin (ERM) axis and on the opposite, inhibition of ezrin phosphorylation prevented mineralization. Another study investigated the role of S1P in trans-differentiation and calcification of valve interstitial cells, which are involved in valve calcification <sup>(109)</sup>. Although valve calcification is a different disease, valve interstitial cell metaplasia into mineralizing cells appears to share similarities with that of VSMCs. In valve interstitial cells, S1P exerted pro-inflammatory effects that were accompanied by increased levels of bone morphogenetic protein 2 (BMP2), stimulation of alkaline phosphatase activity and calcification <sup>(109)</sup>. Collectively, these rare studies seem to indicate that S1P accelerates VC. However, considerable work remains to be done to better characterize SK/S1P involvement in VC associated with atherosclerosis, CKD, and T2DM.

### **3. POTENTIAL OF TARGETING SPHINGOSINE KINASES/SPHINGOSINE 1-PHOSPHATE METABOLIC PATHWAY FOR THE TREATMENT OF BONE AND JOINT PATHOLOGIES AND ECTOPIC CALCIFICATION**

Considering its role in the pathogenesis of various diseases, SK/S1P signaling has been originally suggested to be targeted at the level of its production by using SK inhibitors <sup>(110)</sup>. The targeting of SK is somewhat complicated by the fact that SK1 and SK2 may have opposite actions. To date, although a number of “specific” inhibitors have been reported <sup>(111,112)</sup>, yet none of them have reached clinical drug development. Another valuable strategy is to target the ligand with antibodies preventing S1P binding to all cognate receptors. This approach has been developed with a highly specific mouse antibody (mAb) directed to S1P, Sphingomab<sup>TM</sup>, which have demonstrated strong efficacy in several preclinical studies in cancer <sup>(113,114)</sup> and OA (O Cuvillier, personal data). Unfortunately, the clinical development of the humanized anti-S1P mAb was stopped due to lack of evidence of efficacy in renal cell carcinoma despite a good safety profile.

The effects of S1P are mostly mediated by the S1P receptors, thus the majority of drugs developed to target S1P signaling are directed against these receptors. Antagonists or agonists may display a specificity towards a given receptor subtype <sup>(111)</sup>. Among them, FTY720 or Fingolimod, an analogue of sphingosine, is currently prescribed for the treatment of relapsing-remitting multiple sclerosis <sup>(115,116)</sup>. FTY720 can be phosphorylated *in vivo* mainly by SK2, and by SK1 to a lesser extent <sup>(117)</sup>, to form FTY720-phosphate (FTY720-P), a mimetic of S1P, interacting with all S1P receptors excepted S1P<sub>2</sub> and preferentially inducing internalization and degradation of S1P<sub>1</sub> <sup>(118)</sup> (**Figure 4**). This effect is mainly due to S1P<sub>1</sub> polyubiquitination by FTY720-P <sup>(119)</sup>. Some of its actions are also attributed to its unphosphorylated form, notably via inhibition of SK1 activity, as suggested by several independent studies <sup>(120–122)</sup>. Overall, FTY720 is considered as a general inhibitor of S1P metabolism <sup>(123)</sup>.

Although systemic treatment with Fingolimod did not improve fracture healing in mice <sup>(124)</sup>, many other studies have demonstrated a beneficial role of Fingolimod in different articular or bone-related diseases. In osteoporosis, it has been shown that Fingolimod was able to ameliorate bone loss both by reducing osteoclast activity and by stimulating osteoblast activity. In a murine model of postmenopausal osteoporosis, it prevented bone density loss after ovariectomy and restored osteoclast number to normal levels <sup>(83)</sup>. Another study showed that Fingolimod suppresses bone resorption in female patients with multiple sclerosis <sup>(125)</sup>. On the other hand, Fingolimod stimulated osteogenic differentiation of bone marrow mesenchymal stem cells in ovariectomized rats <sup>(126)</sup>. It enhanced Runx2, Sp7, alkaline phosphatase and osteocalcin gene and protein expression in these cells <sup>(126)</sup>. Similar results were obtained in the context of periodontitis, which is a common infection that damages the soft tissue and the bony periodontal structures supporting the tooth. In this case, Fingolimod was capable to inhibit osteoclast formation in rats with ligature-induced periodontitis <sup>(127)</sup>.



Moreover, Fingolimod could facilitate re-migration of osteoclasts precursors from alveolar bone surface into blood vessels <sup>(127)</sup>.

The relevance of using Fingolimod as a therapeutic drug has also been shown in the context of inflammatory arthritis. It has been reported that Fingolimod reduced inflammation of ankle joints in animal models of induced-arthritis CIA <sup>(128,129)</sup>. The drug had similar benefic effects in the model of SKG mice, which spontaneously develop chronic autoimmune arthritis, resembling to RA <sup>(130)</sup>. In all these three studies, the immunosuppressive effect of Fingolimod seems to be the major factor improving arthritis. Fingolimod inhibited dendritic cell migration <sup>(129)</sup>, reduced the number of autoimmune CD4(+) T cells in the inflamed synovium via their sequestration in the thymus <sup>(128,130)</sup>, and certainly diminished the local production of IL-6, IL-12, TNF- $\alpha$  and PGE<sub>2</sub> <sup>(129,130)</sup>. By down-regulating the inflammation, Fingolimod reduced the incidence and severity of the arthritis, decreased joint damage and limited bone destruction <sup>(128–130)</sup>.

Finally, we recently showed that Fingolimod reduced S1PR-induced activation by SpA patient's synovial fluids in cultured murine chondrocytes <sup>(58)</sup>. On the other hand, Fingolimod decreased alkaline phosphatase activity, matrix mineralization, and bone formation markers in osteoblasts and hypertrophic chondrocytes <sup>(58)</sup>.

#### **4. CONCLUSION**

Altogether, the studies described in this review suggest the potential of inhibiting/blocking SK/S1P metabolic pathway for the treatment or at least improvement of some articular and bone-related diseases, specially RA, SpA and osteoporosis. The analogue of sphingosine FTY720 or Fingolimod, accepted by US and European agencies for the treatment of relapsing-remitting multiple sclerosis appears to be a promising compound for drug repurposing. By targeting S1P metabolism at several levels, its mode of action is twofold. On

the one hand, it could promote the restoration of bone homeostasis. On the other hand, as an immunosuppressive agent, it could reduce the deleterious inflammatory process. In conclusion, *in vivo* or pre-clinical studies need to be developed in order to validate SK/S1P as a target of reference for the treatment of bone and joint pathologies and ectopic calcification.

## 5. FIGURE LEGENDS

**Figure 1. View of sphingolipid pathway.** Ceramide can be synthesized by *de novo* synthesis or obtained from sphingomyelin (SM) degradation. Ceramide is converted to sphingosine and sphingosine is phosphorylated by the action of sphingosine kinase 1 or 2 (SK1, SK2) to produce sphingosine 1-phosphate (S1P). S1P can be degraded reversibly by S1P phosphatases (SPP1, SPP2) or irreversibly by S1P lyase (SPL). PC: phosphatidylcholine, DAG: diacylglycerol.

**Figure 2. Intracellular and extracellular S1P signaling.** S1P is produced nearby intracellular plasma membrane leaflet. S1P can act intracellularly targeting different proteins and metabolic pathways (ex: HDACs, histone deacetylases). S1P can either get out of the cell *via* ABC transporters or S1P-specific transporter, Spns2. S1P can act in an autocrine or paracrine way through its 5 surface receptors (S1P<sub>1-5</sub>), which are coupled to different G proteins.

**Figure 3. SK/S1P actions in bone and joint diseases.** A) In RA, fibroblast-like synoviocytes (FLS) present elevated SK1 activity enhancing S1P production. S1P is secreted and has both autocrine and paracrine effect through S1P<sub>1-3</sub>. S1P stimulates FLS' migration and invasion provoking synovium thickening. S1P can also enhance osteoclastogenesis and synovitis. B) In SpA, chondrocytes or osteoblasts from enthesis increase SK1 and SK2 activity, consequently augmenting S1P production and secretion. S1P can act intracellularly or locally in the enthesis through S1P<sub>1/3</sub>, favoring ectopic ossification. Its role in enthesitis is still unclear. C) In OA, S1P produced by chondrocytes *via* SK2 activity acts locally in the cartilage through S1P<sub>3</sub>, stimulating apoptosis. Whether the S1P generated from SK1 activity, which interacts with S1P<sub>2</sub>, lead to matrix catabolism is still controversial. D) In osteoporosis, S1P plays on the

balance between bone formation and bone loss. Its action on osteoblasts through S1P<sub>1/2</sub> stimulates mineralization, while its action on osteoclasts, mediated by the overexpressed S1P<sub>2</sub>, favors osteoclast precursor's migration and maturation, thus bone resorption.

**Figure 4. FTY720 metabolism.** FTY720 can enter in the cell and is converted to FTY720-phosphate (FTY720-P) by the action of SKs, preferentially SK2. FTY720-P can go out of the cell through Spns2 transporter, and interact with all S1P receptors except S1P<sub>2</sub>. When FTY720-P binds to S1P<sub>1</sub>, it provokes its internalization, ubiquitinylation and degradation through the proteasome.

## **6. ACKNOWLEDGEMENTS**

Authors wish to thank Université Claude Bernard Lyon 1 EDISS doctoral school for supporting AEJ thesis and CNRS UMR 5246 ICBMS for financial support.

Authors' roles: AEJ, CB, SM, DM and OC contributed to the writing and revision of the manuscript. LBM contributed to the conception, writing and revision of the manuscript.

## REFERENCES

1. Kunkel GT, Maceyka M, Milstien S, Spiegel S. Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. *Nature Reviews Drug Discovery*. 2013 Sep;12(9):688–702.
2. Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. *Nature Reviews Molecular Cell Biology*. 2018;19(3):175–91.
3. Ogretmen B. Sphingolipid metabolism in cancer signalling and therapy. *Nature Reviews Cancer*. 2017 Nov 17;18(1):33–50.
4. Meshcheryakova A, Mechtcheriakova D, Pietschmann P. Sphingosine 1-phosphate signaling in bone remodeling: multifaceted roles and therapeutic potential. *Expert Opinion on Therapeutic Targets*. 2017 Jul 3;21(7):725–37.
5. Khavandgar Z, Murshed M. Sphingolipid metabolism and its role in the skeletal tissues. *Cellular and Molecular Life Sciences*. 2015 Mar;72(5):959–69.
6. Xiao L, Zhou Y, Friis T, Beagley K, Xiao Y. S1P-S1PR1 Signaling: the “Sphinx” in Osteoimmunology. *Frontiers in Immunology* [Internet]. 2019 Jun 25 [cited 2019 Aug 26];10. Available from: <https://www.frontiersin.org/article/10.3389/fimmu.2019.01409/full>
7. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. *Trends in Cell Biology*. 2012 Jan;22(1):50–60.
8. Cuvillier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind S, Spiegel S. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature*. 1996 Jun 27;381(6585):800–3.
9. Perez GI, Knudson CM, Leykin L, Korsmeyer SJ, Tilly JL. Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. *Nat. Med.* 1997 Nov;3(11):1228–32.
10. Morita Y, Perez GI, Paris F, Miranda SR, Ehleiter D, Haimovitz-Friedman A, Fuks Z, Xie Z, Reed JC, Schuchman EH, Kolesnick RN, Tilly JL. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. *Nat. Med.* 2000 Oct;6(10):1109–14.
11. Gault CR, Obeid LM, Hannun YA. An Overview of Sphingolipid Metabolism: From Synthesis to Breakdown. In: Chalfant C, Poeta MD, editors. *Sphingolipids as Signaling and Regulatory Molecules* [Internet]. New York, NY: Springer New York; 2010 [cited 2019 Mar 24]. p. 1–23. Available from: [http://link.springer.com/10.1007/978-1-4419-6741-1\\_1](http://link.springer.com/10.1007/978-1-4419-6741-1_1)
12. Milhas D, Clarke CJ, Hannun YA. Sphingomyelin metabolism at the plasma membrane: Implications for bioactive sphingolipids. *FEBS Letters*. 2010 May 3;584(9):1887–94.
13. Huitema K, van den Dikkenberg J, Brouwers JFHM, Holthuis JCM. Identification of a family of animal sphingomyelin synthases. *The EMBO Journal*. 2004 Jan 14;23(1):33–44.

14. Liu H, Sugiura M, Nava VE, Edsall LC, Kono K, Poulton S, Milstien S, Kohama T, Spiegel S. Molecular Cloning and Functional Characterization of a Novel Mammalian Sphingosine Kinase Type 2 Isoform. *Journal of Biological Chemistry*. 2000 Jun 30;275(26):19513–20.
15. Pitson SM. Regulation of sphingosine kinase and sphingolipid signaling. *Trends in Biochemical Sciences*. 2011 Feb;36(2):97–107.
16. Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nature Reviews Molecular Cell Biology*. 2003 May;4(5):397–407.
17. Melendez AJ, Carlos-Dias E, Gosink M, Allen JM, Takacs L. Human sphingosine kinase: molecular cloning, functional characterization and tissue distribution. *Gene*. 2000 Jun;251(1):19–26.
18. Pyne NJ, McNaughton M, Boomkamp S, MacRitchie N, Evangelisti C, Martelli AM, Jiang H-R, Ubhi S, Pyne S. Role of sphingosine 1-phosphate receptors, sphingosine kinases and sphingosine in cancer and inflammation. *Advances in Biological Regulation*. 2016 Jan;60:151–9.
19. Neubauer HA, Pitson SM. Roles, regulation and inhibitors of sphingosine kinase 2. *FEBS Journal*. 2013 Nov;280(21):5317–36.
20. Cuvillier O, Ader I, Bouquerel P, Brizuela L, Malavaud B, Mazerolles C, Rischmann P. Activation of sphingosine kinase-1 in cancer: implications for therapeutic targeting. *Curr Mol Pharmacol*. 2010 Jun;3(2):53–65.
21. Strub GM, Maceyka M, Hait NC, Milstien S, Spiegel S. Extracellular and Intracellular Actions of Sphingosine-1-Phosphate. In: Chalfant C, Poeta MD, editors. *Sphingolipids as Signaling and Regulatory Molecules* [Internet]. New York, NY: Springer New York; 2010 [cited 2018 Sep 12]. p. 141–55. Available from: [http://link.springer.com/10.1007/978-1-4419-6741-1\\_10](http://link.springer.com/10.1007/978-1-4419-6741-1_10)
22. Pitson SM. Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *The EMBO Journal*. 2003 Oct 15;22(20):5491–500.
23. Pitson SM, Xia P, Leclercq TM, Moretti PAB, Zebol JR, Lynn HE, Wattenberg BW, Vadas MA. Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling. *The Journal of Experimental Medicine*. 2005 Jan 3;201(1):49–54.
24. Hait NC, Bellamy A, Milstien S, Kordula T, Spiegel S. Sphingosine Kinase Type 2 Activation by ERK-mediated Phosphorylation. *Journal of Biological Chemistry*. 2007 Apr 20;282(16):12058–65.
25. Maceyka M, Sankala H, Hait NC, Le Stunff H, Liu H, Toman R, Collier C, Zhang M, Satin LS, Merrill AH, Milstien S, Spiegel S. SphK1 and SphK2, Sphingosine Kinase Isoenzymes with Opposing Functions in Sphingolipid Metabolism. *Journal of Biological Chemistry*. 2005 Nov 4;280(44):37118–29.
26. Mandala SM. Sphingosine-1-Phosphate Phosphatases. *Prostaglandins & Other Lipid Mediators*. 2001 Apr;64(1–4):143–56.
27. Mandala SM, Thornton R, Galve-Roperh I, Poulton S, Peterson C, Olivera A, Bergstrom J, Kurtz MB, Spiegel S. Molecular cloning and characterization of a lipid phosphohydrolase that

- degrades sphingosine-1-phosphate and induces cell death. *Proceedings of the National Academy of Sciences*. 2000 Jul 5;97(14):7859–64.
28. Ogawa C, Kihara A, Gokoh M, Igarashi Y. Identification and Characterization of a Novel Human Sphingosine-1-phosphate Phosphohydrolase, hSPP2. *Journal of Biological Chemistry*. 2003 Jan 10;278(2):1268–72.
29. Kumar A, Saba JD. Lyase to live by: Sphingosine phosphate lyase as a therapeutic target. *Expert Opinion on Therapeutic Targets*. 2009 Aug;13(8):1013–25.
30. Veldhoven PPV, Brys V. Human sphingosine-1-phosphate lyase: cDNA cloning, functional expression studies and mapping to chromosome 10q22. *Biochimica et Biophysica Acta*. 2000;1487(2–3):7.
31. Brindley DN, Pilquill C. Lipid phosphate phosphatases and signaling. *Journal of Lipid Research*. 2009 Apr;50(Supplement):S225–30.
32. Serra M, Saba JD. Sphingosine 1-phosphate lyase, a key regulator of sphingosine 1-phosphate signaling and function. *Advances in Enzyme Regulation*. 2010;50(1):349–62.
33. Brizuela L, Ader I, Mazerolles C, Bocquet M, Malavaud B, Cuvillier O. First Evidence of Sphingosine 1-Phosphate Lyase Protein Expression and Activity Downregulation in Human Neoplasm: Implication for Resistance to Therapeutics in Prostate Cancer. *Molecular Cancer Therapeutics*. 2012 Sep 1;11(9):1841–51.
34. Ceccom J, Loukh N, Lauwers-Cances V, Touriol C, Nicaise Y, Gentil C, Uro-Coste E, Pitson S, Maurage CA, Duyckaerts C, Cuvillier O, Delisle M-B. Reduced sphingosine kinase-1 and enhanced sphingosine 1-phosphate lyase expression demonstrate deregulated sphingosine 1-phosphate signaling in Alzheimer's disease. *Acta Neuropathologica Communications* [Internet]. 2014 Dec [cited 2019 Mar 30];2(1). Available from: <http://actaneurocomms.biomedcentral.com/articles/10.1186/2051-5960-2-12>
35. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S, Spiegel S. Regulation of Histone Acetylation in the Nucleus by Sphingosine-1-Phosphate. *Science*. 2009 Sep 4;325(5945):1254–7.
36. Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, Maceyka M, Jiang H, Luo C, Kordula T, Milstien S, Spiegel S. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature*. 2010 Jun;465(7301):1084–8.
37. Panneer Selvam S, De Palma RM, Oaks JJ, Oleinik N, Peterson YK, Stahelin RV, Skordalakes E, Ponnusamy S, Garrett-Mayer E, Smith CD, Ogretmen B. Binding of the sphingolipid S1P to hTERT stabilizes telomerase at the nuclear periphery by allosterically mimicking protein phosphorylation. *Science Signaling*. 2015 Jun 16;8(381):ra58.
38. Parham KA, Zebol JR, Tooley KL, Sun WY, Moldenhauer LM, Cockshell MP, Gliddon BL, Moretti PA, Tigyi G, Pitson SM, Bonder CS. Sphingosine 1-phosphate is a ligand for peroxisome proliferator-activated receptor- $\gamma$  that regulates neoangiogenesis. *The FASEB Journal*. 2015 Sep;29(9):3638–53.



39. Rosen H, Stevens RC, Hanson M, Roberts E, Oldstone MBA. Sphingosine-1-Phosphate and Its Receptors: Structure, Signaling, and Influence. *Annu. Rev. Biochem.* 2013 Jun 2;82(1):637–62.
40. Takabe K, Paugh SW, Milstien S, Spiegel S. “Inside-Out” Signaling of Sphingosine-1-Phosphate: Therapeutic Targets. *Pharmacological Reviews.* 2008 May 15;60(2):181–95.
41. Vu TM, Ishizu A-N, Foo JC, Toh XR, Zhang F, Whee DM, Torta F, Cazenave-Gassiot A, Matsumura T, Kim S, Toh S-AES, Suda T, Silver DL, Wenk MR, Nguyen LN. Mfsd2b is essential for the sphingosine-1-phosphate export in erythrocytes and platelets. *Nature.* 2017 Oct;550(7677):524–8.
42. Nishi T, Kobayashi N, Hisano Y, Kawahara A, Yamaguchi A. Molecular and physiological functions of sphingosine 1-phosphate transporters. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids.* 2014 May;1841(5):759–65.
43. Spiegel S, Maczys MA, Maceyka M, Milstien S. New insights into functions of the sphingosine-1-phosphate transporter SPNS2. *Journal of Lipid Research.* 2019 Mar;60(3):484–9.
44. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *The Lancet.* 2016 Oct;388(10055):2023–38.
45. Triaille C, Lauwerys BR. Synovial Tissue: Turning the Page to Precision Medicine in Arthritis. *Front. Med.* 2019 Mar 21;6:46.
46. Lai W-Q, Irwan AW, Goh HH, Howe HS, Yu DT, Valle-Onate R, McInnes IB, Melendez AJ, Leung BP. Anti-Inflammatory Effects of Sphingosine Kinase Modulation in Inflammatory Arthritis. *The Journal of Immunology.* 2008 Dec 1;181(11):8010–7.
47. Baker DA, Barth J, Chang R, Obeid LM, Gilkeson GS. Genetic Sphingosine Kinase 1 Deficiency Significantly Decreases Synovial Inflammation and Joint Erosions in Murine TNF- - Induced Arthritis. *The Journal of Immunology.* 2010 Aug 15;185(4):2570–9.
48. Hutami IR, Izawa T, Mino-Oka A, Shinohara T, Mori H, Iwasa A, Tanaka E. Fas/S1P 1 crosstalk via NF- $\kappa$ B activation in osteoclasts controls subchondral bone remodeling in murine TMJ arthritis. *Biochemical and Biophysical Research Communications.* 2017 Sep;490(4):1274–81.
49. Kitano M, Hla T, Sekiguchi M, Kawahito Y, Yoshimura R, Miyazawa K, Iwasaki T, Sano H. Sphingosine 1-phosphate/sphingosine 1-phosphate receptor 1 signaling in rheumatoid synovium: Regulation of synovial proliferation and inflammatory gene expression. *Arthritis & Rheumatism.* 2006 Mar;54(3):742–53.
50. Takeshita H, Kitano M, Iwasaki T, Kitano S, Tsunemi S, Sato C, Sekiguchi M, Azuma N, Miyazawa K, Hla T, Sano H. Sphingosine 1-phosphate (S1P)/S1P receptor 1 signaling regulates receptor activator of NF- $\kappa$ B ligand (RANKL) expression in rheumatoid arthritis. *Biochemical and Biophysical Research Communications.* 2012 Mar;419(2):154–9.
51. Zhao C, Moreno-Nieves U, Di Battista JA, Fernandes MJ, Touaibia M, Bourgoin SG. Chemical Hypoxia Brings to Light Altered Autocrine Sphingosine-1-Phosphate Signalling in Rheumatoid Arthritis Synovial Fibroblasts. *Mediators of Inflammation.* 2015;2015:1–12.

52. Zhao C, Fernandes MJ, Turgeon M, Tancredi S, Di Battista J, Poubelle PE, Bourgoin SG. Specific and overlapping sphingosine-1-phosphate receptor functions in human synoviocytes: impact of TNF- $\alpha$ . *Journal of Lipid Research*. 2008 Nov;49(11):2323–37.
53. Yuan H, Yang P, Zhou D, Gao W, Qiu Z, Fang F, Ding S, Xiao W. Knockdown of sphingosine kinase 1 inhibits the migration and invasion of human rheumatoid arthritis fibroblast-like synoviocytes by down-regulating the PI3K/AKT activation and MMP-2/9 production in vitro. *Mol Biol Rep*. 2014 Aug;41(8):5157–65.
54. Sandler RD, Dunkley L. Osteoarthritis and the inflammatory arthritides. *Surgery (Oxford)*. 2018 Jan;36(1):21–6.
55. McGonagle D, Benjamin M, Marzo-Ortega H, Emery P. Advances in the understanding of enthesal inflammation. *Current Rheumatology Reports*. 2002 Dec;4(6):500–6.
56. Bougault C, El Jamal A, Briolay A, Mebarek S, Boutet M-A, Garraud T, Le Goff B, Blanchard F, Magne D, Brizuela L. Involvement of sphingosine kinase/sphingosine 1-phosphate metabolic pathway in spondyloarthritis. *Bone*. 2017 Oct;103:150–8.
57. Barra G, Lepore A, Gagliardi M, Somma D, Matarazzo MR, Costabile F, Pasquale G, Mazzoni A, Gallo C, Nuzzo G, Annunziato F, Fontana A, Leonardi A, De Palma R. Sphingosine Kinases promote IL-17 expression in human T lymphocytes. *Scientific Reports*. 2018 Dec;8(1):13233.
58. El Jamal A, Briolay A, Mebarek S, Le Goff B, Blanchard F, Magne D, Brizuela L, Bougault C. Cytokine- and stretch-induced sphingosine 1-phosphate production by enthesis cells could favor abnormal ossification in spondyloarthritis. *J. Bone Miner. Res*. 2019 Aug 2;
59. Schiphof D, van den Driest JJ, Runhaar J. Osteoarthritis year in review 2017: rehabilitation and outcomes. *Osteoarthritis and Cartilage*. 2018 Mar;26(3):326–40.
60. Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. *Best Practice & Research Clinical Rheumatology*. 2014 Feb;28(1):5–15.
61. Markstedt K, Mantas A, Tournier I, Martínez Ávila H, Hägg D, Gatenholm P. 3D Bioprinting Human Chondrocytes with Nanocellulose-Alginate Bioink for Cartilage Tissue Engineering Applications. *Biomacromolecules*. 2015 May 11;16(5):1489–96.
62. Mobasheri A, Kalamegam G, Musumeci G, Batt ME. Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions. *Maturitas*. 2014 Jul;78(3):188–98.
63. Fan X, Yuan J, Xie J, Pan Z, Yao X, Sun X, Zhang P, Zhang L. Long non-protein coding RNA DANCR functions as a competing endogenous RNA to regulate osteoarthritis progression via miR-577/SphK2 axis. *Biochemical and Biophysical Research Communications*. 2018 Jun;500(3):658–64.
64. Fitzpatrick LR, Green C, Maines LW, Smith CD. Experimental Osteoarthritis in Rats Is Attenuated by ABC294640, a Selective Inhibitor of Sphingosine Kinase-2. *Pharmacology*. 2011;87(3–4):135–43.

65. Mori H, Izawa T, Tanaka E. Smad3 Deficiency Leads to Mandibular Condyle Degradation via the Sphingosine 1-Phosphate (S1P)/S1P3 Signaling Axis. *The American Journal of Pathology*. 2015 Oct;185(10):2742–56.
66. Moon M-H, Jeong J-K, Lee Y-J, Seol J-W, Park S-Y. Sphingosine-1-phosphate inhibits interleukin-1 $\beta$ -induced inflammation in human articular chondrocytes. *International Journal of Molecular Medicine*. 2012 Dec;30(6):1451–8.
67. Masuko K, Murata M, Beppu M, Nakamura H, Kato T, Yudoh K. Sphingosine-1-phosphate modulates expression of vascular endothelial growth factor in human articular chondrocytes: a possible new role in arthritis. *International Journal of Rheumatic Diseases*. 2012 Aug;15(4):366–73.
68. Masuko K, Murata M, Nakamura H, Yudoh K, Nishioka K, Kato T. Sphingosine-1-phosphate attenuates proteoglycan aggrecan expression via production of prostaglandin E2 from human articular chondrocytes. *BMC Musculoskeletal Disorders*. 2007 Dec;8(1):29.
69. Kihara Y, Maceyka M, Spiegel S, Chun J. Lysophospholipid receptor nomenclature review: IUPHAR Review 8: Lysophospholipid receptor nomenclature. *British Journal of Pharmacology*. 2014 Aug;171(15):3575–94.
70. B. Marcu K, Otero M, Olivotto E, Maria Borzi R, B. Goldring M. NF- $\kappa$ B Signaling: Multiple Angles to Target OA. *CDT*. 2010 May 1;11(5):599–613.
71. Minashima T, Campbell KA, Hadley SR, Zhang Y, Kirsch T. The role of ANK interactions with MYBBP1a and SPHK1 in catabolic events of articular chondrocytes. *Osteoarthritis and Cartilage*. 2014 Jun;22(6):852–61.
72. Stradner MH, Gruber G, Angerer H, Huber V, Setznagl D, Kremser M-L, Moazedi-Fürst FC, Windhager R, Graninger WB. Sphingosine 1-Phosphate Counteracts the Effects of Interleukin-1 $\beta$  in Human Chondrocytes: S1P Counteracts IL-1 $\beta$  in Human OA Chondrocytes. *Arthritis & Rheumatism*. 2013 Aug;65(8):2113–22.
73. Anthamatten A, Parish A. Clinical Update on Osteoporosis. *Journal of Midwifery & Women's Health* [Internet]. 2019 Mar 14 [cited 2019 Apr 18]; Available from: <http://doi.wiley.com/10.1111/jmwh.12954>
74. Pekkinen M, Terhal PA, Botto LD, Henning P, Mäkitie RE, Roschger P, Jain A, Kol M, Kjellberg MA, Paschalis EP, van Gassen K, Murray M, Bayrak-Toydemir P, Magnusson MK, Jans J, Kausar M, Carey JC, Somerharju P, Lerner UH, Olkkonen VM, Klaushofer K, Holthuis JCM, Mäkitie O. Osteoporosis and skeletal dysplasia caused by pathogenic variants in SGMS2. *JCI Insight* [Internet]. 2019 Apr 4 [cited 2019 Jul 24];4(7). Available from: <https://insight.jci.org/articles/view/126180>
75. Stoffel W, Hammels I, Jenke B, Schmidt-Soltau I, Niehoff A. Neutral Sphingomyelinase 2 (SMPD3)-Deficiency in Mice Causes Chondrodysplasia with Unimpaired Skeletal Mineralization. *The American Journal of Pathology* [Internet]. 2019 Jun [cited 2019 Jul 25]; Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0002944018309611>

76. Ahn SH, Koh J-M, Gong EJ, Byun S, Lee S-Y, Kim B-J, Lee SH, Chang JS, Kim GS. Association of Bone Marrow Sphingosine 1-phosphate Levels with Osteoporotic Hip Fractures. *Journal of Bone Metabolism*. 2013;20(2):61.
77. Bae SJ, Lee SH, Ahn SH, Kim H-M, Kim B-J, Koh J-M. The circulating sphingosine-1-phosphate level predicts incident fracture in postmenopausal women: a 3.5-year follow-up observation study. *Osteoporos Int*. 2016 Aug 1;27(8):2533–41.
78. Ardawi M-SM, Rouzi AA, Al-Senani NS, Qari MH, Elsamanoudy AZ, Mousa SA. High Plasma Sphingosine 1-phosphate Levels Predict Osteoporotic Fractures in Postmenopausal Women: The Center of Excellence for Osteoporosis Research Study. *Journal of Bone Metabolism*. 2018;25(2):87.
79. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proceedings of the National Academy of Sciences*. 2008 Dec 30;105(52):20764–9.
80. Ryu J, Kim HJ, Chang E-J, Huang H, Banno Y, Kim H-H. Sphingosine 1-phosphate as a regulator of osteoclast differentiation and osteoclast–osteoblast coupling. *The EMBO Journal*. 2006 Dec 13;25(24):5840–51.
81. Weske S, Vaidya M, Reese A, von Wnuck Lipinski K, Keul P, Bayer JK, Fischer JW, Flögel U, Nelsen J, Epple M, Scatena M, Schwedhelm E, Dörr M, Völzke H, Moritz E, Hannemann A, Rauch BH, Gräler MH, Heusch G, Levkau B. Targeting sphingosine-1-phosphate lyase as an anabolic therapy for bone loss. *Nature Medicine*. 2018 May;24(5):667–78.
82. Weske S, Vaidya M, von Wnuck Lipinski K, Keul P, Manthe K, Burkhart C, Haberhauer G, Heusch G, Levkau B. Agonist-induced activation of the S1P receptor 2 constitutes a novel osteoanabolic therapy for the treatment of osteoporosis in mice. *Bone*. 2019 Aug;125:1–7.
83. Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, Proia RL, Germain RN. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature*. 2009 Mar 26;458(7237):524–8.
84. Ishii M, Kikuta J, Shimazu Y, Meier-Schellersheim M, Germain RN. Chemorepulsion by blood S1P regulates osteoclast precursor mobilization and bone remodeling in vivo. *The Journal of Experimental Medicine*. 2010 Dec 20;207(13):2793–8.
85. D’Oronzo S, Coleman R, Brown J, Silvestris F. Metastatic bone disease: Pathogenesis and therapeutic options. *Journal of Bone Oncology*. 2019 Apr;15:S2212137418302586.
86. Macedo F, Ladeira K, Pinho F, Saraiva N, Bonito N, Pinto L, Gonçalves F. Bone metastases: an overview. *Oncol Rev [Internet]*. 2017 May 9 [cited 2019 Apr 30];11(1). Available from: <http://www.oncologyreviews.org/index.php/or/article/view/321>
87. Fornetti J, Welm AL, Stewart SA. Understanding the Bone in Cancer Metastasis: UNDERSTANDING THE BONE IN CANCER METASTASIS. *J Bone Miner Res*. 2018 Dec;33(12):2099–113.
88. Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nat Rev Cancer*. 2011 Jun;11(6):411–25.

89. Sowder ME, Johnson RW. Bone as a Preferential Site for Metastasis: BONE AS A PREFERENTIAL SITE FOR METASTASIS. *JBMR Plus*. 2019 Mar;3(3):e10126.
90. Stayrook KR, Mack JK, Cerabona D, Edwards DF, Bui HH, Niewolna M, Fournier PG, Mohammad KS, Waning DL, Guise TA. TGF $\beta$ -Mediated induction of SphK1 as a potential determinant in human MDA-MB-231 breast cancer cell bone metastasis. *BoneKey Reports* [Internet]. 2015 Jul 8 [cited 2019 Apr 30];4. Available from: <http://www.portico.org/Portico/article?article=pgk2ph9tdkm>
91. Filipenko I, Schwalm S, Reali L, Pfeilschifter J, Fabbro D, Huwiler A, Zangemeister-Wittke U. Upregulation of the S1P 3 receptor in metastatic breast cancer cells increases migration and invasion by induction of PGE 2 and EP 2 /EP 4 activation. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*. 2016 Nov;1861(11):1840–51.
92. Brizuela L, Martin C, Jeannot P, Ader I, Gstalder C, Andrieu G, Bocquet M, Laffosse J-M, Gomez-Brouchet A, Malavaud B, Sabbadini RA, Cuvillier O. Osteoblast-derived sphingosine 1-phosphate to induce proliferation and confer resistance to therapeutics to bone metastasis-derived prostate cancer cells. *Molecular Oncology*. 2014 Oct;8(7):1181–95.
93. Bao Y, Guo Y, Zhang C, Fan F, Yang W. Sphingosine Kinase 1 and Sphingosine-1-Phosphate Signaling in Colorectal Cancer. *IJMS*. 2017 Oct 8;18(10):2109.
94. Liu S, Xu C, Wu W, Fu Z, He S, Qin M, Huang J. Sphingosine kinase $\diamond$ 1 promotes the metastasis of colorectal cancer by inducing the epithelial-mesenchymal transition mediated by the FAK/AKT/MMPs axis. *Int J Oncol* [Internet]. 2018 Oct 25 [cited 2019 May 1]; Available from: <http://www.spandidos-publications.com/10.3892/ijo.2018.4607>
95. Bae GE, Do S-I, Kim K, Park JH, Cho S, Kim H-S. Increased Sphingosine Kinase 1 Expression Predicts Distant Metastasis and Poor Outcome in Patients With Colorectal Cancer. *Anticancer Res*. 2019 Feb;39(2):663–70.
96. Detrano R, Bild DE, Liu K, Bluemke DA, Watson K. Coronary Calcium as a Predictor of Coronary Events in Four Racial or Ethnic Groups. *n engl j med*. 2008;10.
97. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM. Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension*. 2001 Oct;38(4):938–42.
98. Sigrist MK, Taal MW, Bungay P, McIntyre CW. Progressive Vascular Calcification over 2 Years Is Associated with Arterial Stiffening and Increased Mortality in Patients with Stages 4 and 5 Chronic Kidney Disease. *CJASN*. 2007 Nov;2(6):1241–8.
99. Schlieper G, Krüger T, Djuric Z, Damjanovic T, Markovic N, Schurgers LJ, Brandenburg VM, Westenfeld R, Dimkovic S, Ketteler M, Grootendorst DC, Dekker FW, Floege J, Dimkovic N. Vascular access calcification predicts mortality in hemodialysis patients. *Kidney International*. 2008 Dec;74(12):1582–7.
100. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol. Dial. Transplant*. 2003 Sep;18(9):1731–40.

101. Rosenfeld ME, Polinsky P, Virmani R, Kauser K, Rubanyi G, Schwartz SM. Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse. *Arterioscler. Thromb. Vasc. Biol.* 2000 Dec;20(12):2587–92.
102. Rattazzi M, Bennett BJ, Bea F, Kirk EA, Ricks JL, Speer M, Schwartz SM, Giachelli CM, Rosenfeld ME. Calcification of advanced atherosclerotic lesions in the innominate arteries of ApoE-deficient mice: potential role of chondrocyte-like cells. *Arterioscler. Thromb. Vasc. Biol.* 2005 Jul;25(7):1420–5.
103. Herisson F, Heymann M-F, Chétiveaux M, Charrier C, Battaglia S, Pilet P, Rouillon T, Krempf M, Lemarchand P, Heymann D, Gouëffic Y. Carotid and femoral atherosclerotic plaques show different morphology. *Atherosclerosis.* 2011 Jun;216(2):348–54.
104. Shanahan CM, Cary NR, Salisbury JR, Proudfoot D, Weissberg PL, Edmonds ME. Medial localization of mineralization-regulating proteins in association with Mönckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. *Circulation.* 1999 Nov 23;100(21):2168–76.
105. Qiao J-H, Mertens RB, Fishbein MC, Geller SA. Cartilaginous metaplasia in calcified diabetic peripheral vascular disease: morphologic evidence of enchondral ossification. *Hum. Pathol.* 2003 Apr;34(4):402–7.
106. Neven E, Dauwe S, De Broe ME, D'Haese PC, Persy V. Endochondral bone formation is involved in media calcification in rats and in men. *Kidney Int.* 2007 Sep;72(5):574–81.
107. Neven E, Persy V, Dauwe S, De Schutter T, De Broe ME, D'Haese PC. Chondrocyte rather than osteoblast conversion of vascular cells underlies medial calcification in uremic rats. *Arterioscler. Thromb. Vasc. Biol.* 2010 Sep;30(9):1741–50.
108. Morris TG, Borland SJ, Clarke CJ, Wilson C, Hannun YA, Ohanian V, Canfield AE, Ohanian J. Sphingosine 1-phosphate activation of ERM contributes to vascular calcification. *J. Lipid Res.* 2018 Jan;59(1):69–78.
109. Fernández-Pisonero I, López J, Onecha E, Dueñas AI, Maeso P, Crespo MS, Román JAS, García-Rodríguez C. Synergy between Sphingosine 1-Phosphate and Lipopolysaccharide Signaling Promotes an Inflammatory, Angiogenic and Osteogenic Response in Human Aortic Valve Interstitial Cells. Mohanraj R, editor. *PLoS ONE.* 2014 Oct 2;9(10):e109081.
110. Cuvillier O. Downregulating sphingosine kinase-1 for cancer therapy. *Expert Opin. Ther. Targets.* 2008 Aug;12(8):1009–20.
111. Chew WS, Wang W, Herr DR. To fingolimod and beyond: The rich pipeline of drug candidates that target S1P signaling. *Pharmacological Research.* 2016 Nov;113:521–32.
112. Pitman MR, Costabile M, Pitson SM. Recent advances in the development of sphingosine kinase inhibitors. *Cellular Signalling.* 2016 Sep;28(9):1349–63.
113. Visentin B, Vekich JA, Sibbald BJ, Cavalli AL, Moreno KM, Matteo RG, Garland WA, Lu Y, Yu S, Hall HS, Kundra V, Mills GB, Sabbadini RA. Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages. *Cancer Cell.* 2006 Mar;9(3):225–38.

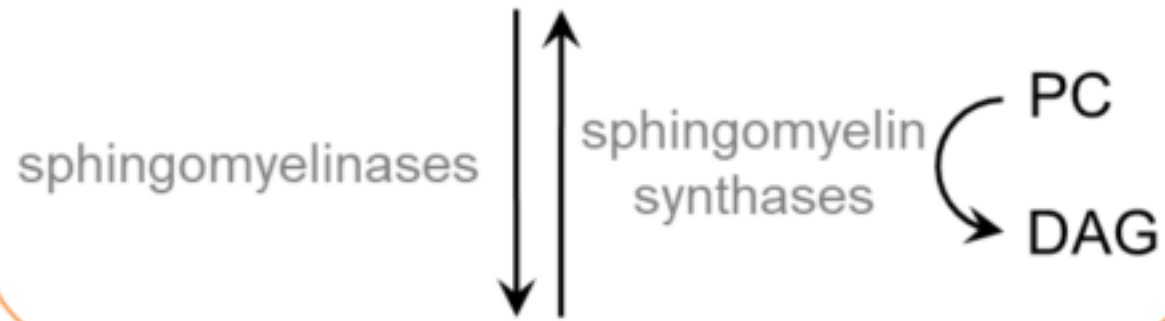
114. Ader I, Gstalder C, Bouquerel P, Golzio M, Andrieu G, Zalvidea S, Richard S, Sabbadini RA, Malavaud B, Cuvillier O. Neutralizing S1P inhibits intratumoral hypoxia, induces vascular remodelling and sensitizes to chemotherapy in prostate cancer. *Oncotarget* [Internet]. 2015 May 30 [cited 2019 May 1];6(15). Available from: <http://www.oncotarget.com/fulltext/3144>
115. Cohen JA, Barkhof F, Comi G, Hartung H-P, Khatri BO, Montalban X, Pelletier J, Capra R, Gallo P, Izquierdo G, Tiel-Wilck K, de Vera A, Jin J, Stites T, Wu S, Aradhye S, Kappos L, TRANSFORMS Study Group. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N. Engl. J. Med.* 2010 Feb 4;362(5):402–15.
116. Kappos L, Radue E-W, O'Connor P, Polman C, Hohlfeld R, Calabresi P, Selmaj K, Agoropoulou C, Leyk M, Zhang-Auberson L, Burtin P, FREEDOMS Study Group. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N. Engl. J. Med.* 2010 Feb 4;362(5):387–401.
117. Kharel Y, Lee S, Snyder AH, Sheasley-O'Neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR. Sphingosine Kinase 2 Is Required for Modulation of Lymphocyte Traffic by FTY720. *Journal of Biological Chemistry.* 2005 Nov 4;280(44):36865–72.
118. Gräler MH, Goetzl EJ. The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. *FASEB J.* 2004 Mar;18(3):551–3.
119. Cuvillier O. Les récepteurs de la sphingosine 1-phosphate: De la biologie à la physiopathologie. *médecine/sciences.* 2012 Nov;28(11):951–7.
120. Pchejetski D, Bohler T, Brizuela L, Sauer L, Doumerc N, Golzio M, Salunkhe V, Teissie J, Malavaud B, Waxman J, Cuvillier O. FTY720 (Fingolimod) Sensitizes Prostate Cancer Cells to Radiotherapy by Inhibition of Sphingosine Kinase-1. *Cancer Research.* 2010 Nov 1;70(21):8651–61.
121. Vessey DA, Kelley M, Zhang J, Li L, Tao R, Karliner JS. Dimethylsphingosine and FTY720 inhibit the SK1 form but activate the SK2 form of sphingosine kinase from rat heart. *J. Biochem. Mol. Toxicol.* 2007;21(5):273–9.
122. Lim KG, Tonelli F, Li Z, Lu X, Bittman R, Pyne S, Pyne NJ. FTY720 Analogues as Sphingosine Kinase 1 Inhibitors: ENZYME INHIBITION KINETICS, ALLOSTERISM, PROTEASOMAL DEGRADATION, AND ACTIN REARRANGEMENT IN MCF-7 BREAST CANCER CELLS. *Journal of Biological Chemistry.* 2011 May 27;286(21):18633–40.
123. Huwiler A, Zangemeister-Wittke U. The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: Recent findings and new perspectives. *Pharmacology & Therapeutics.* 2018 May;185:34–49.
124. Heilmann A, Schinke T, Bindl R, Wehner T, Rapp A, Haffner-Luntzer M, Liedert A, Amling M, Ignatius A. Systemic treatment with the sphingosine-1-phosphate analog FTY720 does not improve fracture healing in mice: FTY720 TREATMENT IN FRACTURE HEALING. *J. Orthop. Res.* 2013 Jun;n/a-n/a.

125. Miyazaki Y, Niino M, Kanazawa I, Suzuki M, Mizuno M, Hisahara S, Fukazawa T, Takahashi E, Amino I, Ochi R, Nakamura M, Akimoto S, Minami N, Fujiki N, Doi S, Shimohama S, Terayama Y, Kikuchi S. Fingolimod suppresses bone resorption in female patients with multiple sclerosis. *Journal of Neuroimmunology*. 2016 Sep;298:24–31.
126. Huang C, Ling R, Li F-J, Li E-C, Huang Q-K, Liu B-G, Ding Y, You S-W. FTY720 enhances osteogenic differentiation of bone marrow mesenchymal stem cells in ovariectomized rats. *Molecular Medicine Reports*. 2016 Jul;14(1):927–35.
127. Lee D-E, Kim J-H, Choi S-H, Cha J-H, Bak E-J, Yoo Y-J. The sphingosine-1-phosphate receptor 1 binding molecule FTY720 inhibits osteoclast formation in rats with ligature-induced periodontitis. *Journal of Periodontal Research*. 2017 Feb;52(1):33–41.
128. Wang F, Tan W, Guo D, He S. Reduction of CD4 positive T cells and improvement of pathological changes of collagen-induced arthritis by FTY720. *European Journal of Pharmacology*. 2007 Nov;573(1–3):230–40.
129. Han Y, Li X, Zhou Q, Jie H, Lao X, Han J, He J, Liu X, Gu D, He Y, Sun E. FTY720 Abrogates Collagen-Induced Arthritis by Hindering Dendritic Cell Migration to Local Lymph Nodes. *The Journal of Immunology*. 2015 Nov 1;195(9):4126–35.
130. Tsunemi S, Iwasaki T, Kitano S, Imado T, Miyazawa K, Sano H. Effects of the novel immunosuppressant FTY720 in a murine rheumatoid arthritis model. *Clinical Immunology*. 2010 Aug;136(2):197–204.



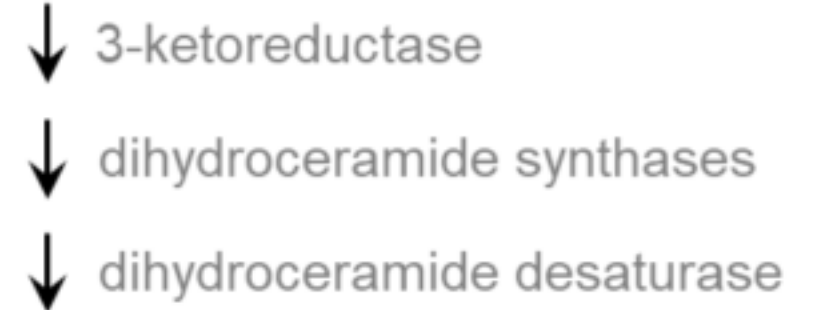
## Sphingomyelin pathway

sphingomyelin

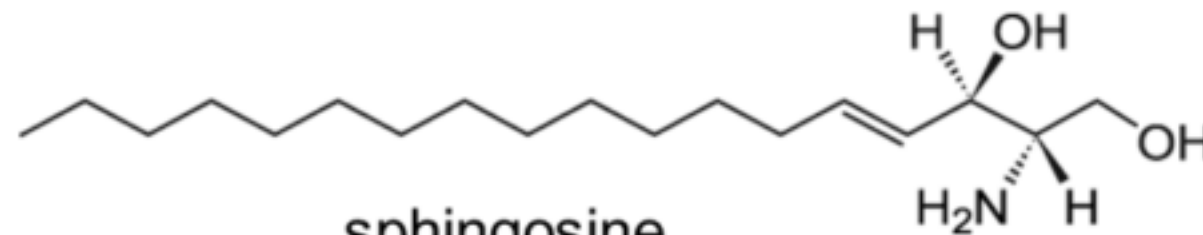
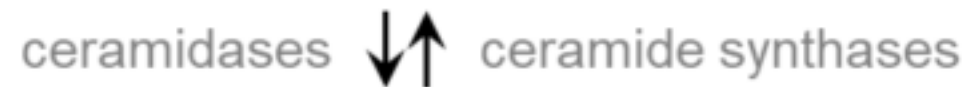


## De novo pathway

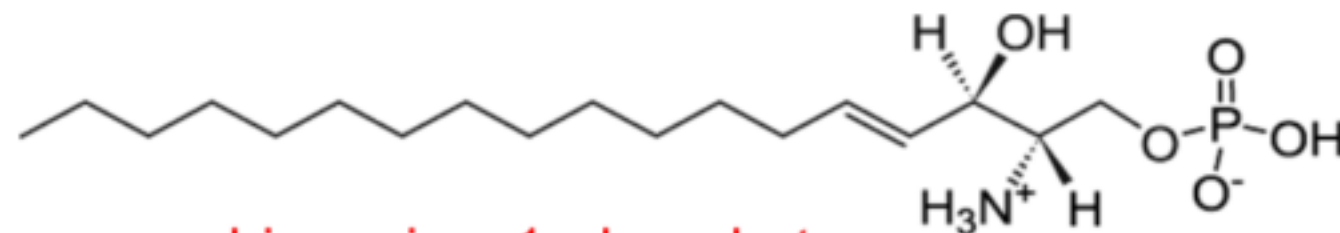
serine + palmitoyl-CoA



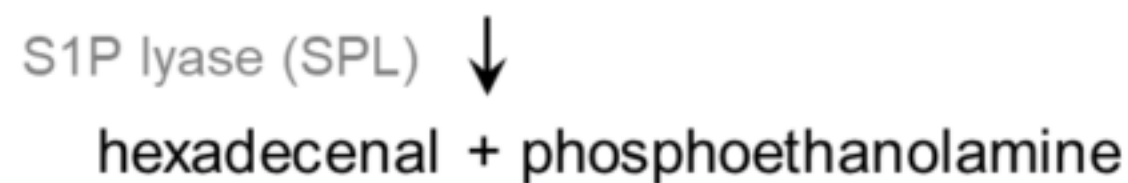
ceramide



sphingosine



sphingosine 1-phosphate



Sphingolipid rheostat

