Advance in the Study of the Mechanisms Regulated by Sphingosine-1-Phosphate

Fei Ye, Xiangqian Kong, Cheng Luo*

(Drug Discovery and Design Center (DDDC), State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China)

Abstract *Sphingosine-1-phosphate (S1P) is a bioactive lipid messenger in the cells that regulate gene expression and NF-κB signal pathway through unknown mechanisms. Recently, Cheng Luo, associate professor of DDDC in Shanghai Institute of Materia Medica, whose project was funded by the National Natural Science Foundation of China, joined in a research team led by Professor Sarah Spiegel of Virginia Commonwealth University. The team continuously made significant breakthroughs in understanding the regulation mechanism of Sphingosine-1-Phosphate. In September 2009, in a paper published on SCIENCE magazine (Science 2009, 325: 1254-7), they firstly demonstrated that S1P is a physiologically important regulator of histone deacetylases (HDACs), HDACs are direct intracellular targets of S1P. Furthermore, they identified the mechanism that S1P regulates gene expression through regulating the activity of HDACs. In June 24th, 2010, in another paper to be published on NATURE magazine (Nature 2010, June 24th, advance online publication, (doi:10.1038/nature09128)) which reports the regulation of NF-κB signaling pathway by S1P. They demonstrate that S1P is the missing cofactor for TRAF2 (tumour-necrosis factor receptor-associated factor 2) and indicate a new paradigm for the regulation of lysine-63-linked poly-ubiquitination. The study also highlight the key role of SphK1 and its product S1P in TNF-α signalling and the canonical NF-κB activation pathway, and then play crucial role in inflammatory, antiapoptotic and immune processes. The identification of new mechanisms by which S1P regulates gene expression and TNF and NF-κB signaling pathway will light up the road to develop novel inhibitors that might be useful for treatment of cancer and inflammatory diseases.*

Key words S1P，SphK1, SphK2, HDAC，TRAF2, NF-κB signaling pathway

Phospholipid and sphingolipid metabolites have important roles in signal transduction pathways initiated by activation of cell surface receptors. The recent identification of nuclear lipid metabolism has demonstrated

*Correspondence: C.L., telephone: 86-21-50271399; email: cluo@mail.shcnc.ac.cn

a new signaling paradigm for phospholipids. The inositol lipids, the best characterized of the intranuclear lipids, have critical roles in nuclear functions, such as pre-mRNA splicing, mRNA export, transcriptional regulation, and chromatin remodeling. Additionally, sphingomyelin is a component of the nuclear matrix, and enzymes controlling sphingolipid metabolism, including neutral sphingomyelinase and ceramidase, are also present in the nucleus, these observations identified that sphingolipids are also metabolized within the nucleus.

Sphingosine-1-phosphate (S1P) is a sphingolipid metabolite present in the nucleus which is generated by Sphingosine kinase I and II (SphK1 and SphK2). It's a bioactive lipid messenger that regulates many cellular physiological processes, including cell growth, survival, movement, angiogenesis, vascular maturation, immunity, and lymphocyte trafficking. Most of its actions are mediated by binding to a family of five hetero-trimeric guanine nucleotide–binding protein-coupled receptors (GPCR). S1P can also act on gene expression and transcription inside the nucleus independently of cell surface receptors. However, the regulations mechanisms and direct intracellular targets of S1P have not been identified. In addition, it has been demonstrated that the interaction of SphK1 with TRAF2 and subsequent activation of SphK1 links TNF-α signals to the activation of NF-κB, yet the mechanism of the involvement of SphK1 and its product S1P in TNF-α signalling and in the canonical NF-κB pathway has not been elucidated.

Recently, significant progresses have been made to elucidate the two mechanisms aforementioned. Cheng Luo, associate professor of DDDC in Shanghai Institute of Materia Medica, whose project was supported by the National Natural Science Foundation of China, joined in a research team led by Prof. Sarah Spiegel of University of Pennsylvania. The team made significant breakthroughs in the understanding of the regulation mechanisms of S1P, and their studies have been published on *SCIENCE*[1] and *NATURE*[2], respectively.

Gene expression and transcription regulated by S1P

The team first mentioned that S1P is a physiologically key regulator of histone deacetylases (HDACs), HDACs are direct intracellular targets of S1P. S1P appears to be an endogenous inhibitor of HDAC, regulates gene expression by inhibiting the enzymatic activity of HDAC. Luo and his colleagues identified the regulation mechanisms and uncovered the secret about its novel function to regulate gene expression. SphK2, one of the isoenzymes that generates S1P, was selectively enriched at the promoters of the genes encoding the cyclin-dependent kinase inhibitor p21 or the transcriptional regulator c-fos, where SphK2 produced S1P. S1P specifically bound to the histone deacetylase HDAC1 and HDAC2 and inhibited their enzymatic activity, preventing the removal of acetyl groups from lysine residues within histone tails, thus enhanced local histone

H3 acetylation and transcription.

Acetylation and deacetylation of core histone protein can influence the transcription activity in chromatin, which is closely related to gene regulation. That is very important in the understanding of Epigenetics. Histone acetylase (HATs) and deacetylases (HDACs) function antagonistically to control histone acetylation.

In this study, firstly, S1P was found to inhibit HDACs activities in cell nuclear extracts. Furthermore, experiments confirmed that S1P functions as an endogenous inhibitor of HDACs by interacting with the catalytic sites of HDAC1 and HDAC2. A common response to inhibition or depletion of HDAC1 or HDAC2 will selectively enriched at the promoters of the genes encoding the cyclin-dependent kinase inhibitor, p21, or the transcriptional regulator, c-fos, where it enhanced local histone H3 acetylation and transcription. Overexpression of SphK2 increased acetylation of histone H3-lysine9 associated with the proximal p21 promoter and further enhanced the response to PMA (PMA is an activator of protein kinase C, which enhances phosphorylation and catalytic activity of SphK2 ,ultimately increases amounts of S1P in the nucleus). They also examined another target gene that shows increased transcription in cells treated with PMA, c-fos. SphK2 was associated with c-fos promoter, over-expression of SphK2 enhanced H3 acetylation at the c-fos promoter.

Together, they concluded that S1P regulated gene expression through HDACs. SphK2, was selectively enriched at the promoters of the genes encoding the cyclin-dependent kinase inhibitor p21 or the transcriptional regulator c-fos, where SphK2 produced S1P. S1P specifically bound to the histone deacetylases HDAC1 and HDAC2 and inhibited their enzymatic activity, preventing the removal of acetyl groups from lysine residues within histone tails, thus enhanced local histone H3 acetylation and transcription.

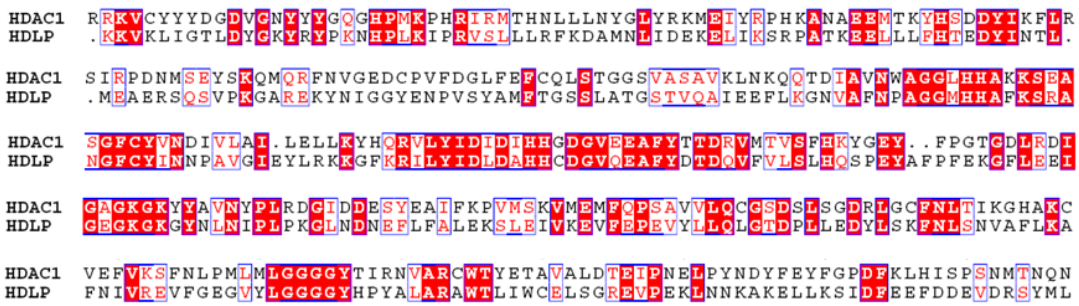
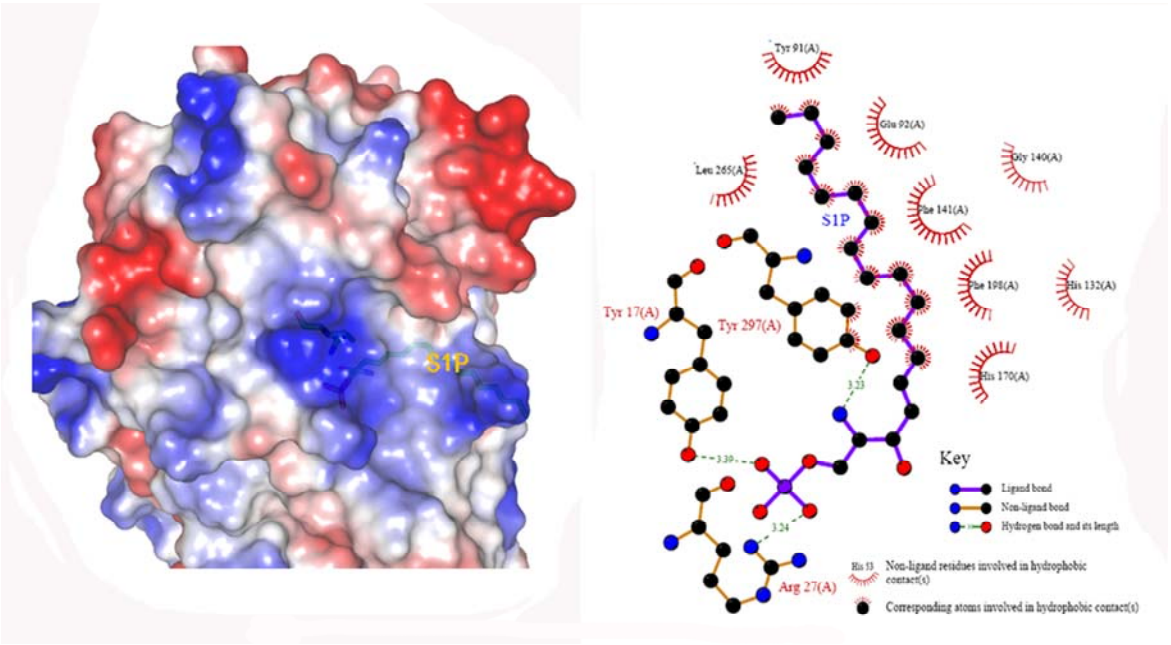


Fig.1. *Sequence alignment of catalytic domain in HDAC1 and HDLP*



A B

Fig.2. *Docking of S1P into the pocket of the HDAC1 homologue from Aquifex Aeolicus, HDLP.(**A,** Surface contour of the binding site with S1P; **B,** Hydrogen bonds and hydrophobic interactions of S1P with the HDLP)*

Funded by the Ministry of Science and Technology of China, National Natural Science Foundation of China and Chinese Academy of Sciences, researchers of Shanghai Institute of Materia Medica, actively participated in this project with their expertise advantages. They predicted that HDACs might be the biological targets of S1P by computational biological approaches. The S1P complexes with HDAC1 homologue (Histone deacetylase like protein，HDLP, that shares 35.2% identity with human HDAC1 over 375 residues, shown in Fig.1) modeled by computational biological approaches, revealed the binding mode and crucial residues of HDLP interaction with S1P (shown in Fig. 2). They also predicted the binding affinity of S1P with HDAC is almost comparable potent as that of SAHA (a wellknown inhibitor of HDACs). Finally, the whole study was greatly accelerated by the guide from the accurate theoretical study.

TNF-α signalling and the NF-κB activation pathway regulated by S1P

Tumour-necrosis factor (TNF) receptor-associated factor 2 (TRAF2) is a key component in NF-κB signalling triggered by TNF-α. Genetic evidence indicates that TRAF2 is necessary for the polyubiquitination of receptor interacting protein 1 (RIP1) that then serves as a platform for recruitment and stimulation of IκB kinase, leading to activation of the transcription factor NF-κB. Although TRAF2 is a RING domain ubiquitin ligase, direct evidence that TRAF2 catalyses the ubiquitination of RIP1 is lacking. TRAF2 binds to sphingosine

kinase 1 (SphK1), one of the isoenzymes that generates the pro-survival lipid mediator sphingosine-1-phosphate (S1P) inside cells.

In this study, the team identified that SphK1 and the production of S1P is necessary for lysine-63-linked polyubiquitination of RIP1, phosphorylation of IκB kinase and IκBα, and IκBα degradation, leading to NF-κB activation. These responses were mediated by intracellular S1P independently of its cell surface GPCRs. S1P specifically binds to TRAF2 at the amino-terminal RING domain and stimulates its E3 ligase activity. S1P, but not dihydro-S1P, markedly increased recombinant TRAF2-catalysed lysine-63-linked, but not lysine-48-linked, polyubiquitination of RIP1 in vitro in the presence of the ubiquitin conjugating enzymes (E2) UbcH13 or UbcH5a. The data showed that TRAF2 is a novel target of S1P, and that S1P is the missing cofactor for TRAF2 E3 ubiquitin ligase activity, describing a new paradigm for the regulation of lysine-63-linked polyubiquitination. These results also highlight the key role of SphK1 and its product S1P in TNF-α signalling and the canonical NF-κB activation pathway important in inflammatory, antiapoptotic and immune processes.

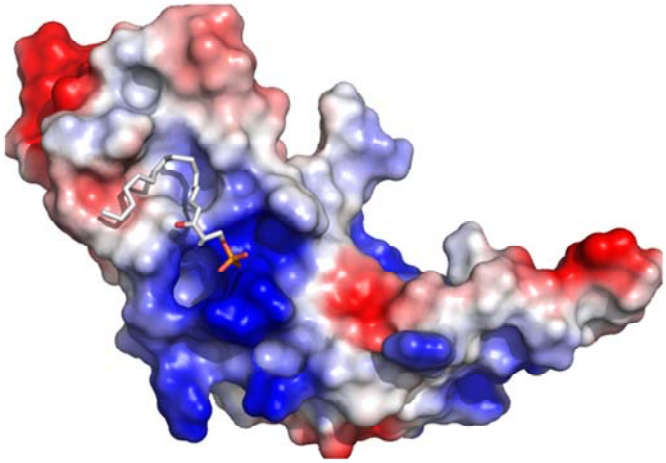


Fig.3. *The binding mode of S1P with the RING domain of TRAF2*

Base on the hypothesis that S1P can interact with TRAF2, researchers of DDDC in Shanghai Institute of Materia Medica constructed the structure of TRAF2 by molecular modeling methods. Modeling of S1P into the RING domain of the crystal structure of TRAF2 revealed that it docked remarkably well in a 16-Å-long binding cavity consisting of a hydrophobic region (Phe 45 Leu 58, Ala 59, Leu 62, Ala 90, Phe 91 and Phe 92, shown in Fig.3) and positively charged region (Arg 43 and Arg 97), which may stabilize the phosphate group of S1P (The modeled structures are very similar to the crystal structure of TRAF2 which was reported at the end of 2009). Further molecular dynamic simulation and free energy prediction indicates the binding affinity of S1P with TRAF2 is -8.07 kcal/mol, whereas dihydro-S1P shows instability in binding with TRAF2 during the

simulation process, consistent with the inability of dihydro-S1P to bind or activate TRAF2. These results provided crucial information for this study. Furthermore, they constructed 3D structures of the whole TRAF family and predicted their binding affinities to S1P. The results indicated that TRAF6 also can bind to S1P weakly, however, there was no obvious binding pocket in TRAF3 which can't be regulated by S1P. These predictions from theoretical studies light up the road for the experimental study and therefore significantly accelerate the study in this project.

Together, the successful strategy used by the research team demonstrates that it will be an crucial strategy to combine various computational biology and experimental approaches to explore other complicated problems in life science.

Acknowledgment. This work was supported by Natural Science Foundation of China (Grant No. 20972174)

References

1. Hait N, Allegood J, et al. Regulation of Histone Acetylation in the Nucleus by Sphingosine-1-Phosphate, Science, 2009, **325**: 1254-1257
2. Alvarez1 S, Harikumar K, et al., Sphingosine-1-phosphate is a missing Cofactor for the E3 Ubiquitin Ligase TRAF2, Nature, 2010, **10**: doi:1038/nature09128.

（20 March 2010;accepted 25 May 2010）