磷脂爬行酶的基因结构、进化、分布和功能

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摘要 磷脂爬行酶(phospholipid scramblase, PLSCR)基因家族是编码一组Ca^{2+}依赖的多棕榈酰化II型膜蛋白, 存在于所有的真核生物中。在高浓度的Ca^{2+}环境中, 磷脂爬行酶会使膜磷脂(phospholipid, PL)发生无规则的翻转运动, 最终造成磷酯酰丝氨酸(phosphatidylserine, PS)外翻, 从而参与调控细胞激活、损伤或者凋亡过程中膜磷脂的分布。PLSCR的功能除参与细胞的增殖与分化外, 还在体内多种过程中发挥作用, 如脂质代谢、线粒体结构功能维护、血液凝固和免疫应答等过程。为更全面系统地了解PLSCR基因家族, 该文对近年来*PLSCR*基因的结构、进化、分布和功能的相关研究进行了综述。

关键词 磷脂爬行酶; 基因结构; 进化; 功能

Gene Structure, Evolution, Distribution and Function of Phospholipid Scramblase

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Abstract Phospholipid scramblase (PLSCR) gene family encodes a kind of Ca^{2+}-dependent multiple-palmitoylated type II endofacial plasma membrane protein, which exists in all eukaryotes. PLSCR is activated in a high concentration of calcium ion, which involved in the collapse of phospholipid (PL) asymmetry at the plasma membrane leading to externalization of phosphatidylserine (PS). Under this activated state, PLSCR mainly regulates the transbilayer movement of the phospholipid in the process of cell activation, damage and apoptosis. More and more evidences revealed that PLSCR not only played an important role in cell proliferation and differentiaton, but also contributed to other physiological pathways, including lipid metabolism, blood coagulation cascade, structure and function maintenance of mitochondrial and immune response. For a more comprehensive understanding of PLSCR gene families, this paper reviewed the gene structure, evolution, distribution and function of *PLSCR* based on the recent studies.

Keywords PLSCR; gene structure; evolution; function

1997年, Sims等[1]从人类红细胞膜上分离出了分子量为35 kDa的蛋白质, 并从白血病细胞系K-562中克隆得到编码该蛋白质的基因。最初的研究发现, 该蛋白质参与Ca^{2+}依赖的膜磷脂爬行运动, 故将其命名为磷脂爬行酶1(phospholipid scramblase 1, PLSCR1)。2000年, Wiedmer等[2]从人细菌人工染色体基因组文库中克隆得到29.7 Kb的人*PLSCR1*基因(*hPLSCR1*), 并利用PCR技术获得了三个与*hPLSCR1*

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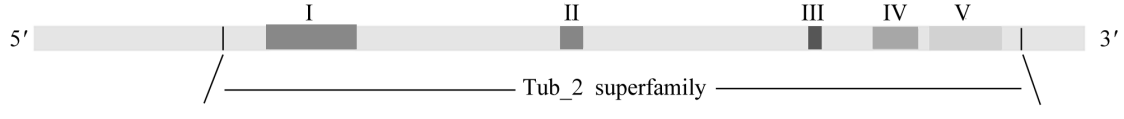
高度同源的核酸序列, 即*hPLSCR2*(编码297个氨基酸, 59%), *hPLSCR3*(编码295个氨基酸, 47%)和*hPLSCR4*(编码329个氨基酸, 46%)。此外, 在家鼠、大猩猩、果蝇、非洲爪蟾和斑马鱼等中都克隆得到了*hPLSCR*不同亚型的同源基因。因此, 研究发现, 磷脂爬行酶是一个基因家族, 包括*hPLSCR1*、*hPLSCR2*、*hPLSCR3*和*hPLSCR4* 4个主要成员。

PLSCR是一组同源且保守的Ca^{2+}结合棕榈酰化II型膜蛋白, 与其他的棕榈酰化蛋白质一样, 棕榈酰化的PLSCRl可锚定在膜上, 而非棕榈酰化的PLSCR1则进入细胞核内发挥作用[3]。不对称的膜磷脂双分子层是构成细胞膜的基本骨架, 氨基类磷脂如磷脂酰丝氨酸(phosphatidylserine, PS)和磷脂酰乙醇胺(phosphatidylethanolamine, PE)分布在膜磷脂双分子层的内侧, 胆碱类磷脂如磷脂酰胆碱(phosphatidylcholine, PC)和鞘磷脂(sphingomyelin, SM)分布在膜外侧[4]。正常情况下, 膜磷脂分子间存在比较缓慢地侧向扩散、旋转、伸缩、翻转等运动, 但在细胞增殖、分化、损伤或凋亡的早期, 胞内高浓度Ca^{2+}将促使细胞膜上的PLSCR1启动细胞膜磷脂分子发生无规则翻转运动, 最终使PS暴露于细胞表面[4]。存在于秀丽隐杆线虫中的*hPLSCR*同源基因*scrm*(scramblase)也参与了细胞凋亡早期PS的外翻[5]。此外, PLSCR在体内还参与了脂质代谢、线粒体结构与功能维护、血液凝固、细胞凋亡、癌变和免疫等过程。虽然PLSCR在机体中参与多种反应过程, 但对该基因的了解还不够系统和全面。本文就*PLSCR*基因的结构与进化、表达、定位以及功能进行了综述。

1　*PLSCR*基因结构与进化分析

Wiedmer等[2]和Kasukabe等[6]鉴定发现, *hPLSCR1*由9个外显子、8个内含子和5′侧翼序列组成, 其中从5′侧翼序列的–95 bp到第1个外显子的+60 bp为启动子区。启动子区包括2个GC盒(位于–79 bp~–66 bp和–59 bp~–46 bp)、1个CCAAT盒(位于–111 bp~–101 bp)和一些转录激活因子如激活蛋白4(activator protein 4, AP4)、上游刺激因子(upstream stimulating factor, USF)、真核转录调控因子1(eukaryotic transcriptional regulator 1, ETRl)、干扰素刺激反应元件(interferon-stimulated response element, ISRE)和干扰素调节因子(interferon regulatory factor, IRF)等的结合区[4]。外显子1属于非编码区, 外显子2~9编码1个含318个氨基酸的蛋白质[2]。PLSCR家族成员, 除了PLSCR2, 均具有1个富含脯氨酸的N-端区域, 其内有多个保守的PXXP和PPXY模体(motif), 可与含有SH3和WW结构域的蛋白质相互作用[7]。除此之外, 如图1所示, 该家族蛋白质还含有1个十分保守的Tub_2超家族(superfamily)结构域, 该结构域是由12个β折叠和C-端的α螺旋组成。此α螺旋是一个跨膜螺旋结构, 类似于Tubby蛋白质的C-端结构域。相关报道称, Tubby蛋白在植物中参与了病原体的防御作用[8]。Tub_2超家族结构域内有5个保守的基序: 可与DNA相互作用的DNA结合结构域(M-E)、富含半胱氨酸的区域(CCCPCC)、将蛋白质导入细胞核的非经典的核定位信号(nuclear localization signal, NLS)(GKISKHWTGI)、保守的Ca^{2+}结合EF手性环形结构域(DADNFGIQFPLD)和富含疏水性氨基酸的跨膜区(KMKAVMIGACFLIDFMFFE)[7,9]。

对不同物种*PLSCR*基因的不同亚型蛋白质序列进行分子进化树分析, 该进化树采用MEGA4软件, 利用邻接法构建发现, PLSCR3、PLSCR4和PLSCR5有规律的成簇分布(图2), 而PLSCR1和PLSCR2的分布比较杂乱。推测这可能由于两者基因结构比较相似造成, 例如人*PLSCR1*基因(*hPLSCR1*)与*hPLSCR2*(59%)的

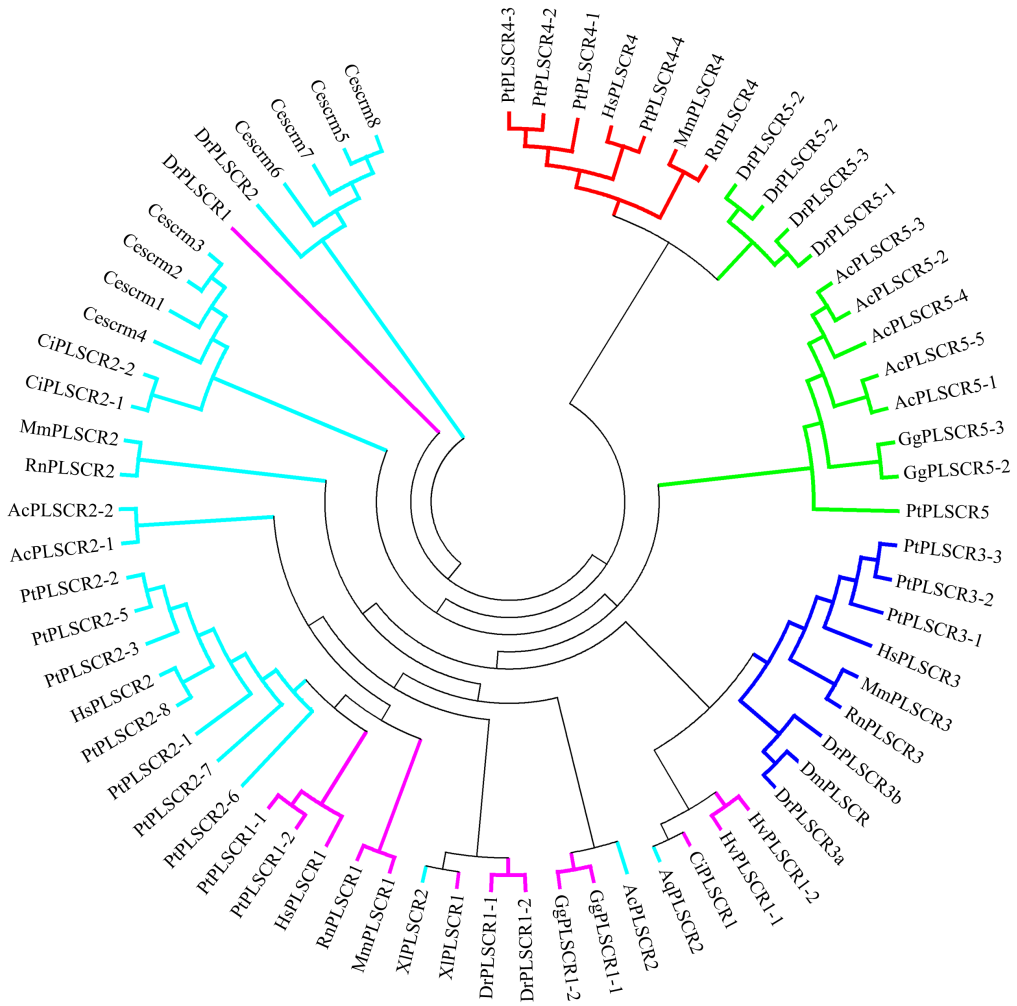


磷脂爬行酶基因含有1个十分保守的Tub_2超家族结构域, 其内有5个保守且关键的模体[7,9]。I: DNA结合结构域; II: 半胱氨酸的区域; III: 核定位信号; IV: Ca^{2+}结合EF手性环形结构域; V: 跨膜区。

The gene of PLSCR family contains a conservative domain of Tub_2 superfamily which has 5 key and conserved motifs marked in roman numerals[7,9]. I: DNA binding motif; II: cysteine palmitoylation motif; III: nuclear localization signal motif; IV: Ca^{2+} binding EF-hand-like motif; V: transmembrane region.

图1　磷脂爬行酶基因结构

Fig.1　Structure of phospholipid scramblase gene



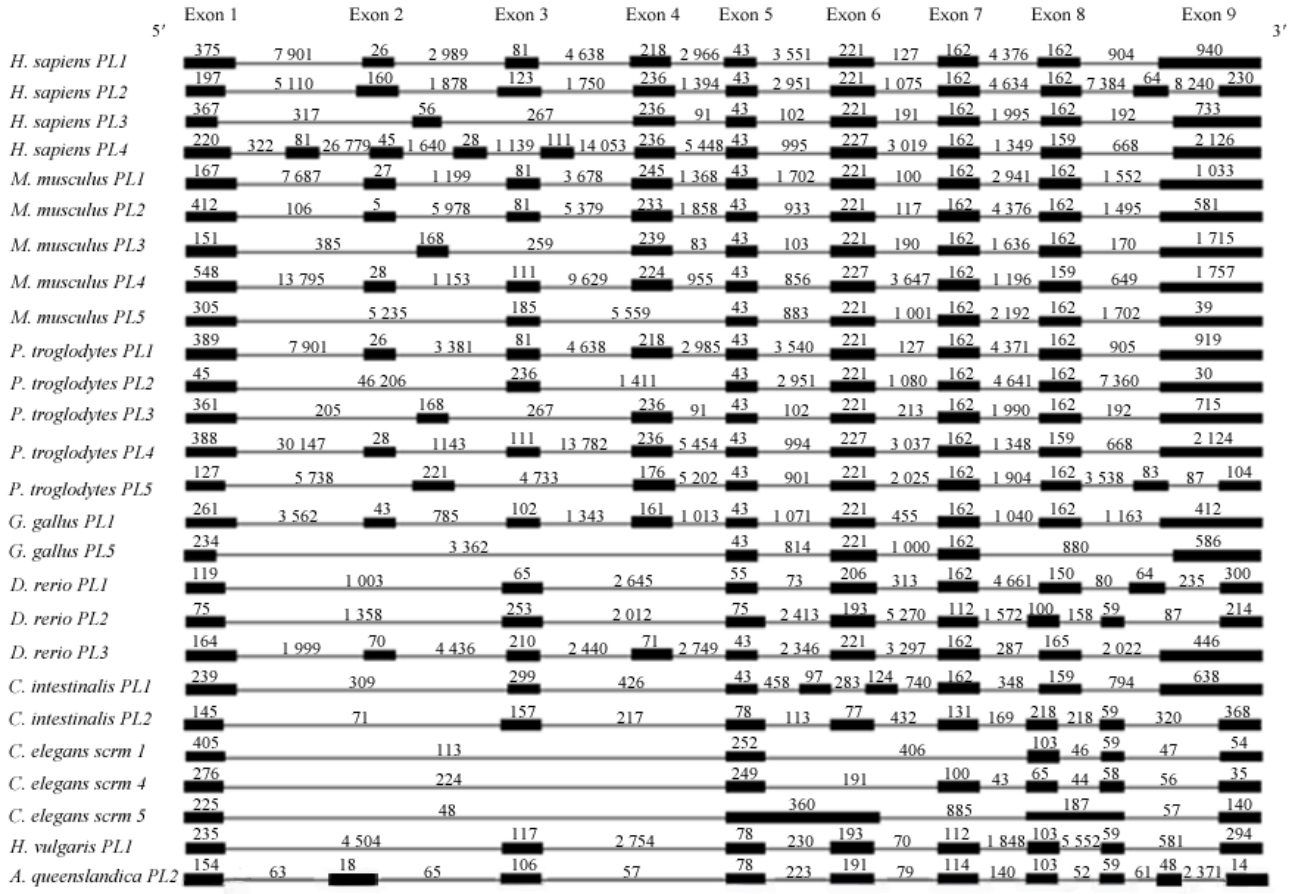
该进化树采用MEGA4软件, 利用邻接法构建。紫色、浅蓝、蓝色、红色、绿色分别代表PLSCR1、PLSCR2、PLSCR3、PLSCR4和PLSCR5不同亚型蛋白质。

The evolutionary tree was constructed with the method of adjacent building by MEGA4 soft. Purple, shallow blue, blue, red, green represent the different subtype proteins of the PLSCR1, PLSCR2, PLSCR3, PLSCR4 and PLSCR5 respectively.

The original tree of PLSCR proteins is from species as follows: HsPLSCR1 (智人磷脂爬行酶, *Homo sapiens phospholipid scramblase*, NP_066928.1), HsPLSCR2 (NC_000003.12), HsPLSCR3 (NP_001188505.1), HsPLSCR4 (NP_001121776.1); PtPLSCR1-1 (黑猩猩, *Pan troglodytes*, XP_001135229.2), PtPLSCR1-2 (XP_009444920.1), PtPLSCR2-1 (XP_009444911.1), PtPLSCR2-2 (XP_009444912.1), PtPLSCR2-3 (XP_009444913.1), PtPLSCR2-5 (XP_009444915.1), PtPLSCR2-6 (XP_009444916.1), PtPLSCR2-7 (XP_009444917.1), PtPLSCR2-8 (XP_009444919.1), PtPLSCR3-1 (XP_001174792.2), PtPLSCR3-2 (XP_003315398.1), PtPLSCR3-3 (XP_009430014.1), PtPLSCR4-1 (XP_003310155.1), PtPLSCR4-2 (XP_003310156.1), PtPLSCR4-3 (XP_009444909.1), PtPLSCR4-4 (XP_003310158.1), PtPLSCR5 (XP_003310100.1); MmPLSCR1 (小家鼠, *Mus musculus*, NP_035766.2), MmPLSCR2 (NP_001182013.1), MmPLSCR3 (NP_001161969.1), MmPLSCR4 (NP_848826.1); RnPLSCR1 (褐家鼠, *Rattus norvegicus*, XP_006243600.1), RnPLSCR2 (NP_001014116.1), RnPLSCR3 (NP_001012139.1), RnPLSCR4 (NP_001012000.1), GgPLSCR1-1 (原鸡, *Gallus gallus*, XP_001231237.1), GgPLSCR1-2 (XP_422696.2), GgPLSCR5-2 (XP_422697.4), GgPLSCR5-3 (XP_004943368.1); XlPLSCR1 (非洲爪蟾, *Xenopus laevis*, NP_001089425.1), XlPLSCR2 (NP_001090508.1); DmPLSCR (黑腹果蝇, *Drosophila melanogaster*, Q8IQD8); AcPLSCR2 (安乐尔蜥, *Anolis carolinensis*, XP_003230373.2), AcPLSCR2-1 (XP_003227037.1), AcPLSCR2-2 (XP_008118262.1), AcPLSCR5-1 (XP_008118266.1), AcPLSCR5-2 (XP_008118267.1), AcPLSCR5-3 (XP_008118270.1), AcPLSCR5-4 (XP_008118271.1), AcPLSCR5-5 (XP_003227040.1); DrPLSCR1 (斑马鱼, *Danio rerio*, XP_003201533.3), DrPLSCR1-1 (XP_693207.5), DrPLSCR1-2 (XP_009295907.1), DrPLSCR2 (XP_003197974.1), DrPLSCR3a (NP_001098583.1), DrPLSCR3b (NP_998031.1), DrPLSCR5-1 (XP_009301436.1), DrPLSCR5-2 (XP_009301437.1), DrPLSCR5-3 (XP_009301438.1), DrPLSCR5-4 (XP_009301439.1); CiPLSCR1 (玻璃海鞘, *Ciona intestinalis*, XP_002121993.3), CiPLSCR2-1 (XP_002131364.1), CiPLSCR2-2 (XP_009858073.1); Cescrm1 (秀丽隐杆线虫, *Caenorhabditis elegans*, NP_001251705.1), Cescrm2 (NP_493321.1), Cescrm3 (NP_503934.2), Cescrm4 (NP_492975.3), Cescrm5 (NP_500500.1), Cescrm6 (NP_492890.2), Cescrm7 (NP_506646.1), Cescrm8 (NP_500501.1); HvPLSCR1-1 (水螅, *Hydra vulgaris*, XP_002163447.2), HvPLSCR1-2 (XP_012565297.1); AqPLSCR2 (海绵, *Amphimedon queenslandica*, XP_003382485.1).

图2 PLSCR的分子进化树

Fig.2 The molecular evolutionary tree of PLSCR



黑色框代表外显子, 连接线代表内含子。框和线上面的数字分别代表外显子和内含子的大小。

Black boxes and the linking lines represent the exons and introns respectively. The numbers above the boxes and lines indicate the size of the introns and exons, respectively.

The genomic structure of PLSCR genes is from the following species: *H. sapiens PL1* (智人磷脂爬行酶, *Homo sapiens phospholipid scramblase*, NM_021105.2); *P. troglodytes PL1* (黑猩猩, *Pan troglodytes*, XM_001135229.4); *M. musculus PL1* (小家鼠, *Mus musculus*, NM_011636.2), *H. sapiens PL2* (NM_001199978.1), *P. troglodytes PL2* (XM_009446636.1), *M. musculus PL2* (NM_001195084.1), *H. sapiens PL3* (NM_001201576.1), *P. troglodytes PL3* (XM_009431739.1), *M. musculus PL3* (NM_001168497.1), *H. sapiens PL4* (NM_001128304.1), *P. troglodytes PL4* (XM_003310108.3), *M. musculus PL4* (NM_178711.3), *P. troglodytes PL5* (XM_003310052.2), *M. musculus PL5* (NM_001195693.1); *G. gallus PL1* (原鸡, *Gallus gallus*, XM_001231236.3), *G. gallus PL5* (XM_422697.4); *D. rerio PL1* (斑马鱼, *Danio rerio*, XM_003201485.3), *D. rerio PL2* (XM_003197926.3), *D. rerio PL3* (NM_212866.1); *C. intestinalis PL1* (玻璃海鞘, *Ciona intestinalis*, XM_002121957.3), *C. intestinalis PL2* (XM_009859771.1); *C. elegans scrm1* (秀丽隐杆线虫, *Caenorhabditis elegans*, NM_001264776.1), *C. elegans scrm4* (NM_060574.4), *C. elegans scrm5* (NM_068099.4); *H. vulgaris PL1* (水螅, *Hydra vulgaris*, XM_002163411.3), *A. queenslandica PL2* (海绵, *Amphimedon queenslandica*, XM_003382437.2).

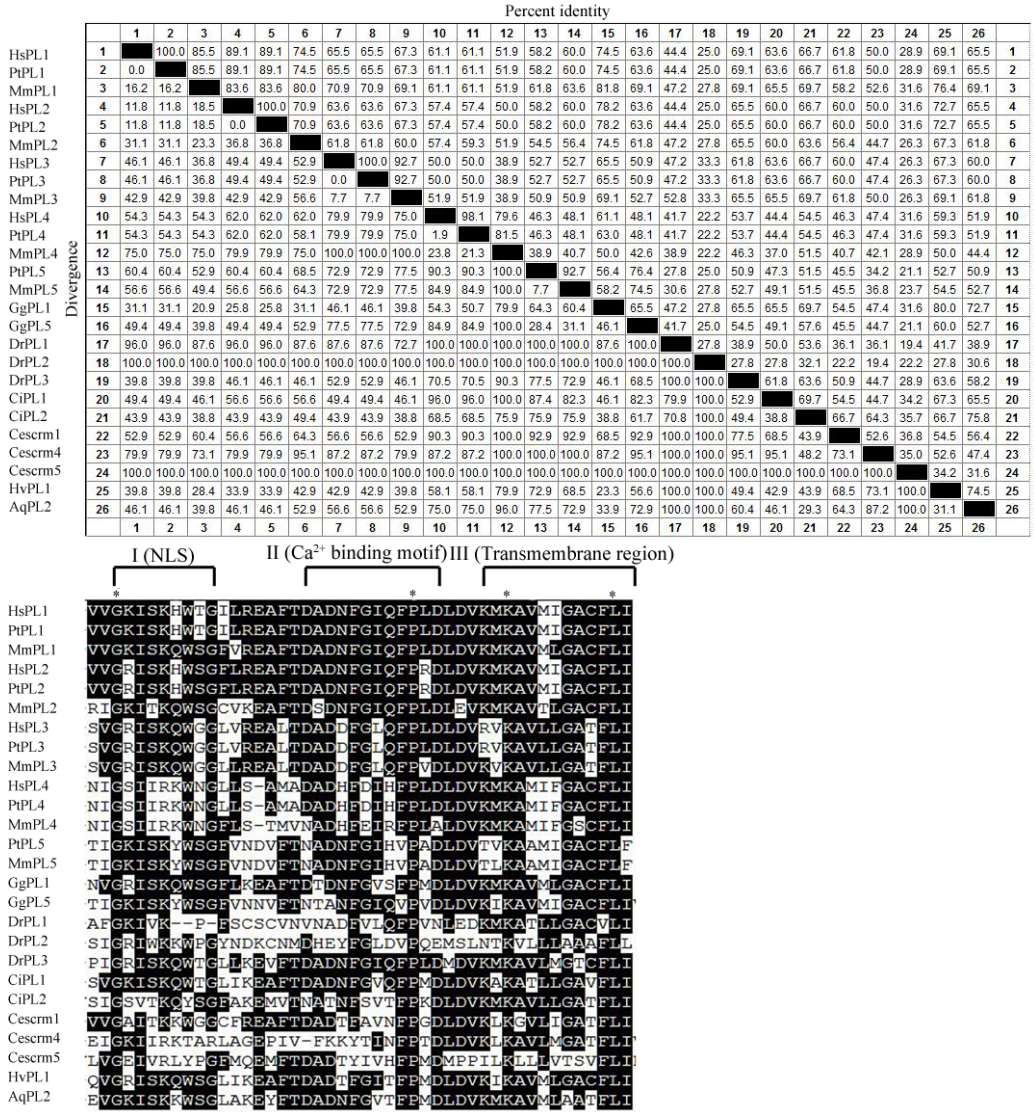
图3　各物种*PLSCR*基因的内含子外显子分析图

Fig.3　The analysis graph of introns and extrons of *PLSCR* in different species

同源性就高于它与*hPLSCR3*(47%)和*hPLSCR4*(46%)的同源性。进一步的序列比对分析发现, 各物种*PLSCR1-5*基因的多亚型是由于不同的可变剪接造成的。选择各物种中核苷酸序列最长的*PLSCR*基因进行结构进化分析发现, *PLSCR*基因的外显子与内含子数目在物种进化过程中差异明显, 在低等动物中更为突出(图3)。从海绵动物到哺乳动物, 外显子的数目逐渐趋于稳定, 基本稳定在9个左右, 且C-端外显子5、6、7、8十分保守(图3), 推测可能由于在这些外显子中存在功能位点。因此, 对比较保守的外显子8(该区域含有保守的功能基序III、IV和V)通过DNASTAR软件的Megalign作进一步的多序列比对分析, 结果如图4所示, 各物种之间该部分氨基酸序列相对保守, 同一性(identity)基本在35%以上(线虫*PLSCR5*和斑马鱼*PLSCR2*除外), 特别是富含疏水性氨基酸的跨膜区最为保守。

2　PLSCR的表达与定位

目前, 对于PLSCR的研究主要集中在哺乳动物中, 特别是对*hPLSCR1*的研究比较深入。除了



各物种中相对保守的氨基酸用不同颜色的阴影标出, 星号表示在各物种中完全保守的氨基酸, 折线代表不同基序的位置。

Comparatively conservative amino acids of each species were marked with different color shadows, an asterisk represent the completely conservative amino acids in each species, the position of the broken line shows the different functional motifs.

The exon 8 of PLSCR proteinsis from species as follows: HsPL1 (智人磷脂爬行酶, *Homo sapiens phospholipid scramblase*, NP_066928.1); PtPL1 (黑猩猩, *Pan troglodytes*, XP_001135229.2); MmPL1 (小家鼠, *Mus musculus*, NP_035766.2), HsPL2 (NC_000003.12), PtPL2 (XP_009444911.1), MmPL2 (NP_001182013.1), HsPL3 (NP_001188505.1), PtPL3 (XP_009430014.1), MmPL3 (NP_001161969.1), HsPL4 (NP_001121776.1), PtPL4 (XP_003310156.1), MmPL4 (NP_848826.1), PtPL5 (XP_003310100.1), MmPL5 (NP_001182622.1); GgPL1 (原鸡, *Gallus gallus*, XP_001231237.1), GgPL2 (XP_422697.4); DrPL1 (斑马鱼, *Danio rerio*, XP_003201533.3), DrPL2 (XP_003197974.1), DrPL3 (NP_998031.1); CiPL1 (玻璃海鞘, *Ciona intestinalis*, XP_002121993.3), CiPL2 (XP_009858073.1); Cescrm1 (秀丽隐杆线虫, *Caenorhabditis elegans*, NP_001251705.1), Cescrm4 (NP_492975.3), Cescrm5 (NP_500500.1); HvPL1 (水螅, *Hydra vulgaris*, XP_002163447.2); AqPL2 (海绵, *Amphimedon queenslandica*, XP_003382485.1).

图4 PLSCR外显子8蛋白质的保守性分析

Fig.4 Aligment of PLSCR exon 8 proteins by Megalign in DNASTAR soft

*hPLSCR3*定位于17号染色体上, 其余的*PLSCR*基因都位于3号染色体长臂的3q23/3q24位点上[2]。人类组织的Northern blot结果显示, 除脑组织外, PLSCR1和PLSCR3广泛表达于各组织中, PLSCR2只表达于睾丸中, 而PLSCR4可在多种组织中检测到。随后, 进一步的研究发现, PLSCR1表达于包括红细胞和血小板在内的多种细胞系中, 而PLSCR3主要表达于线粒体中, PLSCR4在外周血淋巴细胞中没有表达[2]。

从亚细胞水平鉴定发现, PLSCR1定位于质膜的低密度膜微区(脂质筏/小窝), 脂质筏作为一个重要蛋

白质停泊平台, 发挥着重要的作用, 包括信号转导、病原体侵入/吸收、细胞内的运输、分泌和内吞作用[10]。棕榈酰化的PLSCR1可与表皮生长因子受体共定位于人口腔表皮癌细胞脂筏上, 有趣的是, 在表皮生长因子刺激下, PLSCR1进入内吞小泡, 表皮生长因子受体在溶酶体中被降解, 而PLSCR1再循环返回到质膜[11]。非棕榈酰化的PLSCR1蛋白质可以进入细胞核内直接与1,4,5-三磷酸肌醇受体1(inositol 1,4,5-triphosphate receptor type 1, *IP$_3$R1*)基因的5′启动子区域结合, 从而增强该基因的表达[12]。另外, 在非激活的肥大细胞脂筏上, PLSCR1与酪氨酸激酶Lyn和Syk(IgE受体信号转导下游调控元件)共定位[13], 在fMLP(甲酰化Met-Leu-Phe)激活的中性粒细胞脂筏中也发现PLSCR1[14]。此外, 在其他细胞器包括高尔基体和胞内基质中也发现存在PLSCR1[15-16]。

3　PLSCR的功能

PLSCR在进化过程中结构上一直很保守, 且分布于多种组织和细胞中, 由此可推断, PLSCR在生物体中发挥了至关重要的作用。虽然目前对该家族的研究涉及的物种并不太多, 但对hPLSCR功能的探索已有较多报道。在体内其主要与脂质代谢、线粒体结构和功能维护、血液凝固、细胞凋亡、癌变与免疫有关(图5)。

3.1　磷脂酰行酶参与脂质代谢

脂肪细胞内油脂过高造成的腹部脂肪堆积已成为2型糖尿病发病的主要危险因素之一[17], 这也是代谢紊乱的一个潜在表征。研究发现, 在人体内脂质代谢活跃的脂肪和肌肉细胞中PLSCR3总是高表达[18]。进一步研究发现, *PLSCR3*$^{-/-}$敲除小鼠表现出异常的腹部脂肪堆积、葡萄糖耐受不良、胰岛素及血脂(胆固醇、甘油三酯、脂肪酸和瘦蛋白)紊乱, 其原代脂肪细胞也显示出对外源性胰岛素反应的缺陷[17-18]。而且在*PLSCR3*$^{-/-}$敲除(以及*PLSCR*(*1&3*)$^{-/-}$双基因敲除)小鼠中, 多种代谢产物的表达量受磷脂酰行酶的调节, 其中促炎脂质和在脂肪组织中参与脂质代谢的基因表现出显著的上调。进一步研究发现, *PLSCR*(*1&3*)$^{-/-}$双基因敲除小鼠比*PLSCR3*$^{-/-}$敲除的小鼠脂肪堆积速率有所提高, 说明PLSCR1与PLSCR3的部分功能相似, 也参与脂质代谢[18]。这些结果表明, PLSCR3在调控小鼠脂肪积累过程中具有重要作用。

3.2　磷脂酰行酶参与线粒体结构和功能的维持

心磷脂(cardiolipin, CL)主要存在于线粒体内膜上, 它通过心磷脂合酶在线粒体内膜(inner membrane, IM)腔面合成, 对线粒体结构、质量、膜电位的稳定性维持和功能的发挥具有重要意义[19-20]。研究表明, PLSCR3参与了线粒体心磷脂的从头合成和再合成过程, 而大部分新合成的CL从线粒体内膜转移

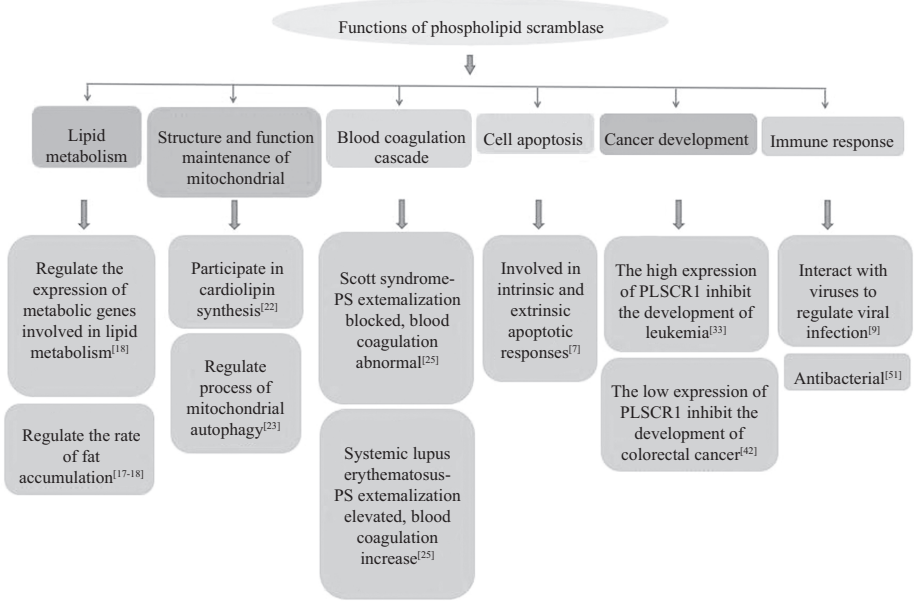


图5　磷脂酰行酶的功能

Fig.5　Functions of phospholipid scramblase

到线粒体外膜(outer membrane, OM)发挥作用[21-22]。当通过siRNA干扰下调PLSCR3的基因表达后, 发现细胞增殖速率减缓, 线粒体质量减小, 细胞色素c、ATP和磷脂含量减少, 线粒体呼吸减弱, 线粒体结构也出现异常[22]。基于这些病理改变, *PLSCR3*突变体细胞对紫外线和tBid诱导凋亡的敏感性减弱, 同时CL的线粒体外膜运输量也相对减少[20]。另有研究报道, 在线粒体自噬进程中, PLSCR3参与的心磷脂的外翻是自噬体和溶酶体靶向损坏线粒体的重要事件, 这说明PLSCR3也参与和调控了自噬过程[23]。PLSCR家族中除了PLSCR3, PLSCR1在自噬体形成过程中也具有重要作用, 然而, 其导致自噬的确切机制还需要进一步研究[9]。

3.3 磷脂爬行酶参与凝血

活化(血小板)或损伤(红细胞、血小板、内皮细胞及其他细胞)的细胞表面磷脂酰丝氨酸的外翻可作为催化剂引起凝血级联反应[24]。斯科特综合征(一种血液疾病)就是由于质膜上的PL爬行运动异常导致PS外翻受阻进而致损伤后的凝血发生异常。该综合征患者体内与PS有特异性亲和力的可诱导型FVa和FVIIIa受体也先天不足[25]。研究还显示, 大约10%的系统性红斑狼疮患者的单核细胞内hPLSCR1以及它的3个剪接变异体表达量增加, 并伴随着PS外翻率的提高, 表现出一种异常的凝血增加[26]。另外, 从抗磷脂综合征患者体内分离出的单核细胞, 其内PLSCR1表达量也升高[27]。在系统性红斑狼疮以及抗磷脂综合征患者的单核细胞中, PLSCR1的表达量比正常人群的高, 因为其参与磷脂酰丝氨酸外翻, 可促进血栓形成导致血栓性疾病发生[26-27]。到目前为止, 磷脂爬行酶家族其他成员在凝血过程中的作用还未见报道。

3.4 磷脂爬行酶参与细胞凋亡

细胞凋亡是多细胞动物为维持内环境稳态, 由基因控制的细胞自主性的有序死亡, 对整个机体的发育起着举足轻重的作用[28]。细胞凋亡过程是一系列蛋白质水解级联反应, 可由许多细胞外和细胞内信号分子或生理和病理诱导剂启动[28-29], 包括外源性和内源性两种途径。外源性途径由膜结合的死亡受体发起, 而内源性途径由破坏DNA的药物或紫外线辐射触发[19]。PLSCR在内源和外源凋亡反应中都具有重要作用, 细胞凋亡早期, PLSCR促使PS暴露导致细胞表面向巨噬细胞发出一个吞噬信号, 从而吞噬和清除凋亡的细胞[7]。有报道称, 转染PLSCR1的CHO-K1细胞(不表达内源性PLSCR1)生长停滞, 形态学发生改变, 细胞死亡率也提高, 且紫外照射后PS合成和外翻速率都加快, 并伴随着大量的胱冬肽酶-3(Caspase-3)被激活[29]。同样, 在白血病细胞株U937中, PLSCR1的高表达也伴有明显的胱冬肽酶-3和蛋白激酶C(protein kinase C, PKC)的活化并增加了依托泊苷诱导的细胞凋亡, 但PLSCR1的诱导不能直接触发细胞凋亡[30]。PLSCR1诱导还可以调节许多凋亡相关基因表达, 如β-连环蛋白1(catenin beta 1, *CTNNB1*)、肿瘤坏死因子超家族成员13(tumor necrosis factor superfamily member 13, *TNFSF13*)、凝溶胶蛋白(gelsolin, *GSN*)以及细胞周期素D3(cyclin D3, *CCND3*)等[30]。除了PLSCR1介导凋亡事件外, PLSCR3在紫外线和tBid诱导下也可促进细胞凋亡[22]。在胱冬肽酶-8介导的细胞凋亡事件中, 活化的胱冬肽酶-8促使胞质蛋白Bid氨基酸末端裂解产生tBid, tBid在磷酸化的PLSCR3的作用下进入线粒体, 并通过与CL相互作用被激活后定位于线粒体, 最终这种活化的tBid激活Bax和Bak蛋白质并形成细胞色素c通道加速细胞凋亡过程[31-32]。不仅在脊椎动物中, 无脊椎动物秀丽隐杆线虫的磷脂爬行酶1(scrm1)突变失活也会导致凋亡细胞表面的PS暴露受阻[5]。因此, PLSCR1与PLSCR3被认为具有促细胞凋亡的作用。

3.5 磷脂爬行酶参与癌变

关于PLSCR在癌症中的作用, 研究较早的是PLSCR与白血病的关系。PLSCR1的诱导表达能有效抑制白血病细胞系U937在体内和体外的增殖生长[33]。与正常细胞株相比, 在某些白血病细胞株中PLSCR1的表达量偏低, 而PLSCR1的过表达又可诱导白血病细胞分化, 例如可以显著促进急性早幼粒白血病细胞的分化, 从而起到治疗作用[34]。这一结果在急性骨髓性白血病(acute myeloid leukemia, AML)的患者中也得到证实。研究发现, 高表达量的PLSCR1可延长病人的存活时间[34]。当AML细胞用汉黄芩苷处理时, PLSCR1的表达量上升且向核内转移并与肌醇1,4,5-三磷酸肌醇受体1(IP_3R1)启动子结合, 从而增加IP_3R1的表达[35]。PLSCR1在几种骨髓性白血病细胞系和原代白血病细胞中的表达受干扰素和分化诱导剂的诱导而上调, 这些诱导剂, 例如全反式维甲酸和佛波酯可引发白血病细胞分化[36-37]。因此, PLSCR1可以作为一种新的潜在的白血病预后

因子[38]。相关的研究显示, PLSCR1还影响细胞周期和分化相关基因的表达, 如促进p21 WAF1/CIP1蛋白质上调和致癌蛋白c-Myc下调[35]。在骨髓细胞中, PLSCR1可与c-Myc的负转录调控因子onzin结合发挥作用[39]。综上所述, PLSCR1可以作为一个肿瘤抑制基因靶向治疗白血病。

然而, 越来越多的证据显示, PLSCRs在结直肠癌、卵巢癌、转移性肝癌等中不具有抑癌作用, 相反PLSCRs的低表达可以抑制癌细胞的生长。例如, 有研究显示, 抗体或siRNA介导的结肠癌和肝癌患者体内PLSCR1的下调可诱使肿瘤细胞数量减少[40-41]。siRNA干扰PLSCR1后, 结直肠癌细胞株LoVo的生长速率变缓, 黏附力下降, 表明PLSCRl表达量的降低能显著抑制肿瘤细胞的增殖和黏附力[42]。同样地, PLSCR1也高表达于卵巢癌细胞, 在该细胞中, PLSCR1的表达水平是通过SnoN(ski related novel protein N)进行调节的, SnoN是TGF-β(transforming growth factor-β)信号通路的负调控因子[43]。MCF7乳腺癌细胞经γ-辐射处理后PLSCR2蛋白质水平会下调[44]。也有相关报道称, PLSCR1在肿瘤进展中发挥重要作用, 如Snail在多种癌症中都显著上调, 而Snail又可以调节PLSCR1的表达, 这可能是PLSCR1参与致癌的机制之一[45]。此外, 在人宫颈癌细胞HeLa中, PLSCRl可与血管生成素(angiogenin, ANG)相互作用, 促进ANG rRNA转录, 并且在肿瘤进展的过程中影响肿瘤的侵袭及转移[46]。ANG具有促进血管新生和肿瘤细胞增殖的双重作用, 它与多种肿瘤的发生、发展以及恶化密切相关[47-48]。

3.6　磷脂酰行酶参与免疫

*PLSCR1*是干扰素(interferon, IFN)刺激基因, IFN可诱导hPLSCR1高表达, 在IFN-α2a诱导的人类肿瘤细胞和脐静脉内皮细胞中, PLSCR1表达量升高了10倍[36]。此外, hPLSCR1还可以被IFN-α、IFN-β和IFN-γ诱导高表达[34]。IFNs在抗病毒、抗增殖、抗肿瘤和免疫调节应答过程中通过JAK(janus kinase)/STAT(signal transducers and activators of transcription)通路来激活表达[49], 有报道称PLSCR1也参与该通路[4]。在抗病毒过程中, IFN-α2a通过顺序激活PKC-δ、JNK、STATl, 上调PLSCRl表达, 进而增强干扰素的抗病毒作用。PLSCR1含有1个干扰素刺激反应元件(ISRE), hPLSCR1的ISRE位于第1个外显子上(+21~+35)[9]。IFN-α2a可以通过ISRE调节PLSCR1的转录表达[50]。由于PLSCR1可通过JAK/STAT通路来参与抗病毒过程, 因此, PLSCR1在抗病毒过程中的作用机理近年来也备受关注[50]。研究显示, 当小鼠纤维母细胞中的PLSCR1表达降低时(通过siRNA或*PLSCR1*$^{-/-}$基因敲除), 水泡性口炎病毒和脑心肌炎病毒的病毒滴度反而增加[9]。也有报道称, IFN-α刺激PLSCR1的高表达可保护细胞免受金黄色葡萄球菌产生的毒素影响[51]。PLSCR1可转录激活人体T细胞白血病病毒1型并与之相互作用, 还可与人类免疫缺陷病毒1型(human immunodeficiency virus-1, HIV-1)相互作用[52-53]。在T淋巴细胞中, PLSCR1和PLSCR4与位于质膜上的具有抗HIV-1病毒活性的CD4受体相互作用[54]。在HepG2和Huh7细胞系以及小鼠中, PLSCR1的表达影响了乙型肝炎病毒的复制[55]。尽管PLSCR1可通过调节IFN-α和IFN-γ使其上调来抑制丙型肝炎病毒的复制[56], 但也有研究发现, PLSCR1可促进丙肝病毒的入侵。在HEK293T和Huh7.5.1细胞中, PLSCR1可与E1和E1丙肝病毒膜蛋白相互作用, 来调节丙肝病毒的感染[57]。因此, PLSCR1不仅可以抑制病毒复制和传播还可以协助病毒感染[9]。

Toll样受体(toll-like receptor, TLR)可作为模式识别受体识别进入细胞的外源病原核酸和蛋白质, 在先天免疫系统中发挥关键作用[58]。尽管PLSCR1调节先天免疫应答和TLR信号途径的详细机制目前还不清楚, 但研究显示, PLSCR1和先天免疫反应调控之间存在着一定的联系[9]。PLSCR1可与TLR9相互作用, 并调节TLR9介导IFN-α的产生[16]。PLSCR表达水平会随着脂多糖、酵母多糖、松节油所诱导的急性先天免疫反应的发生而改变[59]。在肿瘤坏死因子α(tumor necrosis factor α, TNFα)、白细胞介素(interleukin-1β和interleukin-6)和干扰素γ(IFNγ)刺激的肝细胞HepB3和脂肪细胞3T3-L1中, PLSCR1的表达量也发生了上调[59]。同样, 在表皮生长因子(epidermal growth factor, EGF)、干细胞因子(stem cell factor, SCF)和粒细胞集落刺激因子(granulocyte colony stimulating factor, G-CSF)刺激的NB4和HL60细胞中PLSCR1的表达量也会上调[11,60]。然而, 用脂多糖诱导处理小鼠子宫组织后, 除PLSCR4外, 其他的PLSCR家族成员的mRNA和蛋白质却都发生了下调[61]。在中性粒细胞中, PLSCR1可与蛋白酶3(proteinase 3, PR3)相互作用, PR3是韦格纳肉芽肿

病的自身抗体靶分子[9]。还有报道称, PLSCR1作为一个免疫受体成为一种新的肥大细胞信号感受器[7]。

4 结论与前景

综上所述, *PLSCR*是一种结构比较复杂, 但在进化上相对保守的基因。通过参与机体内的脂质代谢、线粒体结构和功能的维持、凝血反应、细胞增殖、分化和凋亡以及免疫应答等过程, PLSCR在生物体多种生理代谢过程中发挥着关键作用。因此, 对该家族基因进行深入的研究对整个机体代谢和稳态调控的研究具有重要意义。目前, 对于PLSCR家族的研究主要集中在人和鼠等哺乳动物中, 虽然在斑马鱼和线虫等低等动物中也有研究, 但对其功能的研究都不够深入。而在该家族成员(PLSCR1-4)中更多的是对PLSCR1和PLSCR3的研究, 其他成员的基因结构和定位虽然都已清楚, 但功能与机制尚不明确。此外, 近年来的报道也只是对该家族单一成员进行研究, 我们不知道这些PLSCRs功能是否相似, 它们彼此之间是相互独立的还是具有代偿作用, 这些问题都需要进一步的探索。随着研究的深入, 相信PLSCR在机体内发挥的功能以及参与的途径会越来越清楚。

参考文献 (References)

1 Zhou Q, Zhao J, Stout JG, Luhm RA, Wiedmer T, Sims PJ. Molecular cloning of human plasma membrane phospholipid scramblase. J Biol Chem 1997; 272(29): 18240-4.

2 Wiedmer T, Zhou Q, Kwoh YD, Sims PJ. Identification of three new members of the phospholipid scramblase gene family. Biochim Biophys Acta 2000; 1467(1): 244-53.

3 Wiedmer T, Zhao J, Nanjundan M, Sims PJ. Palmitoylation of phospholipid scramblase 1 controls its distribution between nucleus and plasma membrane. Biochemistry 2003; 42(5): 1227-33.

4 Zhao KW, Li D, Zhao Q, Huang Y, Silverman RH, Sims PJ, *et al*. Interferon-α-induced expression of phospholipid scramblase 1 through STAT1 requires the sequential activation of protein kinase Cδ and JNK. J Biol Chem 2005; 280(52): 42707-14.

5 Wang X, Wang J, Gengyo-Ando K, Gu L, Sun CL, Yang C, *et al*. *C.elegans* mitochondrial factor WAH-1 promotes phosphatidylserine externalization in apoptotic cells through phospholipid scramblase SCRM-1. Nat Cell Biol 2007; 9(5): 541-9.

6 Kasukabe T, Kobayashi H, Kaneko Y, Okabe-Kado J, Honma Y. Identity of human normal counterpart (MmTRA1b) of mouse leukemogenesis-associated gene (MmTRAla) product as plasma membrane phospholipid scramblase and chromosome mapping of the human MmTRA1b/phospholipid scramblase gene. Biochem Biophys Res Commun 1998; 249(2): 449-55.

7 Sahu SK, Gummadi SN, Manoj N, Aradhyam GK. Phospholipid scramblases: An overview. Arch Biochem Biophys 2007; 462(1): 103-14.

8 魏 健, 李莉云, 曹英豪, 刘雨萌, 巩校东, 刘丽娟, 等. 水稻类Tubby蛋白质在叶片生长和白叶枯病抗性反应中的表达. 植物学报(Wei Jian, Li Liyun, Cao Yinghao, Liu Yumeng, Gong Xiaodong, Liu Lijuan, *et al*. Expression of tubby-like proteins in rice leaves and during interactions between rice and xanthomonas oryzae pv.oryzae. Chinese Bulletin of Botany) 2011; 46(5): 525-33.

9 Kodigepalli KM, Bowers K, Sharp A, Nanjundan M. Roles and regulation of phospholipid scramblases. FEBS 2015; 589(1): 3-14.

10 Frank PG, Lisanti MP. Defining lipid raft structure and function with proximity imaging. Am J Physiol Heart Circ Physiol 2006; 290(6): H2165-6.

11 Sun J, Nanjundan M, Pike LJ, Wiedmer T, Sims PJ. Plasma membrane phospholipid scramblase 1 is enriched in lipid rafts and interacts with the epidermal growth factor receptor. Biochemistry 2002; 41(20): 6338-45.

12 Zhou Q, Ben-Efraim I, Bigcas J, Bigcas J, Wiedmer T, Sims PJ. Phospholipid scramblase 1 binds to the promoter region of the inositol 1,4,5-triphosphate receptor type 1 gene to enhance its expression. J Biol Chem 2005; 280(41): 35062-8.

13 Amir-Moazami O, Alexia C, Charles N, Launay P, Monteiro RC, Benhamou M. Phospholipid scramblase 1 modulates a selected set of IgE receptor-mediated mast cell responses through LAT-dependent pathway. J Biol Chem 2008; 283(37): 25514-23.

14 Frasch SC, Henson PM, Nagaosa K, Fessler MB, Borregaard N, Bratton DL. Phospholipid flip-flop and phospholipid scramblase 1 (PLSCR1) co-localize to uropod rafts in formylated Met-Leu-Phe-stimulated neutrophils. J Biol Chem 2004; 279(17): 17625-33.

15 Kametaka S, Shibata M, Moroe K, Kanamori S, Ohsawa Y, Waguri S, *et al*. Identification of phospholipid scramblase 1 as a novel interacting molecule with beta-secretase (beta-site amyloid precursor protein (APP) cleaving enzyme (BACE)). J Biol Chem 2010; 278(17): 15239-45.

16 Talukder AH, Bao M, Kim TW, Facchinetti V, Hanabuchi S, Bover L, *et al*. Phospholipid scramblase 1 regulates Toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. Cell Res 2012; 22(7): 1129-39.

17 Greenberg AS, McDaniel ML. Identifying the links between obesity, insulin resistance and beta-cell function: Potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest 2002; 32(Suppl 3): 24-34.

18 Wiedmer T, Zhao J, Li L, Zhou Q, Hevener A, Olefsky JM, *et al*. Adiposity, dyslipidemia, and insulin resistance in mice with targeted deletion of phospholipid scramblase 3 (PLSCR3). Proc Natl Acad Sci USA 2004; 101(36): 13296-301.

19 McMillina JB, Dowhan W. Cardiolipin and apoptosis. Biochim Biophys Acta 2002; 1585(2/3): 97-107.

20 He Y, Liu J, Grossman D, Durrant D, Sweatman T, Lothstein L, *et al*. Phosphorylation of mitochondrial phospholipid scramblase 3 by protein kinase C-delta induces its activation and facilitates mitochondrial targeting of tBid. J Cell Biochem 2007; 101(5): 1210-21.

21 Van Q, Liu J, Lu B, Feingold KR, Shi Y, Lee RM, *et al*. Phospholipid scramblase-3 regulates cardiolipin *de novo* biosynthesis and its resynthesis in growing HeLa cells. Biochem J 2007; 401(1): 103-9.

22 Liu J, Dai Q, Chen J, Durrant D, Freeman A, Liu T, *et al*. Phospholipid scramblase 3 controls mitochondrial structure, function, and apoptotic response. Mol Cancer Res 2003; 1(12): 892-902.

23 Chu CT, Ji J, Dagda RK, Dagda RK, Jiang JF, Tyurina YY, *et al*. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat Cell Biol 2013; 15(10): 1197-205.

24 Bevers EM, Comfurius P, Zwaal RF. Platelet procoagulant activity: Physiological significance and mechanisms of exposure. Blood Rev 1991; 5(3): 146-54.

25 Zwaal RF, Comfurius P, Bevers EM. Scott syndrome, a bleeding disorder caused by defective scrambling of membrane phospholipids. Biochim Biophys Acta 2004; 1636(2/3): 119-28.

26 Suzuki E, Amengual O, Atsumi T, Oku K, Hashimoto T, Kataoka H, *et al*. Increased expression of phospholipid scramblase 1 in monocytes from patients with systemic lupus erythematosus. J Rheumatol 2010; 37(8): 1639-45.

27 Amengual O, Atsumi T, Oku K, Suzuki E, Horita T, Yasuda S, *et al*. Phospholipid scramblase 1 expression is enhanced in patients with antiphospholipid syndrome. Mod Rheumato 2013; 23(1): 81-8.

28 Danial NN, Korsmeyer SJ. Cell death: Critical control points. Cell 2004; 116(2): 205-19.

29 Yu A, McMaster CR, Byers DM, Ridgway ND, Cook HW. Stimulation of phosphatidylserine biosynthesis and facilitation of UV-induced apoptosis in Chinese hamster ovary cells overexpressing phospholipid scramblase 1. J Biol Chem 2003; 278(11): 9706-14.

30 Huang Y, Zhao Q, Chen GQ. Phospholipid scramblase 1. Acta Physiologica Sinica 2006; 58(6): 501-10.

31 Zha J, Weiler S, Oh KJ, Wei MC, Korsmeyer SJ. Posttranslational N-myristoylation of BID as a molecular switch for targeting mitochondria and apoptosis. Science 2000; 290(5497): 1761-5.

32 Lutter M, Fang M, Luo X, Nishijima M, Xie X, Wang X. Cardiolipin provides specificity for targeting of tBid to mitochondria. Nat Cell Biol 2002; 2(10): 754-61.

33 Huang Y, Zhao Q, Zhou CX, Gu ZM, Li D, Xu HZ. Antileukemic roles of human phospholipid scramblase 1 gene, evidence from inducible PLSCRl-expressing leukemic cell. Oncogene 2006; 25(50): 6618-27.

34 Nakamaki T, Okabe-Kado J, Yamamoto-Yamaguchi Y, Hino KI, Tomoyasu S, Honma Y, *et al*. Role of MmTRA1b/phospholipid scramblase1 gene expression in the induction of differentiation of human myeloid leukemia cells into granulocytes. Exp Hematol 2002; 30(5): 421-9.

35 Chen Y, Hui H, Yang H, Zhao K, Qin Y, Gu C, *et al*. Wogonoside induces cell cycle arrest and differentiation by affecting expression and subcellular localization of PLSCR1 in AML cells. Blood 2013; 121(18): 3682-91.

36 Zhou Q, Zhao J, Al-Zoghaibi F, Zhou A, Wiedmer T, Robert H, *et al*. Transcriptional control of the human plasma membrane phospholipid scramblase 1 gene is mediated by interferon-alpha. Blood 2000; 95(8): 2593-9.

37 Zhao KW, Li X, Zhao Q, Huang Y, Li D, Peng Z, *et al*. Protein kinase Cδ mediates retinoic acid and phorbol myristate acetate-induced phospholipid scramblase 1 gene expression: Its role in leukemic cell differentiation. Blood 2004; 104(12): 3731-8.

38 Yokoyama A, Yamashita T, Shiozawa E, Nagasawa A, Okabe-Kado J, Nakamaki T, *et al*. MmTRA1b/phospholipid scramblase 1 gene expression is a new prognostic factor for acute myelogenous leukemia. Leuk Res 2004; 28(2): 149-57.

39 Li Y, Rogulski K, Zhou Q, Sims PJ, Prochownik EV. The negative c-Myc target onzin affects proliferation and apoptosis via its obligate interaction with phospholipid scramblase 1. Mol Cell Biol 2006; 26(9): 3401-13.

40 Fan CW, Chen CY, Chen KT, Shen CR, Kuo YB, Chen YS, *et al*. Blockade of phospholipid scramblase 1 with its N-terminal domain antibody reduces tumorigenesis of colorectal carcinomas *in vitro* and *in vivo*. J Transl Med 2012; 10(1): 254.

41 Cui W, Li SY, Du JF, Zhu ZM, An P. Silencing phospholipid scramblase 1 expression by RNA interference in colorectal cancer and metastatic liver cancer. Hepatobiliary Pancreat Dis Int 2012; 11(4): 393-400.

42 崔　伟, 袁　强, 陈彩玲, 陈　纲, 李世拥. 干扰磷脂爬行酶1基因表达对结直肠癌细胞增殖和粘附的影响. 中华普外科手术学杂志(Cui Wei, Yuan Qiang, Chen Cailing, Chen Gang, Li Shiyong. Influence of silencing PLSCR1 expression with RNA interference on the proliferation and adhesion of colorectal cancer cells. Chinese Journal of Operative Procedures of General Surgery) 2014; 8(4): 47-9.

43 Kodigepalli KM, Anur P, Spellman P, Sims PJ, Nanjundan M. Phospholipid scramblase 1, an interferon-regulated gene located at 3q23, is regulated by SnoN/SkiL in ovarian cancer cells. Mol Cancer 2013; 12(1): 32.

44 Jung S, Lee S, Lee J, Li C, Ohk JY, Jeong HK, *et al*. Protein expression pattern in response to ionizing radiation in MCF-7 human breast cancer cells. Oncol Lett 2012; 3(1): 147-54.

45 Francis VG, Padmanabhan P, Gummadi SN. Snail interacts with hPLSCR1 promoter and down regulates its expression in IMR-32. Biochem Biophys Res Commun 2014; 450(1): 172-7.

46 Zhu JQ, Sheng JH, Dong HJ, Kang L, Ang J, Xu ZP. Phospholipid scramblase 1 functionally interacts with angiogenin and regulates angiogenin-enhanced rRNA transcription. Cell Physiol Biochem 2013; 32(6): 1695-706.

47 Lambeth LS, Smith CA．Short hairpin RNA-medialed gene silencing. Methods Mol Biol 2013; 942: 205-32．

48 Guzman-Villanueva D, EI-Sherbiny IM, Herrera-Ruiz D, Vlassov AV, Smyth HD. Formulation approaches to shortinterfering RNA and MicroRNA: Challenges and implications. J Pharm Sci 2012; 101(11): 4046-66.

49 Der SD, Zhou A, Williams BR, Silverman RH. Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Proc Natl Acad Sci USA 1998; 95(26): 15623-8.

50 Dong B, Zhou Q, Zhao J, Zhou A, Harty R, Bose S, *et al*. Phospholipid scramblase 1 potentiates the antiviral activity of interferon. J Virol 2004; 78(17): 8983-93.

51 Lizak M, Yarovinsky TO. Phospholipid scramblase 1 mediates type I interferon-induced protection against staphylococcal

α-toxin. Cell Host Microbe 2012; 11(1): 70-80.
52 Kusano S, Eizuru Y. Human phospholipid scramblase 1 interacts with and regulates transactivation of HTLV-1 Tax. Virology 2012; 432(3): 343-52.
53 Kusano S, Eizuru Y. Interaction of the phospholipid scramblase 1 with HIV-1 Tat results in the repression of Tat-dependent transcription. Biochem Biophys Res Commun 2013; 433(4): 438-44.
54 Py B, Basmaciogullari S, Bouchet J, Zarka M, Moura IC, Benhamou M, *et al*. The phospholipid scramblases 1 and 4 are cellular receptors for the secretory leukocyte protease inhibitor and interact with CD4 at the plasma membrane. PLoS One 2009; 4(3): e5006.
55 Yang J, Zhu X, Liu J, Ding X, Han M, Hu W, *et al*. Inhibition of Hepatitis B virus replication by phospholipid scramblase 1 *in vitro* and *in vivo*. Antiviral Res 2012; 94(1): 9-17.
56 Metz P, Dazert E, Ruggieri A, Mazur J, Kaderali L, Kaul A, *et al*. Identification of type I and type II interferon-induced effectors controlling hepatitis C virus replication. Hepatology 2012; 56(6): 2082-93.
57 Gong Q, Cheng M, Chen H, Liu XY, Si YH, Yang Y, *et al*. Phospholipid scramblase 1 mediates hepatitis C virus entry into host cells. FEBS Lett 2011; 585(17): 2647-52.
58 Barton GM, Kagan JC. A cell biological view of Toll-like receptor function: Regulation through compartmentalization. Nat Rev Immunol 2009; 9(8): 535-42.
59 Lu B, Sims PJ, Wiedmer T, Moser AH, Shigenaga JK, Grunfeld C, *et al*. Expression of the phospholipid scramblase (PLSCR) gene family during the acute phase response. Biochim Biophys Acta 2007; 1771(9): 1177-85.
60 Zhou Q, Zhao J, Wiedmer T, Sims PJ. Normal hemostasis but defective hematopoietic response to growth factors in mice deficient in phospholipid scramblase 1. Blood 2002; 99(11): 4030-8.
61 McLean KC, Oppenheimer KH, Sweet LM, Phillippe M. Phospholipid scramblase expression in the pregnant mouse uterus in LPS-induced preterm delivery. Reprod Sci 2012; 19(11): 1211-8.