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Review

The role of human phospholipid scramblases in apoptosis: An overview

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ABSTRACT

Human phospholipid scramblases (hPLSCRs) are a family of four homologous single pass transmembrane proteins (hPLSCR1-4) initially identified as the proteins responsible for Ca^{2+} mediated bidirectional phospholipid translocation in plasma membrane. Though *in-vitro* assays had provided evidence, the role of hPLSCRs in phospholipid translocation is still debated. Recent reports revealed a new class of proteins, TMEM16 and Xkr8 to exhibit scramblase activity challenging the function of hPLSCRs. Apart from phospholipid scrambling, numerous reports have emphasized the multifunctional roles of hPLSCRs in key cellular processes including tumorigenesis, antiviral defense, protein and DNA interactions, transcriptional regulation and apoptosis. In this review, the role of hPLSCRs in mediating cell death through phosphatidylserine exposure, interaction with death receptors, cardiolipin exposure, heavy metal and radiation induced apoptosis and pathological apoptosis followed by their involvement in cancer cells are discussed. This review aims to connect the multifunctional characteristics of hPLSCRs to their decisive involvement in apoptotic pathways.

1. Introduction

Phospholipids (PL) in the plasma membrane (PM) are asymmetrically distributed in such a way that choline containing PLs such as phosphatidylcholine (PC) and sphingomyelin dominate the outer leaflet, while the aminophospholipids such as phosphatidylethanolamine (PE) and phosphatidylserine (PS) are enriched in the inner leaflet of the lipid bilayer [1–4]. Externalization of aminophospholipids under stress conditions play a vital role in phagocytosis and apoptosis. PM asymmetry is maintained in a cell by a special family of proteins called PL translocators and are classified into two types namely ATP dependent and ATP independent PL translocators. ATP dependent PL translocators maintain PM asymmetry in normal cells at the expense of metabolic energy whereas ATP independent PL translocators include biogenic membrane flippases which mediates ATP independent PL translocation at a rapid pace in biogenic membranes [5]. ATP dependent PL translocators are further sub divided based on their mode of translocation and their specificity for PLs as flippases and floppases [5]. Flippases such as amino phospholipid translocators specifically transport amino PLs such as PS and PE from exoplasmic leaflet to the cytosolic leaflet. Floppases aid in exofacial transport of choline containing PLs such as PC [6–8]. Flippases and floppases together maintains asymmetry in the PM [9,10].

In 1996, Basse and co-workers isolated a 37-kDa integral membrane protein from human erythrocytes capable of energy independent scrambling of PLs in the presence of calcium [11]. Subsequent studies by the same group revealed that this protein is localized in the PM and is involved in the translocation of PL between the membrane leaflets when reconstituted into liposomes in the presence of Ca^{2+} and termed it as human phospholipid scramblase (hPLSCR1) [12]. Recombinant hPLSCR1 showed scramblase activity in artificial liposomes and also displayed Ca^{2+} [12] mediated surface PS exposure when overexpressed in Jurkat cells [13]. hPLSCRs were reported as multifunctional proteins because of its role in several key cellular processes such as cell proliferation [14,15], antiviral response [16–21], tumor suppression [22], protein interactions [23–27], transcriptional regulation [28–31] and apoptosis. This review primarily focuses on addressing the function of hPLSCRs in apoptosis and their deterministic role in different apoptotic pathways.

2. Human phospholipid scramblases

hPLSCRs are a group of type II single pass transmembrane proteins mediating Ca^{2+} induced ATP independent bidirectional trans-bilayer movement of PLs between the two leaflets of PM. Under normal conditions, scramblases are inactive but a thousand-fold increase in the intracellular Ca^{2+} level results in the activation of hPLSCRs along with the inactivation of flippases and floppases thereby disrupting the membrane asymmetry. In humans, scramblases constitute a family of four homologous proteins termed hPLSCR1-hPLSCR4. The predicted

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open reading frames encode proteins of 224 aa (hPLSCR2), 295 aa (hPLSCR3) and 329 aa (hPLSCR4) which are 59%, 47% and 46% identical to hPLSCR1 whereas hPLSCR5 is reported only in mRNA level. hPLSCR1, hPLSCR2, hPLSCR4 and hPLSCR5 are found to be closely clustered on chromosome 3 (3q23, 3q24), whereas hPLSCR3 is located on chromosome 17. Analysis of mRNA level expression of scramblases revealed that hPLSCR1 and hPLSCR3 are expressed in all tissues except in brain. Similarly hPLSCR4 is also expressed in variety of tissues except peripheral blood lymphocytes whereas the expression of hPLSCR2 is restricted only to testis [32]. Within a cell, hPLSCR1 and 4 was found to be localized to PM, cytoplasm and nucleus [26,33–35] whereas hPLSCR2 and hPLSCR3 was exclusively localized to nucleus and mitochondria respectively [34,36]. The differential gene expression in specific tissues needs further investigation as it is unclear and might have unique functions.

Multiple sequence alignment of hPLSCRs revealed several domains conserved from *C. elegans* to humans. Major domains of PLSCRs include (i) a putative EF-hand like calcium binding motif responsible for Ca^{2+} binding and activation [37,38], (ii) cysteine palmitoylation motif that helps in membrane anchoring [39], (iii) DNA binding motif that is involved in protein-DNA interactions during transcriptional regulation [31], (iv) a non-classical nuclear localization signal that aids in nuclear localization [26], (v) C-terminal helix that is essential for membrane insertion and scramblase activity [40], (vi) a cholesterol binding domain [41] and (vii) a proline rich domain (PRD) responsible for oligomerization mediated scramblase activity [34] (for a detailed review of the domain architecture, please refer [8]). Fig. 1 shows the multiple sequence alignment of hPLSCR1-4 from humans, mouse and *C. elegans*. While calcium binding motif, DNA binding motif and C-terminal helix were found to be highly conserved, PRD was not conserved among the homologs and across the species. Though hPLSCRs were initially identified as type II single pass transmembrane proteins, a recent report by Herate and co-workers revealed that overexpression of hPLSCR1 at the cell surface of differentiated macrophages resulted in the modification of the membrane topology of hPLSCR1 explaining that the protein may also adopt a type I transmembrane orientation exposing N-terminal region at the cell surface using the same transmembrane domain [42]. The physiological relevance for this flexible membrane topology is yet to be ascertained.

3. Is hPLSCR1 a true scramblase?

hPLSCRs were initially thought to be the proteins involved in Scott Syndrome, a bleeding disorder characterized by impaired PS exposure in platelets [43,44]. Subsequently, orthologs of hPLSCRs in different organisms were identified and characterized. hPLSCR1 was identified as the protein responsible for PL scrambling activity in erythrocytes and platelets [11]. Purified recombinant hPLSCR1 retained the PL scrambling activity *in vitro* when reconstituted to artificial liposomes [12]. Initial reports claimed the importance of hPLSCRs for elevated PS exposure levels on the cell surface during apoptosis. Zhao and coworkers reported that Raji cells stably transfected with hPLSCR1 cDNA showed higher PS exposure levels compared to untransfected cells [13]. However, subsequent research questioned the role of hPLSCR1 in PS exposure. Transcriptional activation of hPLSCR1 by interferons did not affect the PS exposure and increased expression of hPLSCR1 did not increase the PS exposure in several cell lines [45]. PLSCR1 deficient mice showed altered granulocyte production when treated with growth factors and impaired antiviral response to interferon but did not show any defects on PL scrambling. PLSCR3-null mice and PLSCR1 and PLSCR3 double mutant mice showed dyslipidemia and insulin resistance but did not alter PL scrambling [14]. Cells from PLSCR deficient *Drosophila* exhibited enhanced neurotransmitter secretion at larval neuromuscular synapses but did not show any alterations in lipid scrambling [46].

In 2010, Suzuki and co-workers identified TMEM16F, a member of calcium channels called anoctamins was directly responsible for Ca^{2+} dependent PS exposure in erythrocytes and a mutation in TMEM16F was responsible for Scott Syndrome [47,48]. A recent study identified that Xk-family protein Xkr8 exhibited PL scrambling activity and externalized PS in response to apoptotic stimuli and Xkr8 deficient cells did not exhibit apoptotic PS exposure [49–51]. These reports along with other key factors such as single transmembrane domain and smaller molecular weight compared to other transmembrane proteins questioned the PL scrambling role of PLSCRs.

Contradictorily, recombinant purified hPLSCR1, hPLSCR3 and hPLSCR4 showed scrambling activity *in-vitro* when reconstituted in proteoliposomes. Overexpression of PLSCR ortholog in *C. elegans* exhibited enhanced externalization of PS and apoptosis. PLSCR expression levels in *Dugesia japonica* (djPLSCR) was high when the planarians were stimulated with pathogen-associated molecular patterns suggesting that djPLSCR could play key roles in pathogen defense and apoptosis. Based on the reports, there might be two possibilities: i) hPLSCRs could be one among the many scramblases present in the cell and are triggered specifically at certain conditions, ii) Apart from scrambling activity, hPLSCRs could have several other key functions in the cell. Even after two decades of research revealing promising evidence for the various roles played by hPLSCRs, their exact function in a cell remains unclear.

4. Apoptosis and hPLSCRs

Apoptosis or programmed cell death involves a series of events resulting in regulated destruction and elimination of the targeted cell [52] and is essential for normal development, aging and maintenance of homeostasis by controlling cell populations in tissues. Apoptotic cells exhibit biochemical modifications such as protein cleavage, DNA breakdown and phagocytic recognition [53–55]. Caspases and metal dependent endonucleases mediate protein cleavage and DNA degradation respectively. Phagocytic recognition happens through the expression of cell surface markers *i.e.* exposure of PS, an anionic PL from the inner leaflet to the outer leaflet of the PM [56]. The major pathways leading to apoptosis of the cell include the extrinsic pathway and the intrinsic pathway, all of which converges to effector or execution pathway. Activation of caspases, cytochrome C release, DNA fragmentation and externalization of PS followed by phagocytosis are considered to be the hallmarks of apoptosis.

hPLSCR1 and hPLSCR3 are the most extensively studied proteins in the scramblase family and are reported to have crucial roles in apoptosis. During apoptosis, cells overexpressing hPLSCR1 was shown to have enhanced PS exposure, a hallmark of apoptosis. Upon UV treatment, hPLSCR1 stably transfected CHO-K1 cells and hPLSCR3 overexpressed cells displayed increased PS exposure and increased levels of caspase 3 and cytochrome C release thereby leading to apoptosis. hPLSCR1 plays a significant role in interferon mediated antiviral defense and apoptosis upon viral infection through the JAK-STAT pathway. In this section, a brief overview of the different pathways of apoptosis followed by the role of hPLSCRs in each of the pathways were discussed.

4.1. hPLSCRs in extrinsic pathway

Extrinsic pathway is mainly triggered by tumor necrosis factor receptor superfamily (TNFRSF) of proteins present in the PM and are subsequently mediated by a group of proteases termed caspases. TNFRSF are a group of transmembrane proteins that are mainly characterized by a cysteine rich extracellular domain named as the death domain which binds to cytokines thereby mediating the apoptotic signal from the PM to several downstream apoptotic pathways within the cell [57,58]. The cytokines that trigger apoptosis mainly include tumor necrosis factor (TNF-α), TNF-related apoptosis-inducing ligand (TRAIL), TNF-related weak inducer of apoptosis (TWEAK) and Fas ligand [58–63]. Caspases are a family of cysteine-aspartate proteases that