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Original Article

IN SILICO ANALYSIS OF TECOVIRIMAT A REPURPOSED DRUG AGAINST THE MONKEYPOX VIRUS, ITS OFF-TARGET HUMAN PROTEINS, AND IMPACT ON HUMAN HEALTH

JANVI AGGARWAL[1], POONAM SHARMA[1*]

Department of Zoology, Gargi College, University of Delhi, India
[*]Corresponding author: Poonam Sharma; [*]Email: poonam.sharma@gargi.du.ac.in

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ABSTRACT

Objective: In this study, *in silico* analysis of human off-target proteins of tecovirimat, an investigational drug reported to stop monkey pox virus infection by binding to a protein that the virus uses to enter host cells was performed to better understand its off-target long-term and short-term effects on other important biological processes in patients.

Methods: The target and off-target proteins of the drug, as well as their characteristics, protein-protein interactions, and the pathways they are involved in, were thoroughly analyzed using a number of databases, including Drug Bank, the NCBI Gene Database, BLAST, the UCSC Gene Sorter, Gene MANIA, STRING, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database.

Results: The current study showed that although the repurposing drug tecovirimat aids in the treatment of patients with monkeypox by binding to the viral p37 protein, it can also accidentally interfere with vital biological processes by interacting with off-target proteins or by indirectly interfering with the proteins that interact with these target proteins.

Conclusion: The findings highlight the importance of extensively assessing and evaluating all repurposed drugs for their off-target effects before making them available to the general public.

Keywords: Monkeypox, Tecovirimat, p37 protein, Rheumatoid arthritis, Alzheimer's disease

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INTRODUCTION

Monkeypox is caused by a DNA virus (MPV, MPXV, or hMPXV), which was found in humans in 2003. Monkeypox virus is a zoonotic virus that belongs to the orthopoxvirus family and has a genome size of 190 kb. The monkeypox virus, like other poxviruses, has an oval shape and an outer lipoprotein membrane that shields viral enzymes, DNA, and transcription factors. Monkeypox viruses rely mostly on the protein encoded in their genome that permits them to multiply in the cytoplasm of the host cells [1]. Like smallpox and rabbitpox, monkeypox is a poxvirus with a peculiar life cycle. Despite being DNA viruses, monkeypox totally bypasses the host cell nucleus and instead completes replication, transcription, translation, and virus assembly in the cytoplasm. MPXV transmission can occur through an animal bite or scratch direct contact with an infected animal's blood, biological fluids, tissue, or sores.

As soon as the virus attaches to and fuses with the host cell, it uncoats and begins to produce early genes. At this stage, DNA replication initiates, followed by the transcription of early and late genes. The p37 protein aids in the formation and coating of intracellular mature virions (IMVs), which can either remain inside host cells as cell-associated virus (CEV) particles or migrate outside cells as extracellular enveloped virus (EEV) particles. p37 is a 37 kDa peripheral membrane protein expressed by the F13L gene that plays an important role in IMV particle envelopment. As a result, p37 is responsible for virulence by producing and egressing wrapped virions. The emergence of zoonotic diseases has grown increasingly widespread as a result of a variety of factors such as urbanization, deforestation, tourism, zoos, climate change, and wildlife exploitation. Drug repurposing has effectively found promising candidate drugs that potentially open up new treatment paths for treating these emerging viruses such as SARS-CoV-2, MERS-CoV, and H5N1 [2]. Repurposing existing drugs can be appealing, given the process is often safer, less expensive, and can be carried out in less time. One such repurposed medicine, tecovirimat, was recently licensed by the US Food and medicine Administration (FDA) against poxviruses and targets the critical viral p37 protein [3] (fig. 1).

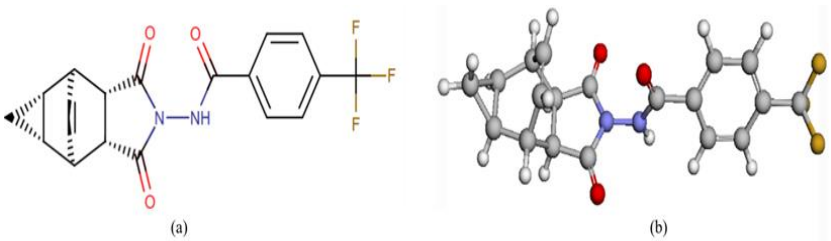


Fig. 1: Structure of tecovirimat drug (a) 2D structure (b) 3D structure

Tecovirimat inhibits the crucial viral p37 protein and consequently prevents the formation and egress of enveloped virions, which are essential for virulence (fig. 2). As most investigational drugs have a range of mild to severe short-and long-term unfavorable side effects, this study aimed to understand tecovirimat's off-target effects by identifying and analyzing structurally comparable off-target proteins. It also aimed to explore the possibility of analyzing tecovirimat drug behavior *in silico* for effective pathological control and after-effect management.

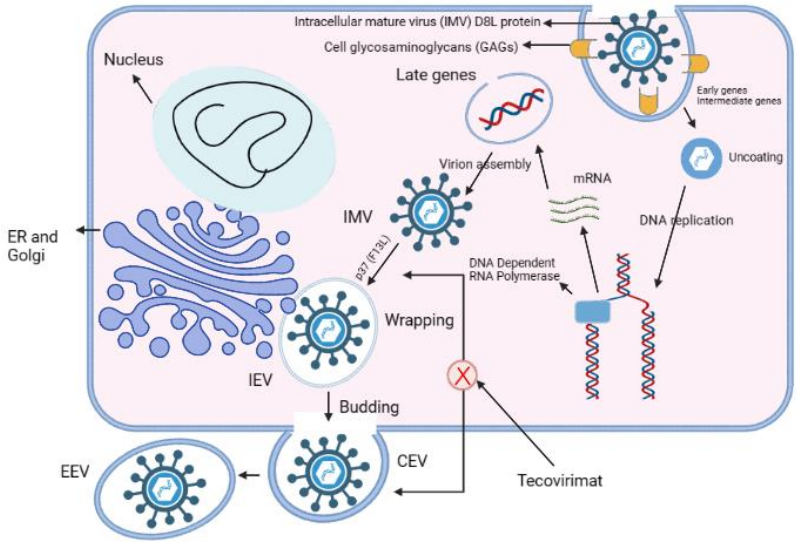


Fig. 2: Schematic illustration of the MPXV replication cycle and the mechanism of action of the tecovirimat drug

MATERIALS AND METHODS

Drug Bank was used to extract detailed information of about tecovirimat, a drug used for monkeypox treatment. It provided information on the Drug Bank accession number, type, groups, weight, chemical formula, structure, mode of action in the body, toxicity, absorption, and metabolism [4]. The NCBI Gene Database was used to learn about the p37 gene, including its symbol, gene type, lineage, and summary. Furthermore, the amino acid sequence of the p37 protein was retrieved. Next, other human proteins with similar amino acid sequences were identified using the NCBI Basic Local Alignment Search Tool (BLAST). The "Distance tree of results" was selected to obtain a visual representation of how different proteins are related based on the sequences they share. The two most closely related off-target proteins were identified by analyzing the Distance tree of results [5]. For a better understanding of the functions of the p37 gene in humans, the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database was used, from which various signaling pathways involving p37 and related proteins were obtained and studied [6]. Moreover, the Amazonia database was used to identify the tissues and organs that will be most disrupted by the drug targeting p37. The expression levels of the off-target proteins that were found to be similar to p37 in different human tissues and organs were also observed using the Amazonia database. The effect of binding the tecovirimat drug to the off-target protein was further studied using various research databases and literature searches. The protein-protein interaction networks of the off-target proteins were studied using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database [7, 8].

RESULTS AND DISCUSSION

The twelve proteins having a similar structure as the p37 protein were found to belong to the phospholipase D superfamily. Four major types of phospholipases (A, B, C, and D) categorized based on where phospholipids are cleaved belong to this superfamily. Each of the phospholipase families contains a large number of isoforms, each of which is expressed in various cell types and organelles and serves a particular purpose. Two isoforms of phospholipase D (PLD) enzymes that hydrolyze the abundant membrane phospholipid, phosphatidylcholine (PC), into the choline headgroup and phosphatidic acid (PA), namely, phosphatidylcholine-hydrolyzing phospholipase D3 (PLD3) and phosphatidylcholine-hydrolyzing phospholipase D4 (PLD4 isoform), show the best homology match with the p37 protein (table 1). Using STRING, protein interactions were generated with off-target proteins (PLD3 and PLD4) that may cause disturbances in the functioning of these genes indirectly.

Phosphatidylcholine-hydrolyzing phospholipase D3 (PLD3)

The *PLD3* gene encodes the phosphatidylcholine-hydrolyzing phospholipase D3 protein. PLD 3 is highly expressed in the brain and, in particular, in cortical neurons. PLD3 is a 5'-3' exonuclease that resides in lysosomes, where it is implicated in the regulation of inflammatory responses by degrading ssDNA [9]. Defects in nuclease activity and ER retention are caused by the PLD3 mutation. Genetic variants in PLD3 affect proteolytic processing, intracellular sorting, and specific acid 5′ exonuclease activity and have been associated with a higher risk of developing Alzheimer's disease [10-12]. PLD3 depletion and exonuclease dysfunction-causing SNPs promote lysosomal impairment and congestion of the degradative route. Moreover, genetic variants in PLD3 have been linked with cerebrospinal fluid total-tau and phosphorylated-tau levels, cognitive function in Alzheimer's disease patients, and are additionally associated with other neurological traits like longevity [13-15].

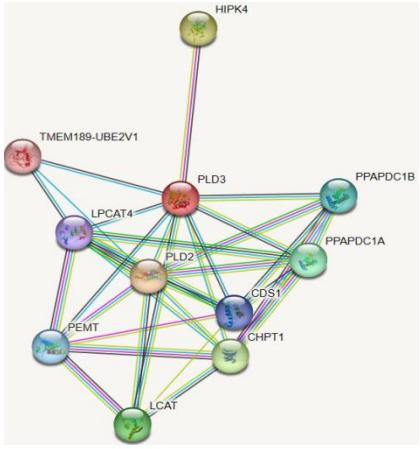
Table 1: Structurally Important off-target proteins with a similar structure to p37

Protein	Query coverage	Blastp E-value	Genome sequence	Genome location
Phospholipase D5 isoform 4	98%	1e-27	Chromosome 1, NC_000001.11	Chr1, 242,089, 854-242,348,148
Phospholipase D5 isoform X1	96%	5e-27	Chromosome 1, NC_000001.11	Chr1, 242,089, 854-242,361,011
Phospholipase D5 isoform 2	96%	5e-27	Chromosome 1, NC_000001.11	Chr1, 242,089, 854-242,449,356
Phospholipase D5 isoform X2	96%	5e-27	Chromosome 1, NC_000001.11	Chr1, 242,089, 854-242,348,158
Phospholipase D5 isoform 1	96%	7e-27	Chromosome 1, NC_000001.11	Chr1, 242,089, 854-242,524,276
Phospholipase D5 isoform X4	84%	2e-21	Chromosome 1, NC_000001.11	Chr1, 242,089, 854-242,288,448
5'-3' exonuclease PLD3	95%	1e-19	Chromosome19, NC_000019.10	Chr19, 40,366, 484-40, 378,173
Phospholipase D5 isoform 3	69%	1e-19	Chromosome 1, NC_000001.11	Chr1, 242,089, 854–242,220,098
Phospholipase D5 isoform X3	69%	3e-19	Chromosome 1, NC_000001.11	Chr1, 242,089, 854–242,251,706
5'-3' exonuclease PLD4 isoform 2	68%	2e-17	Chromosome 14, NC_000014.9	Chr14, 104,927 ,141–104,932,964
5'-3' exonuclease PLD4 isoform 1	68%	2e-17	Chromosome 14, NC_000014.9	Chr14, 104,927, 120–104,932,964
5'-3' exonuclease PLD4 isoform X1	52%	7e-14	Chromosome 14, NC_000014.9	Chr14, 104,927, 120–104,937,212

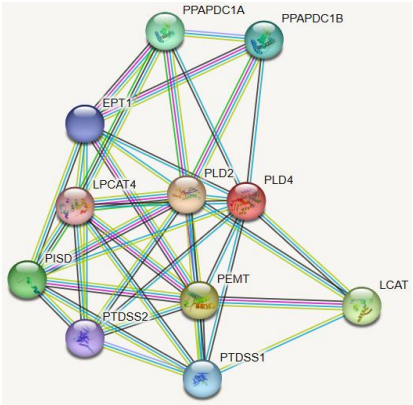
Phosphatidylcholine-hydrolyzing phospholipase D4 (PLD4)

PLD4 is a transmembrane glycoprotein, a member of the phospholipase family without phospholipase D activity [16]. PLD4 is involved in the phagocytosis of microglia and specifically regulates exocytosis [17]. Immunohistochemical analysis revealed that the main expression of PLD4 is located in the colon cancer mesenchymal and lymph nodes compared with normal tissues, where PLD4 promotes M1 macrophages to perform antitumor effects in colon cancer cells [18]. The expression of PLD4 was closely associated with M1-type macrophages, and inhibition of its expression in M1 macrophages by siRNA led to a significant decrease in the secretion of pro-inflammatory cytokines IL-1, IL-6, and TNF-α. PLD4 is reported to be involved in the activation of M1 cells *in vitro* and has an inhibitory effect on colon cancer [18]. PLD4 might play an important role in regulating the activation of M1 macrophages, which are capable of inducing lysis in various types of cancer cells, but the mechanism of action needs further exploration [19]. The manner in which PLD4 impacted macrophage activation still needed further study. According to many studies, PLD4 loci are reported to be associated with rheumatoid arthritis and also associated with systemic lupus erythematosus (SLE) and systemic sclerosis, suggesting the role of PLD4 in autoimmune diseases [20]. Single nuclear polymorphisms (SNPs) associated with PLD4 are associated with susceptibility to rheumatoid arthritis. According to recent studies, two autoimmune disorders with aberrant inflammatory skin lesions, systemic sclerosis and rheumatoid arthritis, are linked to mutations in the PLD4 gene [21, 22].

These findings draw attention to the severe negative effects of tecovirimat drug binding to off-target proteins in patients, including rheumatoid arthritis, systemic sclerosis, hypertension, acute respiratory distress syndrome, congenital muscular dystrophy, intellectual disability, visual impairment, normochromic anemia, renal failure, cancer, and neurodegenerative disorders such as Alzheimer's disease.



(a)



(b)

Fig. 3: (a) Protein-protein interaction networks of PLD3 (b) Protein-protein interaction networks of PLD4

Protein-protein interactions

Protein interactions with off-target proteins of the tecovirimat drug, PLD3 and PLD4, were investigated in order to gain insight and understanding into how they may induce indirect disruptions in the function of these genes (fig. 3a and b).

These interactions also aid in determining the molecular basis of disruptions and the cell's reaction to them. Ten proteins generated by STRING for the protein-protein interaction networks of PLD3 and PLD4 were PLD2, HIPK4, CHPT1, LCAT, PPAPDC1A, PPAPDC1B, PEMT, CDS1, LPCAT4, and TMEM189-UBE2V1.

Phospholipase D2 (PLD2) is involved in signal-induced cytoskeletal regulation and endocytosis. It is involved in various cellular functions, and variation in its expression can lead to a number of health concerns like high blood pressure, oncogenesis, diabetes, and obesity [23, 24]. Any variation in the level of expression of homeodomain-interacting protein kinase 4 (HIPK4), a crucial regulator of sperm head shape and a possible target for male contraception, can result in male infertility [25]. HIPK4 diacylglycerol choline phosphotransferase (CHPT1), a key player in the development and maintenance of vesicular membranes, catalyzes the synthesis of phosphatidylcholine from CDP-choline.

Overexpression of CTL1 is reported in several cancer cell lines and is linked to the development of malignancy, while decreased CTL1 expression is linked to acute respiratory distress syndrome (ARS) [26, 27]. An essential enzyme in the extracellular metabolism of plasma lipoproteins is lecithin cholesterol acyltransferase (LCAT). LCAT is also produced in the brain by primary astrocytes and is involved in the esterification of free cholesterol on nascent APOE (Apolipoprotein E). Any deviation from the normal expression level of LCAT might cause visual impairment, normochromic anemia, and, in rare cases, renal failure [28]. Phospholipid phosphatases 4 and 5 (PPAPDC1A and PPAPDC1B) are engaged in a number of cell-signaling pathways and serve as carcinoma suppressors that belong to the PA-phosphatase-related phosphoesterase family [29]. Phosphatidylethanolamine N-methyltransferase (PEMT) catalyzes the methylation pathway of phosphatidylcholine biosynthesis, and the dysregulation of PEMT activity can cause changes in lipid metabolism [30]. A crucial enzyme in the production of phosphatidylinositol, phosphatidylglycerol, and cardiolipin is phosphatidate cytidylyltransferase 1 (CDS1). In the brain, CDS1 is reported to be involved in the signal transduction mechanism of retinal and neural cells. The dysfunction of CDS1 has been reported to be associated with congenital muscular dystrophy, cataracts, and intellectual disability [31]. A specific group of lysophospholipids serve as substrates for the lysophospholipid acyltransferase (LPCAT4), which exhibits acyl-CoA-dependent lysophospholipid acyltransferase activity. Variations in LPCAT4 expression have been linked to apoptosis, indicating that lysophospholipid esterification is essential for preserving cellular structure and function [32]. Plasmanylethanolamine desaturase (TMEM189-UBE2V1) belongs to the ubiquitin-conjugating enzyme family, which catalyzes the last step in the synthesis of plasmalogens, an abundant group of glycerophospholipids that is deficient in diseases like Alzheimer's [33]. As a result, the protein-protein interactions studies revealed that tecovirimat overdose may cause indirect disruptions in the functioning of these proteins, eventually leading to many severe short-and long-term side effects in patients.

CONCLUSION

This study emphasizes the importance of carefully assessing and evaluating the off-target effects of all approved repurposed drugs before their administration to the general population in order to comprehend their off-target effects. The results of the current study showed that although the repurposing drug tecovirimat aids in the treatment of patients with monkeypox by binding to the viral p37 protein, it can also accidentally interfere with vital biological processes by interacting with off-target proteins or by indirectly interfering with the proteins that interact with these target proteins. This can have severe negative effects on patients, including rheumatoid arthritis, systemic sclerosis, hypertension, diabetes, obesity, acute respiratory distress syndrome, congenital muscular

dystrophy, intellectual disability, visual impairment, normochromic anemia, renal failure, and neurodegenerative disorders like Alzheimer's disease. Long-term exposure to the drug could possibly make consumers susceptible to cancer.

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AUTHORS CONTRIBUTIONS

Both authors have contributed equally.

CONFLICT OF INTERESTS

The authors have no conflicts of interest regarding this investigation.

REFERENCES

1. Farahat RA, Abdelaal A, Shah J, Ghozy S, Sah R, Bonilla Aldana DK. Monkeypox outbreaks during COVID-19 pandemic: are we looking at an independent phenomenon or an overlapping pandemic? Ann Clin Microbiol Antimicrob. 2022 Jun 15;21(1):26. doi: 10.1186/s12941-022-00518-2, PMID 35706004.
2. Patel M, Mazumder R, Kaushik KK, Debnath A, Mishra R, Pal S. A brief description of COVID-19 pulmonary viral infection and repurposing of drugs for its treatment. Int J App Pharm. 2022;14(5):22-31. doi: 10.22159/ijap.2022v14i5.45168.
3. Rabaan AA, Abas AH, Tallei TE, Al-Zaher MA, Al-Sheef NM, Fatimawali A-NEZ. Monkeypox outbreak 2022: what we know so far and its potential drug targets and management strategies. J Med Virol. 2023 Jan;95(1):e28306. doi: 10.1002/jmv.28306, PMID 36372558.
4. Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P. DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. Nucleic Acids Res. 2006 Jan 1;34:D668-72. doi: 10.1093/nar/gkj067, PMID 16381955.
5. Kent WJ, Hsu F, Karolchik D, Kuhn RM, Clawson H, Trumbower H. Exploring relationships and mining data with the UCSC gene sorter. Genome Res. 2005 May;15(5):737-41. doi: 10.1101/gr.3694705, PMID 15867434.
6. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017 Jan 4;45(D1):D353-61. doi: 10.1093/nar/gkw1092, PMID 27899662.
7. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S. The string database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021 Jan 8;49(D1):D605-12. doi: 10.1093/nar/gkaa1074, PMID 33237311.
8. MSS, Dinesh S, Sharma S. Prediction of high-risk NSSNPS associated with WISP3 gene expression: an *in silico* study. Int J App Pharm. 2023;15(5):161-70. doi: 10.22159/ijap.2023v15i5.48269.
9. Gonzalez AC, Schweizer M, Jagdmann S, Bernreuther C, Reinheckel T, Saftig P. Unconventional trafficking of mammalian phospholipase D3 to lysosomes. Cell Rep. 2018 Jan 23;22(4):1040-53. doi: 10.1016/j.celrep.2017.12.100, PMID 29386126.
10. Cappel C, Gonzalez AC, Damme M. Quantification and characterization of the 5′ exonuclease activity of the lysosomal nuclease PLD3 by a novel cell-based assay. J Biol Chem. 2021 Jan-Jun;296(96):100152. doi: 10.1074/jbc.RA120.015867, PMID 33288674. PMCID PMC7857491.
11. Cruchaga C, Karch CM, Jin SC, Benitez BA, Cai Y, Guerreiro R. Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature. 2014 Jan 23;505(7484):550-4. doi: 10.1038/nature12825, PMID 24336208. PMCID PMC4050701.
12. Tan MS, Wang P, Ma FC, Li JQ, Tan CC, Yu JT. Common variant in PLD3 influencing cerebrospinal fluid total tau levels and hippocampal volumes in mild cognitive impairment patients from the ADNI cohort. J Alzheimers Dis. 2018;65(3):871-6. doi: 10.3233/JAD-180431, PMID 30103332.
13. Lambert JC, Grenier-Boley B, Bellenguez C, Pasquier F, Campion D, Dartigues JF. PLD3 and sporadic Alzheimer's disease risk. Nature. 2015 Apr 2;520(7545):E1. doi: 10.1038/nature14036, PMID 25832408.
14. Engelman CD, Darst BF, Bilgel M, Vasiljevic E, Koscik RL, Jedynak B M. The effect of rare variants in TREM2 and PLD3 on longitudinal cognitive function in the Wisconsin registry for Alzheimer's prevention. Neurobiol Aging. 2018 Jun;66(177):177.e1-5. doi: 10.1016/j.neurobiolaging.2017.12.025, PMID 29395285, PMCID PMC5924624.
15. Nygaard HB, Erson Omay EZ, Wu X, Kent BA, Bernales CQ, Evans DM. Whole-exome sequencing of an exceptional longevity cohort. J Gerontol A Biol Sci Med Sci. 2019 Aug 16;74(9):1386-90. doi: 10.1093/gerona/gly098, PMID 29750252. PMCID PMC6696723.
16. Van Acker ZP, Bretou M, Sannerud R, Damme M, Annaert W. Deficiency of the lysosomal exonuclease PLD3 impacts the degradative route. Alzheimers Dem. 2021 Dec;17(S3) Suppl 3:e050868. doi: 10.1002/alz.050868.
17. Otani Y, Yamaguchi Y, Sato Y, Furuichi T, Ikenaka K, Kitani H. PLD$ is involved in phagocytosis of microglia: expression and localization changes of PLD4 are correlated with activation state of microglia. PLOS ONE. 2011;6(11):e27544. doi: 10.1371/journal.pone.0027544, PMID 22102906.
18. Tang X, Mo C, Wang Y, Wei D, Xiao H. Anti-tumour strategies aiming to target tumor-associated macrophages. Immunology. 2013 Feb;138(2):93-104. doi: 10.1111/imm.12023, PMID 23113570, PMCID PMC3575762.
19. Gao L, Zhou Y, Zhou SX, Yu XJ, Xu JM, Zuo L. PLD4 promotes M1 macrophages to perform antitumor effects in colon cancer cells. Oncol Rep. 2017 Jan;37(1):408-16. doi: 10.3892/or.2016.5216, PMID 27840999.
20. Terao C, Ohmura K, Kawaguchi Y, Nishimoto T, Kawasaki A, Takehara K. PLD4 as a novel susceptibility gene for systemic sclerosis in a Japanese population. Arthritis Rheum. 2013 Feb;65(2):472-80. doi: 10.1002/art.37777, PMID 23124809.
21. Chen WC, Wang WC, Okada Y, Chang WP, Chou YH, Chang HH. rs2841277 (*PLD4*) is associated with susceptibility and rs4672495 is associated with disease activity in rheumatoid arthritis. Oncotarget. 2017 Jul 18;8(38):64180-90. doi: 10.18632/oncotarget.19419, PMID 28969061. PMCID PMC5609993.
22. Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genet. 2012 Mar 25;44(5):511-6. doi: 10.1038/ng.2231, PMID 22446963.
23. Nelson RK, Ya Ping J, Gadbery J, Abedeen D, Sampson N, Lin RZ. Phospholipase D2 loss results in increased blood pressure via inhibition of the endothelial nitric oxide synthase pathway. Sci Rep. 2017 Aug 22;7(1):9112. doi: 10.1038/s41598-017-09852-4, PMID 28831159. PMCID PMC5567230.
24. Bradshaw RA, Dennis EA. editors. Handbook of cell signaling. Academic press; 2009 Nov 3.
25. Liu X, Zang C, Wu Y, Meng R, Chen Y, Jiang T. Homeodomain-interacting protein kinase HIPK4 regulates phosphorylation of manchette protein RIMBP3 during spermiogenesis. J Biol Chem. 2022;298(9):102327. doi: 10.1016/j.jbc.2022.102327, PMID 35931115, PMCID PMC9440445.
26. Stoica C, Ferreira AK, Hannan K, Bakovic M. Bilayer forming phospholipids as targets for cancer therapy. Int J Mol Sci. 2022 May 9;23(9):35563655. doi: 10.3390/ijms23095266, PMID 35563655, PMCID PMC9100777.
27. Dushianthan A, Cusack R, Grocott MPW, Postle AD. Abnormal liver phosphatidylcholine synthesis revealed in patients with acute respiratory distress syndrome. J Lipid Res. 2018 Jun;59(6):1034-45. doi: 10.1194/jlr.P085050, PMID 29716960. PMCID PMC5983399.
28. Griffith JR. Williams textbook of endocrinology: P. Reed Larsen, MD, Henry M. Kronenberg, MD, Shlomo Melmed MD, Kenneth S, Polonsky MD, Philadelphia WB. Saunders Company, 2003. J Pediatr Adolesc Gyneco. 2004 Jun 1;17(3):217-8.
29. Bernard Pierrot I, Gruel N, Stransky N, Vincent Salomon A, Reyal F, Raynal V. Characterization of the recurrent 8p11-12 amplicon identifies PPAPDC1B, a phosphatase protein, as a new therapeutic target in breast cancer. Cancer Res. 2008 Sep 1;68(17):7165-75. doi: 10.1158/0008-5472.CAN-08-1360, PMID 18757432.

30. Tollefsbol T, editor. Handbook of epigenetics: the new molecular and medical genetics. Academic Press; 2017 Jul 10.
31. Li J, Xin Y, Li J, Chen H, Li H. Phosphatidylethanolamine N-methyltransferase: from functions to diseases. Aging Dis. 2023;14(3):879-91. doi: 10.14336/AD.2022.1025, PMID 37191416.
32. Jain S, Zhang X, Khandelwal PJ, Saunders AJ, Cummings BS, Oelkers P. Characterization of human lysophospholipid acyltransferase 3. J Lipid Res. 2009 Aug;50(8):1563-70. doi: 10.1194/jlr.M800398-JLR200, PMID 19351971. PMCID PMC2724057.
33. Werner ER, Keller MA, Sailer S, Lackner K, Koch J, Hermann M. The *TMEM189* gene encodes plasmanylethanolamine desaturase, which introduces the characteristic vinyl ether double bond into plasmalogens. Proc Natl Acad Sci USA. 2020 Apr 7;117(14):7792-8. doi: 10.1073/pnas.1917461117, PMID 32209662. PMCID PMC7149458.