Understanding the Multifaceted Role of Ectonucleotide Pyrophosphatase/Phosphodiesterase 2 (ENPP2) and its Altered Behaviour in Human Diseases

R.P. Cholia[1], H. Nayyar[3], R. Kumar[3] and A.K. Mantha[*,1,2]

[1]*Center for Animal Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, Punjab, India*

[2]*Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX-77555, USA*

[3]*Laboratory for Drug Design and Synthesis, Centre for Pharmaceutical Sciences and Natural Products, School of Basic and Applied Sciences, Central University of Punjab, Bathinda 151 001, Punjab India*

A.K. Mantha

Abstract: Ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2) also known as Autotaxin, is a secreted lysophospholipase D, which hydrolyzes lysophosphatidylcholine (LPC) into Lysophosphatidic acid (LPA). LPA is the bioactive product of ENPP2 enzyme, which induces diverse signalling pathways *via* six LPA-G-protein coupled receptors (GPCRs). ENPP2 is an essential protein for normal development and its altered expression is associated with various human diseases. Cellular ENPP2 silencing results in lethality at the embryonic stage in mice. Initially, it is identified as an autocrine factor in melanoma cells. Different research groups are currently exploring to understand the multifaceted role of ENPP2 in various processes such as embryonic and neural development, migration, invasion, differentiation, proliferation, angiogenesis, and survival. Altered expression of ENPP2 is also associated with various diseases like inflammation, cancer, fibrosis, rheumatoid arthritis and neural defects. In this article, we have summarized structural aspects of ENPP2 and biochemical functions associated with its diverse cellular roles in various human diseases including cancer and Alzheimer's disease (AD). In addition, keeping in view and advocating findings, a note on various phytochemicals and synthetic inhibitors, which are currently explored as therapeutic agents targeting functions of ENPP2 for the treatment of various human diseases is also presented.

Keywords: ATX, ENPP2, cell proliferation, LPA, LPC, lysophospholipase D.

INTRODUCTION

Ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2) is an extracellular secreted enzyme. ENPP2 (also known as autotaxin, ATX) is the member of ectonucleotide pyrophosphatase/ phosphodiesterases family. In the extracellular fluids, ENPP2 catalyzes the conversion of lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) [1, 2]. Stracke *et al.* in 1992, identified ENPP2 as an autocrine factor in A2058 melanoma cells. They purified ENPP2 from the conditioned medium of A2058 melanoma cells. ENPP2 is a 125 kDa glycoprotein. They named this glycoprotein as autotaxin. ENPP2 has pI of 7.7±0.2 [3]. ENPP2 is also involved in the initiation of the metastatic cascade in primary tumor cells (Stracke *et al.*, 1993). In 1994, Murata *et al.* identified 45% amino acid homology in ENPP2/autotaxin with pyrophosphatase/type1 phosphodiesterase (PC-1), which is expressed on the surface of activated B cells and plasma cells [4]. Database searches confirm phosphodiesterase-I alpha (PD1α), and human ENPP2 are as spliced products of the same gene. Nucleotide and amino acid sequence analysis revealed that PD1α lacks 156 nucleotides (1022-1177) and 52 amino acids (325-376) from human ENPP2 [5]. The oligosaccharide chain linked to the ENPP2 is an asparagine linkage and decreases the molecular mass of ENPP2 to 100-105 kDa and was found not to be associated with its motility stimulating activity [6].

ENPP2 is a well characterized second of seven known members of the ENPP family and the only secreted protein from this family with additional lysophospholipase D activity [7]. Other synonyms for ATX are: nucleotide pyrophosphatase/phosphodiesterase 2 (NPP2), PD1 alpha, lysophospholipase D, and phosphodiesterase nucleotide pyrophosphatase 2 (PDNP2) [8]. ENPP2 is known to be involved in various cellular processes such as embryonic development [3], blood vessel formation during the development [9], inflammation, and progression of cancer [1]. LPA is the bioactive product of ENPP2 and it acts *via* the LPA receptors coupled with G-protein; which results in cytokine production, inflammation, hyperplasia, tumor formation and metastasis [1]. It was found that ENPP2

*Address correspondence to this author at the Center for Animal Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, Pin Code: 151 001, Punjab, India; Tel: + 91-164 2864142; E-mail: anilmantha@gmail.com or Anil.kumar@cup.ac.in

1566-5240/15 $58.00+.00

also produces another bioactive lipid, cyclic phosphatidic acid (CPA), which is an analog of LPA. CPA has a cyclic phosphate at the sn-2 and -3 position of glycerol [10], which functions opposite to LPA. CPA has anti-mitogenic action and elevates cAMP level, inhibits the platelet aggregation, promotes the neurite outgrowth and enhances the survival of the neuronal cells. In addition, CPA is also known to inhibit the cell invasion and acts as an anti-metastatic agent [11].

ENPP2 mediates many physiological and patho-physiological processes to promote embryonic development involving vascular and neural development, cell survival, proliferation, and migration. Alteration in ENPP2 expression accelerates the progression of various human diseases such as cancer, neurological disorders, liver diseases, pulmonary fibrosis, and rheumatoid arthritis. Intense research being conducted in the area of ENPP2, especially regarding cancer. However, ENPP2 exploration in other human diseases demands research focus too. Keeping in view of advantage with the inhibition of ENPP2 activities, which will open up the new therapeutic field of treatment for various chronic diseases. Here, in this review, we have summarized both structural and functional aspects associated with diverse signalling of ENPP2/LPA axis, and their role in various human diseases.

STRUCTURE OF HUMAN ENPP2

Structurally, ENPP2 consists of different domains: N-terminal hydrophobic domain, two somatomedin B (SMB) like domains, a catalytic phosphodiesterase (PDE) domain, and C-terminal nuclease (NUC) like domain (Fig. **1**) [7]. ENPP2 shows structural similarity with ENPP1 and ENPP3 of ENPP family members [12]. ENPP2 shows interaction with other members of ENPP family, ENPP6 and various groups of phospholipase A2 (PLA2) as predicted by the String 10 software under confident view (Fig. **2**).

ENPP2 has an N-terminal hydrophobic domain that functions as a signal peptide. During secretion of active ENPP2 enzyme, the signal peptide is cleaved from the full-length ENPP2 [13]. The amino acid sequence in this region is highly conserved in various species such as rat, mouse, human and chicken. ENPP2 can be cleaved at two N-terminal sites that match the consensus sequence of signal peptide and furin. The presence of a signal peptide confirmed that ENPP2 is not a transmembrane protein [14]. Initially, it was thought that ENPP2 is a type II transmembrane metalloenzyme and an extracellular domain containing a conserved catalytic site [15]. The N-terminal signal peptide is consecutive by two somatomedin B-like (SMB) domains (SMB1 and SMB2). These SMB

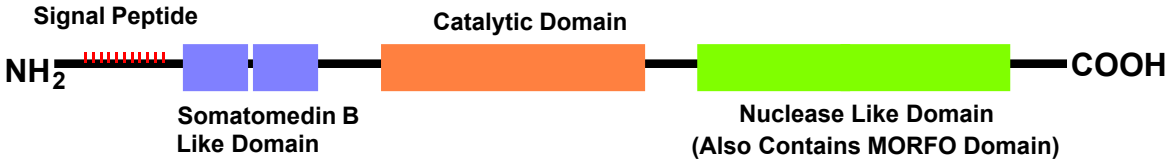

Fig. (1). Structural organization of ENPP2.

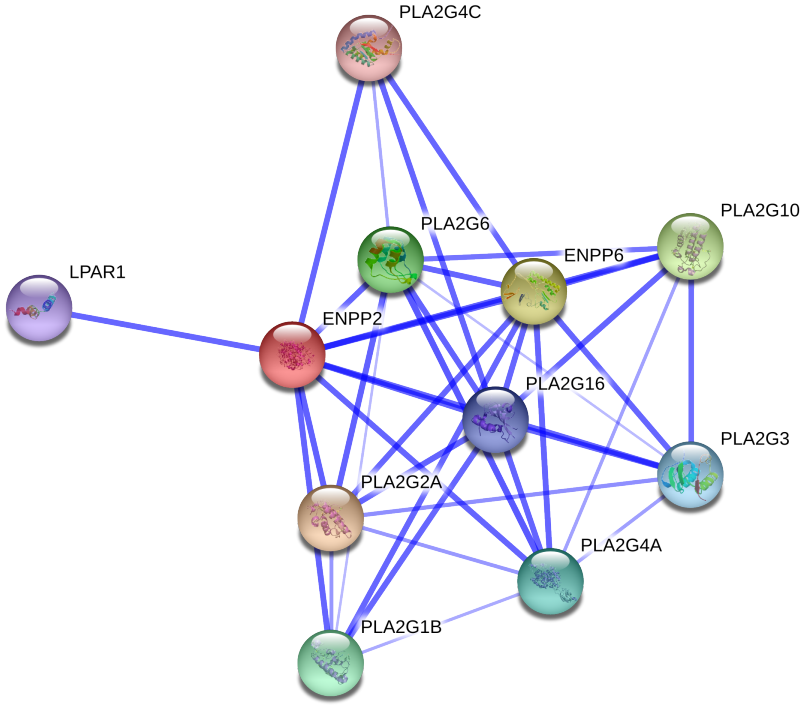

Fig. (2). Predicted functional partners of ENPP2 using String 10 under confident view. ENPP2 showed interaction with its family member ENPP6 and various groups of phospholipase A2 (PLA2).

domains are rich in cysteine residues. Functionally these domains are involved in intramolecular disulfide bridge formation [13, 16]. Initially, it was thought that these domains are involved in homodimerization, however now it is confirmed that these domains are involved in the intramolecular disulfide bridge formation [17]. Therefore, these domains functionally act as protein interaction domains. ENPP2 also contains Arg-Gly-Asp (RGD) tripeptide motif in its SMB2 domain. RGD motif is involved in protein-protein interaction [12]. The PDE domain has catalytic function and consists of approximately 400 amino acid residues [17]. Phosphodiesterase catalytic site (Y^{201}MRPVYPTKTFPN^{213}) is essential for the motility stimulating activity of ENPP2 [18]. The PDE domain is indispensable for catalytic activity of ENPP2 [19]. The NUC like domain is necessary for the correct folding, intracellular localization and secretion of the enzyme [20]. Molecular dynamic (MD) simulation of ENPP2 confirmed that the nuclease like domain and Asn524 glycan are essential for the catalytic activity of ENPP2 [21]. In the C-terminal region, there is an another functionally active modulator of oligodendrocyte remodeling and focal adhesion organization (MORFO) domain. It has been observed that MORFO domain is involved in the regulation of morphological/structural plasticity of differentiating oligodendrocytes. This activity of ENPP2 is independent of its catalytic LysoPLD activity [22-24].

The human *ENPP2* gene is identical to the mouse *ENPP2* gene [25]. ENPP2's localization in the genome is identified to be on the chromosome 8q23-24 [26]. The *ENPP2* gene is 81,754 bp in length with 26 exons. However, exon 12 is alternatively spliced into 3276 bp mRNA fragment with exon 12 that encodes 105.2 kDa protein composed of 915 aa and other spliced fragment of 3120 bp without exon 12, which encodes 863 aa, which is a 99.0 kDa precursor protein [8].

Isoforms of ENPP2

ENPP2 is produced initially as a pre-proenzyme. Upon removal of signal peptide moiety by the signal peptidases, ENPP2 is subsequently cleaved by the pro-protein convertases like furin before it converts to mature ENPP2 [27]. ENPP2 has four alternative forms α, β, γ [28] and δ that are recently identified [29]. These isoforms result from the alternate splicing of *ENPP2* gene. ENPP2α binds specifically to heparin with high affinity but, ENPP2β does not, which enhances the lysophospholipase D activity of ENPP2α [30]. All isoforms have the same domain architecture. All of the ENPP2 isoforms differ from each other by the absence or presence of exon 12 or exon 21. Human ENPP2 α, β, γ, and δ consists of 915/863/888/859 aa [29]. ENPP2α lacks exon 21, ENPP2β lacks exon 12 and 21, and ENPP2γ lacks exon 12 [25]. ENPP2β is widely expressed in the peripheral tissues and less in the brain. ENPP2γ is highly expressed in the brain and marginally expressed in the peripheral tissues. In addition, ENPP2α showed lower expression patterns, both in the brain and peripheral tissues [25].

Mechanism of Action and Functions of ENPP2

All cellular activities of ENPP2 are operated through its bioactive product LPA, which is a simple lipid. LPA is produced by the action of ENPP2 on the membrane LPC, which is the physiological substrate for the phosphodiesterase activity of ENPP2 and also reported to produce LPA. As studied in mice, circulating LPA has a half-life of about 3 min. LPC is present at up to 200 μM in blood plasma in a predominantly in albumin-bound form. Circulating LPA has a concentration between 10^{-7} to 10^{-6} M. Most of the biological functions of ENPP2 are operated *via* LPA [31]. LPA regulates itself, and its biosynthesis occurs in the extracellular environment [32]. LPA has multifaceted bioactivities displayed *via* G-protein coupled (GPCRs) LPA receptors [1]. As reviewed by Berdichevets *et al*. membrane phospholipids get hydrolyzed *via* lysoPLD activity which produces LPA. It has been found that LPA performs diverse functions through the six different GPCRs, LPA1 - LPA6 as shown in Fig. (**3**) [20]. LPA signalling results in cytokine production, inflammation, hyperplasia, tumor formation and metastasis [1]. LPA induces cell migration *via* activating Rac through Tiam1 GDP/GTP exchange factor [33]. Lipophilic, xenobiotics such as 2,3,7,8-tetrachloro-dibenzo-para-dioxin (TCDD)-mediated activation of NFAT-1 promotes cell migration *via* ENPP2 signalling in breast cancer MCF-7 cells [34]. As studied in melanoma cells, ENPP2 exerts its motility effect *via* the G-protein coupled phosphoinositide-3-kinase γ [35]. Cell division cycle 42 (Cdc42) and Rac1 are essential for the invasive potential of ENPP2 as studied in A2058 melanoma cells [35]. LPA binds to the receptor for advanced glycation end (RAGE) products, primarily through RAGE V-type immunoglobulin domain that is a member of the immunoglobulin superfamily. The RAGE operated signalling is further involved in activation of AKT signalling in primary murine aortic smooth muscle cells (SMCs), and are involved in phosphorylation of ERK mitogen-activated protein (MAP) kinase in rat C6 astroglial cells [36]. ENPP2 rescue cells from apoptosis during starvation *via* degrading caspase-3, cleaving PARP, and enhancing NIH3TC cell survival *via* activating PI3K and Akt-pathways [37]. Glycoprotein progranuline (PGRN) induces invasion potential in breast cancer cell lines upon LPA stimulation [38]. LPA signalling is not only restricted to proliferation and migration in cancer cells; it is also involved in paracrine actions such as tissue remodeling, angiogenesis, inflammation and tumor progression [39]. Despite the catalytic activity, ENPP2 also performs non-catalytic function inside the cellular periphery; as non-cell autonomous regulator of neuronal cells and affecting the localization and adhesion of neuronal progenitors in a cell-autonomous and non-cell-autonomous manner, independent of its catalytic activity [40].

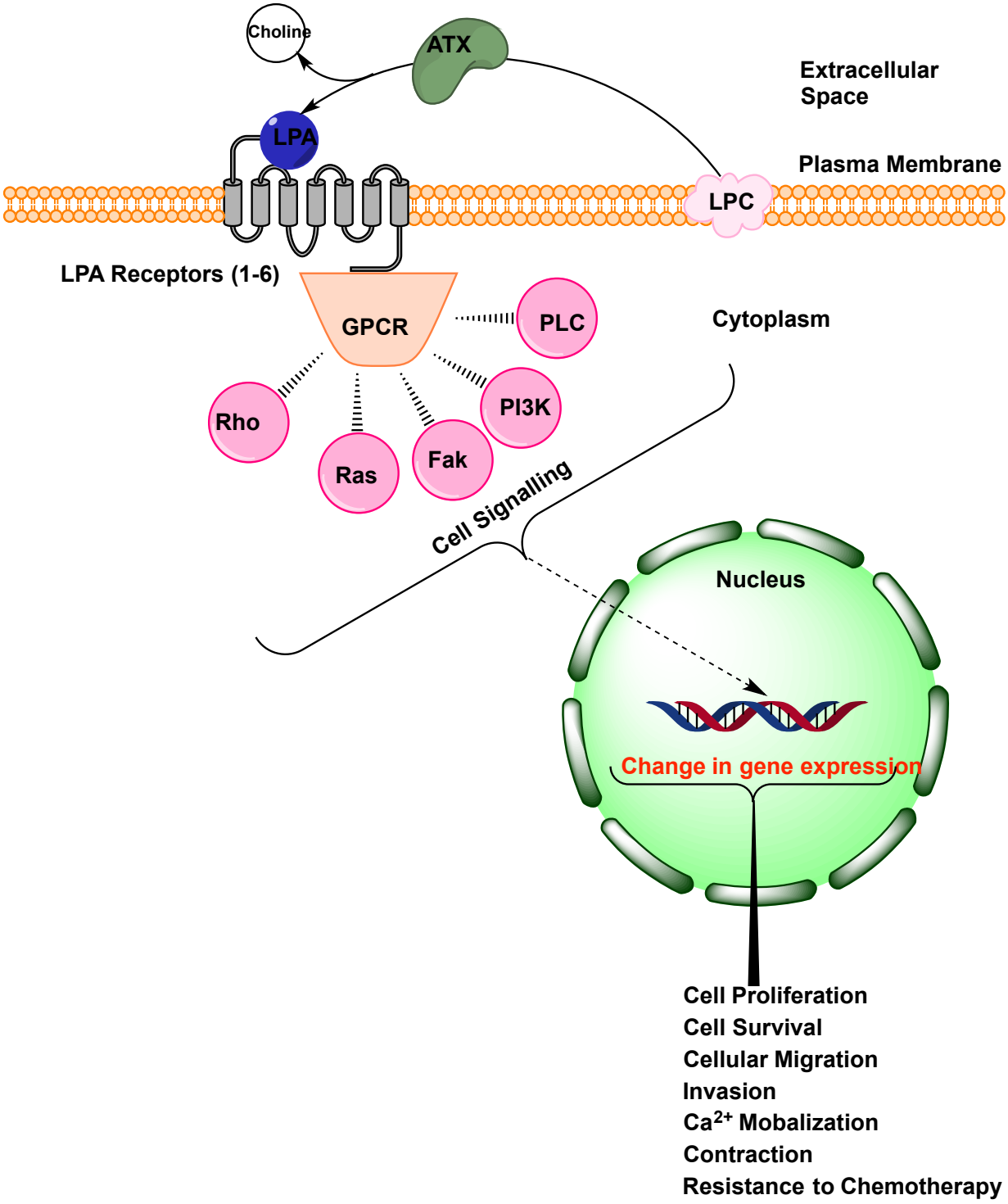

Fig. (3). Signalling mechanism of ENPP2/LPA axis: ENPP2 hydrolyzes the membrane LPC into LPA and choline. LPA induces multifaceted activity *via* various signalling messengers such as Rac, Rho, Ras, PI3K, and PLC *via* six G-protein coupled LPA receptors (LPAR1-LPAR6) and modulates the expression of various genes involves in the various physiological cellular processes.

Regulation of ENPP2 by Key Transcription Factors

Various transcription factors (TFs) can up-regulate/down-regulate the expression of ENPP2 (Table **1**), e.g., Homeobox (Hoxa13) protein, nuclear factor of activated-T cells-1 (NFAT-1) and v-Jun [7]. ENPP2 promoter has four SP1 sites as well as a binding site for the NFAT and nuclear factor kappa B (NF-κB), but no TATA or CAAT boxes [8]. c-Jun enhances the cellular invasion by increasing the expression of ENPP2 [41]. It has been found that proinflammatory cytokine, tumor necrosis factor alpha (TNF-α) promotes ENPP2 expression *via* NF-κB-mediated expression [42]. The integrin α6β4 found to

Table 1. Various interacting partners of ENNP2.

Sr. No.	Interacting Partners	Mechanism/Process Involved	References
1	Cdc42	Cellular Motility	[35]
2	Rac1	Cellular Motility	[27, 35, 107]
3	NF-κB	Promoter binder	[8]
4	RAGE	Signal transduction, Proliferation and Migration	[36]
5	Hoxa13	Transcriptional regulator	[7]
6	NFAT-1	Transcriptional regulator	[7]
7	v-jun	Transcriptional regulator	[7]
8	TNF-α	Upregulation	[42]
9	FGF & EGF	Upregulation	[42]
10	AP-1 & SP	Upregulation	[44]
11	VEGF-A	Upregulation	[45]
12	STAT3	Upregulation	[50]
13	IL-1β & IL-4	Downregulation	[27, 42]
14	IFNγ	Downregulation	[108]

activate the TF NFAT-1, resulting in overexpression of ENPP2 [43]. Fibroblast growth factor (FGF) and epidermal growth factor (EGF) also found to induce ENPP2 expression, whereas cytokine interleukin-1 (IL-1), IL-4 and interferon gamma (IFN-γ) were found to decrease the expression of ENPP2 mRNA in cultured fibroblast-like synoviocytes, SFC [42]. In addition, it has been found that constitutive ENPP2 expression in neuroblastoma cells (SH-SY5Y and SK-NBE) is mediated by TFs like AP-1 and SP through CRE/AP-1-like element [44]. Vascular endothelial factor (VEGF-A) was found to increase the expression of ENPP2, and further modulate the level of LPA that acts *via* the GPCR LPA_4 resulting in change in VEGF responsiveness *via* VEGF receptor 2, finally affecting tumor progression of ovarian cancer cells SKOV3 [45]. ENPP2 and LPA also regulate the migration and homing of CD^+ T cells [46]. In addition, glucose treatment (0.02 M) of prostate cancer cells enhanced the mRNA levels of ENPP2 [47]. ENPP2/LPA also found to induce the expression of osteopontin (OPN), which is further regulated by the PI3K and Akt in hepatic cancer SMMC7721 cell line [48]. ENPP2 was found to promote the secretion of matrix metalloproteinase-3 (MMP3) *via* mitogen-activated protein kinases involving c-Jun and JNK in human fibrosarcoma cell line HT-1080 [49]. In primary breast cancer samples, a positive correlation has been observed between ENPP2 and STAT3, associated with cellular migration, and further suggests that inhibition of STAT3 leads to decrease the level of ENPP2 [50].

ENPP2 IN HUMAN HEALTH

Role in Embryonic Development

Studies in mice and zebrafish uncovered the role of ENPP2 in embryonic development involving vascular and neural development. Disruption of the *ENPP2* gene causes vascular defects in the extraembryonic yolk sac and embryo. These defects result in the intra-uterine death as studied in mice model [51]. ENPP2 deficiency results in the formation of a head cavity in the mouse embryo [52]. ENPP2 is the primary LPA producing enzyme *in vivo* and is also known to be involved in vascular development. ENPP2 deficient mice die at the embryonic day 9.5 with profound vascular defects [9]. ENPP2 also function as a matricellular protein and is reported to be involved in the modulation of oligodendrocyte extracellular interaction and modulation of oligodendrocytes, independent of its catalytic activity, and further this activity of ENPP2 is mediated by the novel functional active MORFO domain [53]. During embryonic development, the ENPP2 expression has been found in the proliferating sub-ventricular and choroid plexus and after birth, ENPP2 is mainly found in white matter areas in the central nervous system (CNS). In the adult brain, ENPP2 is solely expressed in leptomeningeal cells and also in oligodendrocyte precursor cells. Following neurotrauma, ENPP2 is found to be strongly up-regulated in reactive astrocytes adjacent to the lesion [54]. ENPP2 is predominantly present in luteal steroidogenic cells of the corpora luteal follicle of rat ovary [55]. ENPP2 knockdown mice fail to develop the mature blood vessels and resulting in lethality within 10.5 days [56]. ENPP2 inactivation in the early stage of development is also found to cause lethality [57].

Role in Cancer Development

ENPP2 has LysoPLD activity that is primarily involved in the production of bioactive molecule LPA, which is participating in the plethora of physiological and pathophysiological activities through G-protein coupled LPA receptors. LPA signalling plays a crucial

role in breast cancer and cancer related inflammation [1]. Elevated expression of ENPP2 and aberrant activities of LPA are found in several human malignancies in which LPA acts as mitogen and motility factor for several cell types [58]. The ENPP2 overexpression has been observed in non-small lung cancer [59] and in hepatocellular carcinoma [60]. ENPP2 also interacts with hormones, oncogenes, and suppressor genes which are deregulated in exocrine pancreatic cancer and inducing exocrine pancreatic cancer [61]. Molecular studies confirmed that ENPP2 mRNA expression is higher in breast cancer cell lines, such as MDA-MB-435s, MDA-MD-235, MCF7, and HBL-100 as compared to the normal cells contributing to the invasiveness of breast cancer [62]. ENPP2's lysoPLD activity produces LPA, which enhances the cellular motility and invasiveness in tumor cells [63]. Overexpressed ENPP2 was also found to be responsible for enhancing cellular proliferation and migration in thyroid carcinoma cells [64]. Higher serum lysoPLD activity was also observed in the pregnant women during the third trimester [65]. The ENPP2 stimulation causes angiogenesis in ENPP2 mRNA transfected NIH3T3 cells [66]. ENPP2 enhances cellular motility in a Cdc42/Rac1 dependent pathway in which Rac1 and PAK are downstream activators of cell motility that is induced by ENPP2 *via* PI3Kγ signalling pathway. ENPP2 increases the level of Cdc42-GTP, Rac1-GTP and activate PI3K, Rac1 and Cdc42 further phosphorylates the FAK, a non-receptor protein kinase, which plays a critical role in controlling cell migration. However, the necessity of FAK phosphorylation in the motility of tumor cells upon ENPP2 involvement and the exact mechanism of phosphorylation of FAK is not evident [35]. It has been analyzed that LPA activated p21-activated kinase 1 (PAK1) *via* Rac1 and Cdc42 plays a significant role in phosphorylating FAK by LPA as studied in A2058 human melanoma cells and induces cellular motility [67]. A study in rat intestinal epithelial IEC-18 cells found that Ser^{910} is the prominent site of phosphorylation upon LPA induction [68]. LPA stimulated FAK autophosphorylation attributes for cell migration *via* G12/13-RhoA-ROCK signalling pathway [69]. However, there is a contradiction of ROCK involvement in LPA induced cell migration, as another study reported that ROCK is not involved in the LPA induced activation of PAK1 [67].

ENPP2 stimulated LPA signalling was also found to be facilitating the cell motility *via* LPA receptor, LPA_1 [70]. ENPP2 hydrolyzes the phosphospingolipids, producing biologically active S1P that inhibits ENPP2 and LPA mediated cell migration as studied in A2088 and NIH3T3 cells. Sphingosylphosphorylcholine (SPC) also inhibits the ENPP2 induced migration, but not of LPA-mediated. However, SPC inhibits LPA induced migration in the presence of ENPP2. Both S1P and ENPP2+SPC enhances the accumulation of Rho-GTP, which is a cytoskeleton regulator [71]. In addition, ENPP2 also plays an important role in tumorigenesis *via* activation of v-Jun as studied *in vivo* in chick embryo fibroblasts [72]. Furthermore, LPA was also found to regulate the migration behavior of gastric cancer cells in a receptor-specific manner [73]. Overexpressed ENPP2 in glioblastoma contributed for the cell motility *via* LPA1 and LPA receptor signalling cascade [74], and in addition, LPA was also found to induce progression of bone metastasis [75].

Role in Neurological Disorders

Both ENPP2 and LPA play an important role in the development of the nervous system [28]. Mice with *ENPP2* $^{(-/-)}$ embryo showed neural defects [51]. ENPP2 promotes the formation and expansion of oligodendroglial network and play a key role in focal adhesion organization *via* its MORFO domain [24]. TFs AP-1 and SP are involved in the regulation of constitutive expression of ENPP2 in N-*myc* amplified SK-N-BE and non-N-*myc* amplified SH-SY5Y neuroblastoma cells [44]. Neuroblastoma cell lines having N-*myc* locus amplification are prone to higher expression of ENPP2 and upon retinoic acid induced differentiation. However, N-*myc* is not essential for the ENPP2 expression [76]. In primary cultures, LPA induced morphological changes in neuroblastoma morphology is mediated by small GTPase, Rho [77]. LPA induces $G\alpha_{12/13}$ to activate RhoA to induce growth cone collapse and neurite retraction in neuronal cells [78]. LPA signalling involves in the left-right symmetry in the zebrafish embryo through LPA-3 receptor involving Wnt signalling pathway [79]. LPA expression is also found to be increased in multiple sclerosis (MS) patients [80] and higher activity of ENPP2 is also observed in cerebrospinal fluid (CSF) of MS patients [81]. Expression and activity of ENPP2 in the frontal cortices of Alzheimer's type dementia (ATD) indicate ENPP2's role in the pathology of AD [82]. ENPP2 was found to be inducing neuropathic pain *via* LPA and LPA1 receptors [83, 84] and responsible in demyelination in neurons [83]. ENPP2 is also reported to possess counter adhesive property towards the oligodendroglial cells and reported to be involved in the myelination such as oligodendrocyte remodeling *via* modulation of oligodendrocyte-ECM interactions in matricellular fashion [85]. During nerve injury-induced neuropathic pain, ENPP2 mediated levels of LPA production was found to be increased in the ipsilateral sides of the spinal dorsal horn and dorsal roots, but not in the dorsal root ganglion, spinal nerve, or sciatic nerve [86]. However, the demyelination of dorsal root fibers is found to be associated with neuropathic pain [87].

Role in Other Human Diseases

ENPP2 concentration reported to be higher in women than men. In chronic liver disease patients, ENPP2 levels were found to be increased, whereas it was found to be decreased in postoperative prostate cancer patients, but not altered in nephrosis patients [88]. A cohort study conducted on 270 patients indicated that serum ENPP2 levels serve as an indicator of the severity of liver disease and prognosis of cirrhosis patients [89]. Serum ENPP2 activity was also found to be increased in patients of cholestatic and specific for pruritus of cholestatic [90]. ENPP2 also

acts as a novel player in the idiopathic pulmonary fibrosis, and its genetic deletion from bronchial epithelial cells or macrophages was found to attenuate the disease severity [91]. In addition, increased LPA concentrations have been reported in the alveolar space of both idiopathic pulmonary fibrosis patients [91]. It was also reported that LPA induces the motility of high endothelial venule (HEV) and endothelial cells, and promotes the interaction of LPA-HEV and lymphocyte transmigration across the basal lamina of HEVs in steady state [92]. Patients with mild asthma showed an increase in ENPP2 protein in bronchoalveolar lavage fluid and indicating the role of the ENPP2 in allergic lung inflammation [93]. Lipopolysaccharide (LPS), a cell wall component of gram–ve bacteria was found to induce ENPP2 and LPA expression in BV-2 microglial cells [94]. Elevated expression of ENPP2 has been observed in rheumatoid arthritis. ENPP2's mRNA was found to be downregulated by cytokines IL-1β and IL-4, leading to the reduction of 5'-nucleotide phosphodiesterase activity in cell lysates of cultured fibroblast-like synoviocytes (SFC) of patients with rheumatoid arthritis [95]. The metabolically stabilized analog of LPA,1-bromo-3(S)-hydroxy-4-(palmitoyloxy)butyl-phosphonate (BrP-LPA), a dual function inhibitor of ENPP2 and pan-antagonist of LPA receptor was found to attenuate the collagen-induced arthritis development [96].

Overproduction of growth factors, overexpression of genes and hyperactivity of their products lead to some abnormalities and chronic diseases, which eventually lead to health related problems and sometimes decrease the survival life of the organism. ENPP2 expression and activity is also found in a hyper condition in various chronic diseases. Since the ENPP2/LPA axis is involved in tumor progression, angiogenesis, neurological diseases, and other chronic diseases, because of these ENPP2 has been considered as a potential therapeutic target for the treatment of different human diseases. Potent and selective ENPP2 inhibitors would also be useful as research tools for investigating the functions of ENPP2 and LPA. These findings summarized as above advocate for a need to decrease the level of hyperactive ENPP2 using chemical or phytochemical substances/compounds which can be utilized for therapeutic potential.

PHYTOCHEMICAL AND CHEMICAL ENPP2 INHIBITORS

Consistently epidemiological studies are exploring the importance of bioactive non-nutrient plant chemicals (phytochemicals) that are present in fruits, vegetables, and other plant materials in reducing the risk of developing various human chronic diseases such as cancer and cardiovascular diseases [97]. Curcumin is an essential ingredient of Indian diet and known to inhibit the integrin α6β4 [98] and tumor suppressor *CST6,* resulting in decreased transcription level of ENPP2 [1]. Silibinin is a natural flavonoid, which is produced from the milk thistle herb. Silibinin has anti-angiogenic effect [99], and inhibits the Wnt/β-catenin *via* suppressing the LRP6 receptor, a Wnt pathway's co-receptor in prostate and breast cancer cells [100], and decreases the nuclear localization of NFATc1, which is a transcriptional factor of ENPP2 and increases the expression of ENPP2 [101]. Silibinin was also reported for its role in inhibiting angiogenesis [102] and inducible nitric oxide synthase, iNOS [103]. The possible mechanism attributed to this being up-regulation of the p53 level and its downstream product GADD 45α indicating its role in DNA repair [104]. Another phytochemical L-histidine has been reported to inhibit the cellular migration induced by ENPP2, but not of LPA in A2058 and SKOV-3 tumor cells [105].

In addition to phytochemicals as described above various chemical inhibitors for ENPP2 have been developed and studied so far in both *in vitro* and *in vivo* studies such as lipid-based (Fig. **4**), cPA-based (Fig. **5**), Tyrosine-based; small molecule inhibitors (Fig. **6**) [27]. A recent study with a new inhibitor of ENPP2, ONO-8430506 has been reported to be effective in decreasing plasma ENPP2 activity and thus delaying breast tumor growth and lung metastasis in mice [106]. However, more studies are required aiming for the protection against the hyperactive and aggressive behavior and activity of ENPP2 in cancer cells.

SUMMARY AND FUTURE PERSPECTIVE

ENPP2 plays a vital role in various cellular and physiological processes and is crucial for the embryonic development, and *ENPP2* gene deletion also leads to early embryonic lethality. ENPP2 contributes to the vascular development by inducing endothelial migration and invasion. All ENPP2 induced processes are centered around its hydrolytic bioactive signalling product LPA, which is produced by the LysoPLD activity of ENPP2 on the membrane phospholipid, LPC. LPA diversifies the ENPP2 action *via* six LPA receptors that are coupled to G-protein. ENPP2 also produces CPA, which has an antagonistic role to LPA. The altered expression and activity of ENPP2 leads to various human diseases such as inflammation, cancer, fibrosis, rheumatoid arthritis and neuronal defects. Initially, it was discovered as an autocrine factor and identified to play a major role in the cellular migration, proliferation, survival and angiogenesis. ENPP2 induces metastasis in tumor cell mass *via* its invasion potential and causes spreading of tumor cells and angiogenesis in tumor cell mass. Structurally, ENPP2 is composed of the somatomedin domain, phosphodiesterase domain and nuclease like domain that are associated with its diverse cellular functions associated with LysoPLD activity and involved in LPA signalling and role in cellular remolding.

Since its discovery, the functional role of ENPP2 in different homeostatic and pathophysiological conditions, associated with human health has been reported, and its crystal structure is also determined. However, many queries remain to be addressed in its functional network with other accessory interacting

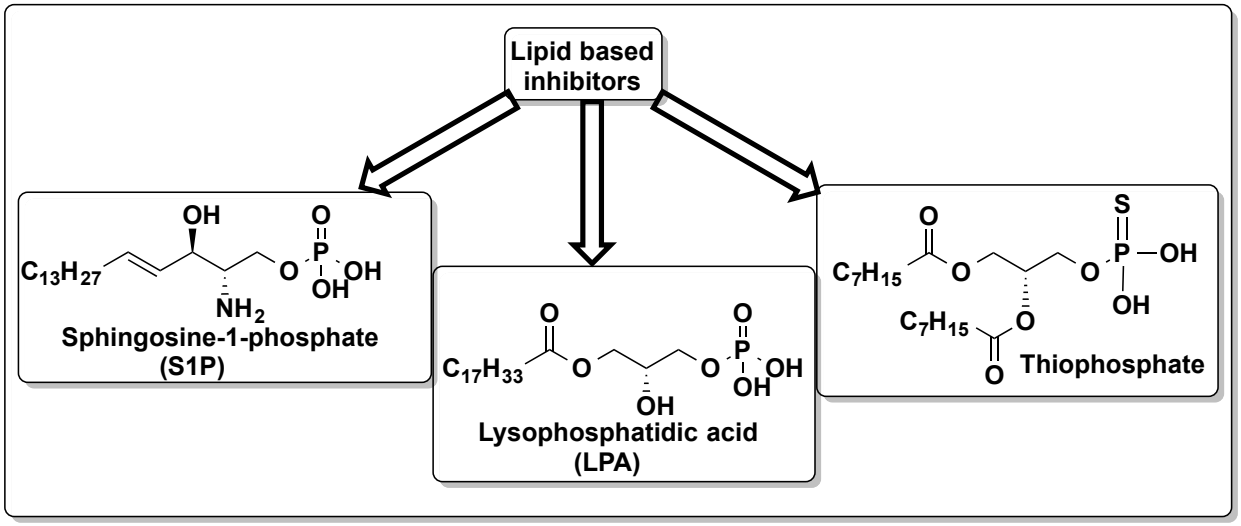

Fig. (4). Lipid based ENPP2 inhibitors.

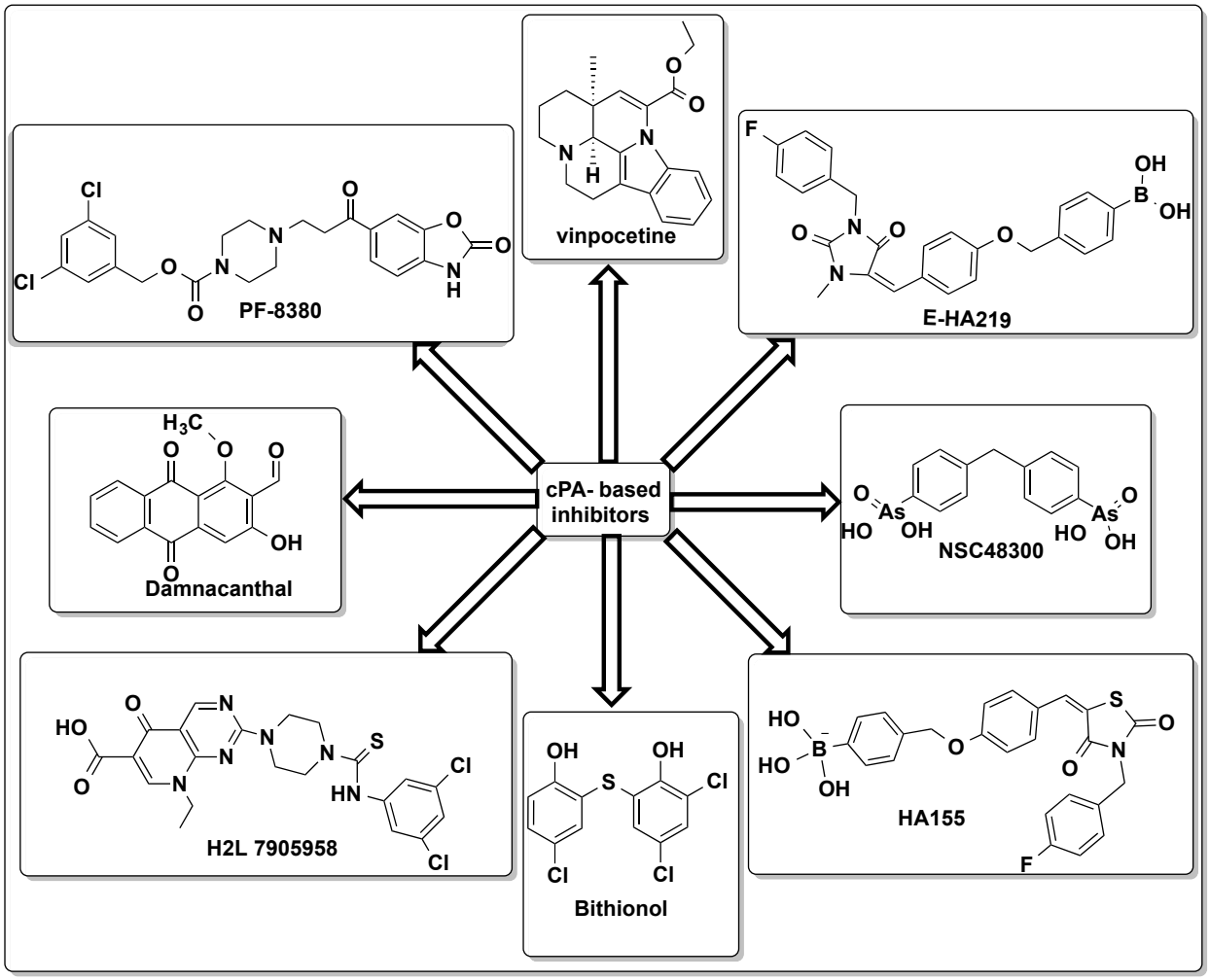

Fig. (5). cPA-based ENPP2 inhibitors.

protein partners, which might be involved in the functional diversification of this protein and the site for therapeutic intervention for various pathophysiological conditions.

In this review, we have discussed recent advances in understanding the structure of ENPP2 and its role in various human diseases. Recent studies have explored the role of ENPP2's bioactive product LPA in physiological and pathological conditions. ENPP2 is

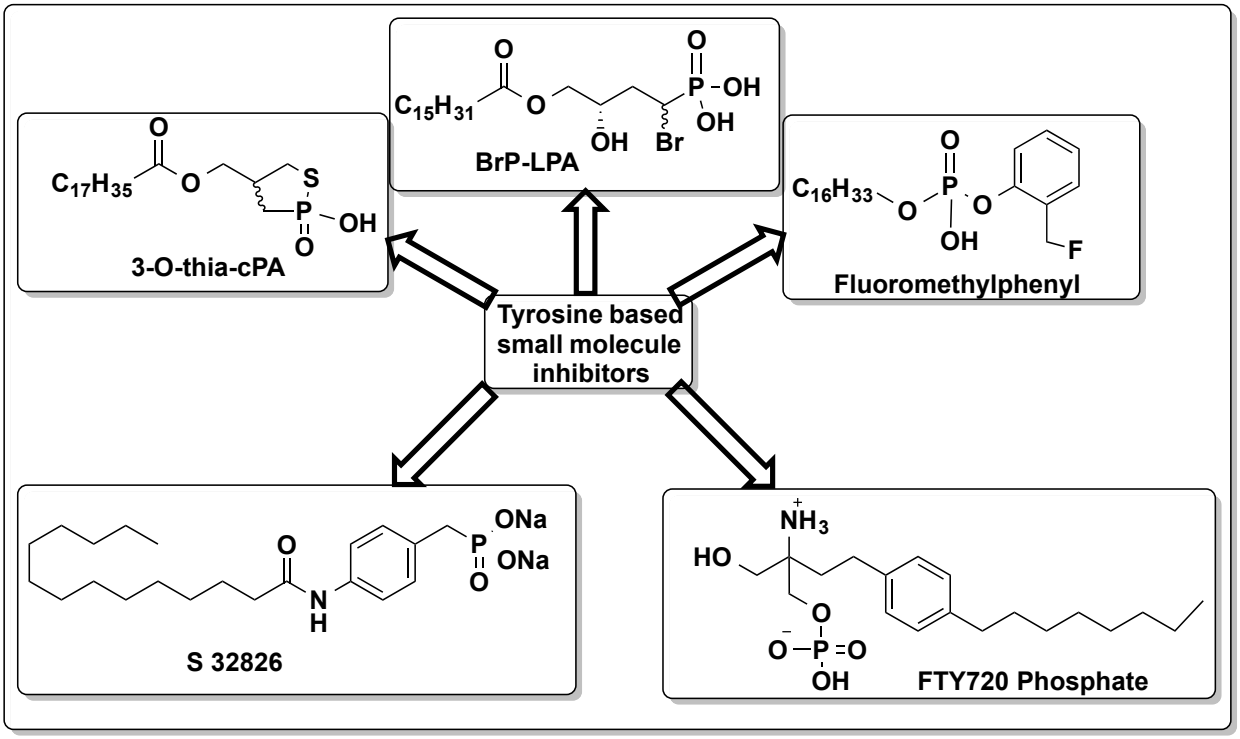

Fig. (6). Tyrosine based small molecule ENPP2 inhibitors.

emerging as an important potential therapeutic target. Protein-protein interaction network of ENPP2 is not fully explored yet during physiological and pathological conditions. There is also a need for the development of specific pharmacological compounds that will inhibit ENPP2's altered expression and activity in various diseases. The development of pharmacological compounds will not only help to understand the role of ENPP2 but also lead to the novel treatment of various human diseases.

ABBREVIATIONS

ATX = Autotaxin

CPA = Cyclic Phosphatidic Acid

EGF = Epidermal growth factor

ENPP2 = Ectonucleotide pyrophosphatase/ phosphodiesterase 2

FGF = Fibroblast growth factor

GPCR = G-protein coupled receptors

Hoxa13 = Homeobox protein

IFNγ = Interferon-gamma

LysoPLD = Lysophospholipase D

MORFO = Modulator of oligodendrocyte remodeling and focal adhesion organization

NF-κB = Nuclear factor kappa B

NFAT-1 = Nuclear factor of activated-T cells-1

NPP2 = Nucleotide pyrophosphatase 2

PD1α. = Phosphodiesterase-1 alpha

Rac 1 = Ras-related C3 botulinum toxin substrate 1

RAGE = Receptor for advanced glycation end products

SMB = Somatomedin B

STAT3 = Signal transducer and activator of transcription 3

TNF-α = Tumor necrosis factor

VEGF = Vascular endothelial growth factor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This research is supported by University Grants Commission (UGC), Govt. of India [under the scheme of start-up grant no. 20-39(12)/2013(BSR)] awarded to A.K.M., and UGC-major grant [F.No.42-676/2013(SR)] awarded to R.K. Because of the limited focus of the article, many relavent and appropriate references could not be included, for which the authors apologize. The CUPB publication number provided for this manuscript is P-151/14.

REFERENCES

[1] Liu S, Murph M, Panupinthu N, Mills GB. ATX-LPA receptor axis in inflammation and cancer. Cell Cycle 2009; 8: 3695-701.

[2] Benesch MG, Zhao YY, Curtis JM, McMullen TP, Brindley DN. Regulation of autotaxin expression and secretion by lysophosphatidate and sphingosine-1-phosphate. J Lipid Res 2015; 56: 1134-44.

[3] Stracke ML, Krutzsch HC, Unsworth EJ*, et al.* Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. J Biol Chem 1992; 267: 2524-9.

[4] Murata J, Lee HY, Clair T*, et al.* cDNA cloning of the human tumor motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases. J Biol Chem 1994; 269: 30479-84.

[5] Kawagoe H, Soma O, Goji J*, et al.* Molecular Cloning and Chromosomal Assignment of the Human Brain-Type Phosphodiesterase I/Nucleotide Pyrophosphatase Gene (PDNP2). Genomics 1995; 30: 380-4.

[6] Stracke M, Arestad A, Levine M, Krutzsch H, Liotta L. Autotaxin is an N linked glycoprotein but the sugar moieties are not needed for its stimulation of cellular motility. Melanoma Res 1995; 5: 203-10.

[7] Hausmann J, Perrakis A, Moolenaar WH. Structure-function relationships of autotaxin, a secreted lysophospholipase D. Adv Biol Regul 2013; 53: 112-7.

[8] Stracke ML, Clair T. Gene Section. http://atlasgeneticsoncology.org 2007; 182-5.

[9] Van Meeteren LA, Ruurs P, Stortelers C*, et al.* Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. Mol Cell Biol 2006; 26: 5015-22.

[10] Tsuda S, Okudaira S, Moriya-Ito K*, et al.* Cyclic phosphatidic acid is produced by autotaxin in blood. J Biol Chem 2006; 281: 26081-8.

[11] Fujiwara Y. Cyclic phosphatidic acid—a unique bioactive phospholipid. Biochim Biophys Acta 2008; 1781: 519-24.

[12] Zimmermann H, Zebisch M, Sträter N. Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 2012; 8: 437-502.

[13] Van Meeteren LA, Moolenaar WH. Regulation and biological activities of the autotaxin–LPA axis. Prog Lipid Res 2007; 46: 145-60.

[14] Koike S, Keino-Masu K, Ohto T, Masu M. The N-terminal hydrophobic sequence of autotaxin (ENPP2) functions as a signal peptide. Genes Cells 2006; 11: 133-42.

[15] Goding JW, Grobben B, Slegers H. Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. Biochim Biophys Acta 2003; 1638: 1-19.

[16] Stefan C, Jansen S, Bollen M. Modulation of purinergic signaling by NPP-type ectophosphodiesterases. Purinergic Signal 2006; 2: 361-70.

[17] Stefan C, Jansen S, Bollen M. NPP-type ectophosphodiesterases: unity in diversity. Trends Biochem Sci 2005; 30: 542-50.

[18] Lee HY, Clair T, Mulvaney PT*, et al.* Stimulation of tumor cell motility linked to phosphodiesterase catalytic site of autotaxin. J Biol Chem 1996; 271: 24408-12.

[19] Aikawa S, Hashimoto T, Kano K, Aoki J. Lysophosphatidic acid as a lipid mediator with multiple biological actions. J Biochem 2014; 157: 81-9.

[20] Berdichevets I, Tyazhelova T, Shimshilashvili KR, Rogaev E. Lysophosphatidic acid is a lipid mediator with wide range of biological activities. Biosynthetic pathways and mechanism of action. Biochemistry (Mosc) 2010; 75: 1088-97.

[21] Koyama M, Nishimasu H, Ishitani R, Nureki O. Molecular dynamics simulation of autotaxin: Roles of the nuclease-like domain and the glycan modification. J Phys Chem B 2012; 116: 11798-808.

[22] Fox MA, Alexander JK, Afshari FS, Colello RJ, Fuss B. Phosphodiesterase-I alpha/autotaxin controls cytoskeletal organization and FAK phosphorylation during myelination. Mol Cell Neurosci 2004; 27: 140-50.

[23] Dennis J, Morgan MK, Graf MR, Fuss B. P2Y12 receptor expression is a critical determinant of functional responsiveness to ATX's MORFO domain. Purinergic Signal 2012; 8: 181-90.

[24] Dennis J, White MA, Forrest AD*, et al.* Phosphodiesterase-Iα/autotaxin's MORFO domain regulates oligodendroglial process network formation and focal adhesion organization. Mol Cell Neurosci 2008; 37: 412-24.

[25] Giganti A, Rodriguez M, Fould B*, et al.* Murine and Human Autotaxin α, β, and γ Isoforms: Gene Organization, Tissue Distribution, and Biochemical Caracterization. J Biol Chem 2008; 283: 7776-89.

[26] Lee HY, Murata J, Clair T*, et al.* Cloning, chromosomal localization, and tissue expression of autotaxin from human teratocarcinoma cells. Biochem Biophys Res Commun 1996; 218: 714-9.

[27] Albers HMHG, Ovaa H. Chemical Evolution of Autotaxin Inhibitors. Chem Rev 2012; 112: 2593-603.

[28] Fotopoulou S, Oikonomou N, Grigorieva E*, et al.* ATX expression and LPA signalling are vital for the development of the nervous system. Dev Biol 2010; 339: 451-64.

[29] Hashimoto T, Okudaira S, Igarashi K*, et al.* Identification and biochemical characterization of a novel autotaxin isoform, ATXδ, with a four-amino acid deletion. J Biochem 2012; 151: 89-97.

[30] Houben AJ, van Wijk XM, van Meeteren LA*, et al.* The polybasic insertion in autotaxin α confers specific binding to heparin and cell surface heparan sulfate proteoglycans. J Biol Chem 2013; 288: 510-9.

[31] Samadi N, Bekele R, Capatos D*, et al.* Regulation of lysophosphatidate signaling by autotaxin and lipid phosphate phosphatases with respect to tumor progression, angiogenesis, metastasis and chemo-resistance. Biochimie 2011; 93: 61-70.

[32] Van Meeteren LA, Ruurs P, Christodoulou E*, et al.* Inhibition of autotaxin by lysophosphatidic acid and sphingosine 1-phosphate. J Biol Chem 2005; 280: 21155-61.

[33] Van Leeuwen F, Giepmans B, Van Meeteren L, Moolenaar W. Lysophosphatidic acid: mitogen and motility factor. Biochem Soc Trans 2003; 31: 1209-12.

[34] Seifert A, Rau S, Küllertz G, Fischer B, Santos AN. TCDD induces cell migration *via* NFATc1/ATX-signaling in MCF-7 cells. Toxicol Lett 2009; 184: 26-32.

[35] Jung ID, Lee J, Yun SY*, et al.* Cdc42 and Rac1 are necessary for autotaxin-induced tumor cell motility in A2058 melanoma cells. FEBS Lett 2002; 532: 351-6.

[36] Rai V, Touré F, Chitayat S*, et al.* Lysophosphatidic acid targets vascular and oncogenic pathways *via* RAGE signaling. J Exp Med 2012; 209: 2339-50.

[37] Song J, Clair T, Noh JH*, et al.* Autotaxin (lysoPLD/NPP2) protects fibroblasts from apoptosis through its enzymatic product, lysophosphatidic acid, utilizing albumin-bound substrate. Biochem Biophys Res Commun 2005; 337: 967-75.

[38] Swamydas M, Nguyen D, Allen LD, Eddy J, Dréau D. Progranulin stimulated by LPA promotes the migration of aggressive breast cancer cells. Cell Commun Adhes 2011; 18: 119-30.

[39] Stortelers C, Kerkhoven R, Moolenaar WH. Multiple actions of lysophosphatidic acid on fibroblasts revealed by transcriptional profiling. BMC Genomics 2008; 9: 1-15.

[40] Greenman R, Gorelik A, Sapir T*, et al.* Non-cell autonomous and non-catalytic activities of ATX in the developing brain. Front Neurosci 2015; 9: 1-17.

[41] Sioletic S, Czaplinski J, Hu L*, et al.* c-Jun promotes cell migration and drives expression of the motility factor ENPP2 in soft tissue sarcomas. J Pathol 2014; 234: 190-202.

[42] Wu JM, Xu Y, Skill NJ*, et al.* Autotaxin expression and its connection with the TNF-alpha-NF-κB axis in human hepatocellular carcinoma. Mol Cancer 2010; 9: 1-14.

[43] Chen M, O'Connor KL. Integrin α6β4 promotes expression of autotaxin/ENPP2 autocrine motility factor in breast carcinoma cells. Oncogene 2005; 24: 5125-30.

[44] Farina AR, Cappabianca L, Ruggeri P*, et al.* Constitutive autotaxin transcription by Nmyc-amplified and non-amplified neuroblastoma cells is regulated by a novel AP-1 and SP-mediated mechanism and abrogated by curcumin. FEBS Lett 2012; 586: 3681-91.

[45] Ptaszynska MM, Pendrak ML, Bandle RW, Stracke ML, Roberts DD. Positive feedback between vascular endothelial growth factor-A and autotaxin in ovarian cancer cells. Mol Cancer Res 2008; 6: 352-63.

[46] Knowlden S, Chapman T, Emo J*, et al.* LPA and autotaxin regulate T cell migration and homing (P5095). J Immunol 2013; 190: 129.12.

[47] Lin C-C, Lee H. High glucose treatment enhances autotaxin and VEGF-C expression in PC-3 human prostate cancer cell (693.20). FASEB J 2014; 28: 693-720.

[48] Zhang R, Zhang Z, Pan X*, et al.* ATX–LPA Axis Induces Expression of OPN in Hepatic Cancer Cell SMMC7721. Anat Rec 2011; 294: 406-11.

[49] Haga A, Nagai H, Deyashiki Y. Autotaxin promotes the expression of matrix metalloproteinase-3 *via* activation of the MAPK cascade in human fibrosarcoma HT-1080 cells. Cancer Invest 2009; 27: 384-90.

[50] Azare J, Doane A, Leslie K*, et al.* Stat3 mediates expression of autotaxin in breast cancer. PLoS One 2011; 6: e27851.

[51] Moolenaar WH, Houben AJ, Lee S-J, van Meeteren LA. Autotaxin in embryonic development. Biochim Biophys Acta 2013; 1831: 13-9.

[52] Koike S, Keino-Masu K, Masu M. Deficiency of autotaxin/lysophospholipase D results in head cavity formation in mouse embryos through the LPA receptor-Rho-ROCK pathway. Biochem Biophys Res Commun 2010; 400: 66-71.

[53] Dennis J, Nogaroli L, Fuss B. Phosphodiesterase-Iα/autotaxin (PD-Iα/ATX): A multifunctional protein involved in central nervous system development and disease. J Neurosci Res 2005; 82: 737-42.

[54] Savaskan N, Rocha L, Kotter M*, et al.* Autotaxin (NPP-2) in the brain: cell type-specific expression and regulation during development and after neurotrauma. Cell Mol Life Sci 2007; 64: 230-43.

[55] Masuda K, Haruta S, Orino K, Kawaminami M, Kurusu S. Autotaxin as a novel, tissue-remodeling-related factor in regressing corpora lutea of cycling rats. FEBS J 2013; 280: 6600-12.

[56] Tanaka M, Okudaira S, Kishi Y*, et al.* Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid. J Biol Chem 2006; 281: 25822-30.

[57] Ferry G, Giganti A, Cogé F, *et al.* Functional invalidation of the autotaxin gene by a single amino acid mutation in mouse is lethal. FEBS Lett 2007; 581: 3572-8.

[58] Houben AJ, Moolenaar WH. Autotaxin and LPA receptor signaling in cancer. Cancer Metastasis Rev 2011; 30: 557-65.

[59] Yang Y, Mou L-j, Liu N, Tsao M-S. Autotaxin expression in non–small-cell lung cancer. Am J Respir Cell Mol Biol 1999; 21: 216-22.

[60] Zhang G, Zhao Z, Xu S, Ni L, Wang X. Expression of autotaxin mRNA in human hepatocellular carcinoma. Chin Med J 1999; 112: 330-2.

[61] Kadekar S, Silins I, Korhonen A*, et al.* Exocrine pancreatic carcinogenesis and autotaxin expression. PLoS One 2012; 7: e43209.

[62] Lee HY, Bae G-U, Jung ID*, et al.* Autotaxin promotes motility *via* G protein-coupled phosphoinositide 3-kinase γ in human melanoma cells. FEBS Lett 2002; 515: 137-40.

[63] Umezu-Goto M, Kishi Y, Taira A*, et al.* Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 2002; 158: 227-33.

[64] Kehlen A, Englert N, Seifert A*, et al.* Expression, regulation and function of autotaxin in thyroid carcinomas. Int J Cancer 2004; 109: 833-8.

[65] Tokumura A, Majima E, Kariya Y*, et al.* Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. J Biol Chem 2002; 277: 39436-42.

[66] Nam SW, Clair T, Kim Y-S*, et al.* Autotaxin (NPP-2), a metastasis-enhancing motogen, is an angiogenic factor. Cancer Res 2001; 61: 6938-44.

[67] Jung ID, Lee J, Lee KB*, et al.* Activation of p21-activated kinase 1 is required for lysophosphatidic acid-induced focal adhesion kinase phosphorylation and cell motility in human melanoma A2058 cells. Eur J Biochem 2004; 271: 1557-65.

[68] Jiang X, Sinnett-Smith J, Rozengurt E. Differential FAK phosphorylation at Ser-910, Ser-843 and Tyr-397 induced by angiotensin II, LPA and EGF in intestinal epithelial cells. Cell Signal 2007; 19: 1000-10.

[69] Bian D, Mahanivong C, Yu J*, et al.* The G12/13-RhoA signaling pathway contributes to efficient lysophosphatidic acid-stimulated cell migration. Oncogene 2005; 25: 2234-44.

[70] Hama K, Aoki J, Fukaya M*, et al.* Lysophosphatidic acid and autotaxin stimulate cell motility of neoplastic and non-neoplastic cells through LPA1. J Biol Chem 2004; 279: 17634-9.

[71] Clair T, Aoki J, Koh E*, et al.* Autotaxin hydrolyzes sphingosylphosphorylcholine to produce the regulator of migration, sphingosine-1-phosphate. Cancer Res 2003; 63: 5446-53.

[72] Black EJ, Clair T, Delrow J, Neiman P, Gillespie DA. Microarray analysis identifies Autotaxin, a tumour cell motility and angiogenic factor with lysophospholipase D activity, as a specific target of cell transformation by v-Jun. Oncogene 2003; 23: 2357-66.

[73] Shida D, Kitayama J, Yamaguchi H*, et al.* Dual mode regulation of migration by lysophosphatidic acid in human gastric cancer cells. Exp Cell Res 2004; 301: 168-78.

[74] Kishi Y, Okudaira S, Tanaka M*, et al.* Autotaxin is overexpressed in glioblastoma multiforme and contributes to cell motility of glioblastoma by converting lysophosphatidylcholine to lysophosphatidic acid. J Biol Chem 2006; 281: 17492-500.

[75] Peyruchaud O, Leblanc R, David M. Pleiotropic activity of lysophosphatidic acid in bone metastasis. Biochim Biophys Acta 2013; 1831: 99-104.

[76] Dufner-Beattie J, Lemons R, Thorburn A. Retinoic acid–induced expression of autotaxin in N-myc–amplified neuroblastoma cells. Mol Carcinog 2001; 30: 181-9.

[77] Fukushima N, Weiner JA, Chun J. Lysophosphatidic acid (LPA) is a novel extracellular regulator of cortical neuroblast morphology. Dev Biol 2000; 228: 6-18.

[78] Kranenburg O, Poland M, van Horck FP*, et al.* Activation of RhoA by lysophosphatidic acid and Gα12/13 subunits in neuronal cells: induction of neurite retraction. Mol Biol Cell 1999; 10: 1851-7.

[79] Lai S-L, Yao W-L, Tsao K-C*, et al.* Autotaxin/Lpar3 signaling regulates Kupffer's vesicle formation and left-right asymmetry in zebrafish. Development 2012; 139: 4439-48.

[80] Balood M, Zahednasab H, Siroos B*, et al.* Elevated serum levels of lysophosphatidic acid in patients with multiple sclerosis. Hum Immunol 2014; 75: 411-3.

[81] Zahednasab H, Balood M, Harirchian MH*, et al.* Increased autotaxin activity in multiple sclerosis. J Neuroimmunol 2014; 273: 120-3.

[82] Umemura K, Yamashita N, Yu X*, et al.* Autotaxin expression is enhanced in frontal cortex of Alzheimer-type dementia patients. Neurosci Lett 2006; 400: 97-100.

[83] Inoue M, Xie W, Matsushita Y*, et al.* Lysophosphatidylcholine induces neuropathic pain through an action of autotaxin to generate lysophosphatidic acid. Neuroscience 2008; 152: 296-8.

[84] Ma L, Matsumoto M, Xie W, Inoue M, Ueda H. Evidence for lysophosphatidic acid 1 receptor signaling in the early phase of neuropathic pain mechanisms in experiments using Ki-16425, a lysophosphatidic acid 1 receptor antagonist. J Neurochem 2009; 109: 603-10.

[85] Fox MA, Colello RJ, Macklin WB, Fuss B. Phosphodiesterase-Iα/autotaxin: a counteradhesive protein expressed by oligodendrocytes during onset of myelination. Mol Cell Neurosci 2003; 23: 507-19.

[86] Ma L, Uchida H, Nagai J*, et al.* Evidence for de novo synthesis of lysophosphatidic acid in the spinal cord through phospholipase A2 and autotaxin in nerve injury-induced neuropathic pain. J Pharmacol Exp Ther 2010; 333: 540-6.

[87] Nagai J, Uchida H, Matsushita Y*, et al.* Autotaxin and lysophosphatidic acid1 receptor-mediated demyelination of dorsal root fibers by sciatic nerve injury and intrathecal lysophosphatidylcholine. Mol Pain 2010; 6: 1-11.

[88] Nakamura K, Igarashi K, Ide K*, et al.* Validation of an autotaxin enzyme immunoassay in human serum samples and its application to hypoalbuminemia differentiation. Clin Chim Acta 2008; 388: 51-8.

[89] Pleli T, Martin D, Kronenberger B*, et al.* Serum Autotaxin Is a Parameter for the Severity of Liver Cirrhosis and Overall Survival in Patients with Liver Cirrhosis--A Prospective Cohort Study. PLoS One 2014; 9: e103532.

[90] Kremer AE, van Dijk R, Leckie P*, et al.* Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions. Hepatology 2012; 56: 1391-400.

[91] Oikonomou N, Mouratis M-A, Tzouvelekis A*, et al.* Pulmonary autotaxin expression contributes to the pathogenesis of pulmonary fibrosis. Am J Respir Cell Mol Biol 2012; 47: 566-74.

[92] Bai Z, Cai L, Umemoto E*, et al.* Constitutive lymphocyte transmigration across the basal lamina of high endothelial venules is regulated by the autotaxin/lysophosphatidic acid axis. J Immunol 2013; 190: 2036-48.

[93] Park GY, Lee YG, Berdyshev E*, et al.* Autotaxin production of lysophosphatidic acid mediates allergic asthmatic inflammation. Am J Respir Crit Care Med 2013; 188: 928-40.

[94] Awada R, Saulnier-Blache JS, Grès S*, et al.* Autotaxin Downregulates LPS–Induced Microglia Activation and Pro-Inflammatory Cytokines Production. J Cell Biochem 2014; 115: 2123-32.

[95] Kehlen A, Lauterbach R, Santos A*, et al.* IL-1β-and IL-4-induced down-regulation of autotaxin mRNA and PC-1 in fibroblast-like synoviocytes of patients with rheumatoid arthritis (RA). Clin Exp Immunol 2001; 123: 147-54.

[96] Nikitopoulou I, Kaffe E, Sevastou I*, et al.* A metabolically-stabilized phosphonate analog of lysophosphatidic acid attenuates collagen-induced arthritis. PLoS One 2013; 8: e70941.

[97] Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 2004; 134: 3479S-85S.

Received: February 23, 2015 Revised: May 18, 2015 Accepted: June 10, 2015

[98] Im Kim H, Huang H, Cheepala S, Huang S, Chung J. Curcumin inhibition of integrin (α6β4)-dependent breast cancer cell motility and invasion. Cancer Prev Res 2008; 1: 385-91.

[99] Vakili MN, Mirzayi H, FH Shirazi N. Studying silibinin effect on human endothelial and hepatocarcinoma cell lines. Res Pharm Sci 2012; 7: S174.

[100] Lu W, Lin C, King TD*, et al.* Silibinin Inhibits Wnt/β-catenin Signaling by Suppressing Wnt Co-receptor LRP6 Expression in Human Prostate and Breast Cancer Cells. Cell Signal 2012; 24: 2291-6.

[101] Kavitha CV, Deep G, Gangar SC*, et al.* Silibinin inhibits prostate cancer cells-and RANKL-induced osteoclastogenesis by targeting NFATc1, NF-κB, and AP-1 Activation in RAW264. 7 cells. Mol Carcinog 2012; 53: 169-80.

[102] Velmurugan B, Gangar SC, Kaur M*, et al.* Silibinin exerts sustained growth suppressive effect against human colon carcinoma SW480 xenograft by targeting multiple signaling molecules. Pharm Res 2010; 27: 2085-97.

[103] Chittezhath M, Deep G, Singh RP, Agarwal C, Agarwal R. Silibinin inhibits cytokine-induced signaling cascades and down-regulates inducible nitric oxide synthase in human lung carcinoma A549 cells. Mol Cancer Ther 2008; 7: 1817-26.

[104] Roy S, Deep G, Agarwal C, Agarwal R. Silibinin prevents ultraviolet B radiation-induced epidermal damages in JB6 cells and mouse skin in a p53-GADD45α-dependent manner. Carcinogenesis 2012; 33: 629-36.

[105] Clair T, Koh E, Ptaszynska M*, et al.* L-histidine inhibits production of lysophosphatidic acid by the tumor-associated cytokine, autotaxin. Lipids Health Dis 2005; 4: 1-13.

[106] Benesch MG, Tang X, Maeda T*, et al.* Inhibition of autotaxin delays breast tumor growth and lung metastasis in mice. FASEB J 2014; 28: 2655-66.

[107] Hoelzinger DB, Nakada M, Demuth T*, et al.* Autotaxin: a secreted autocrine/paracrine factor that promotes glioma invasion. J Neurooncol 2008; 86: 297-309.

[108] Santos AN, Riemann D, Santos AN*, et al.* Treatment of fibroblast-like synoviocytes with IFN-γ results in the down-regulation of autotaxin mRNA. Biochem Biophys Res Commun 1996; 229: 419-24.