Fosfolipasi A$_2$: nuove lezioni dallo studio delle cellule endoteliali

Lectio Magistralis in Occasion of the Opening Ceremony of the Accademia Gioenia CLXXXVI
Year February 26th, 2010

MARIO ALBERGHINA

Department of Biochemistry, University of Catania, Viale Andrea Doria 6, 95125 Catania, Italy.
email address: malber@unict.it

RIASSUNTO

La ricerca sugli enzimi intracellulari fosfolipasici A$_2$ (PLA$_2$) nelle cellule endoteliali (ECs) è interessante per svelare i meccanismi di processi biologici come l’angiogenesi, l’adesione e la tras migrazione di cellule infiammatorie attraverso l’endotelio, l’aterogenesi, la formazione e il mantenimento della barriera emato-encefalica ed emato-retinica, e la progressione dei tumori. Ad oggi, solo informazioni riguardanti la funzione e la regolazione di tre ben caratterizzate fosfolipasi, la PLA$_2$ Ca$^{2+}$-dipendente citosolica (cPLA$_2$), la PLA$_2$ Ca$^{2+}$-independente (iPLA$_2$) e la PLA$_2$ secretoria (sPLA$_2$), lungo i cammini intracellulari di segnalazione biochimica, sono disponibili per le cellule endoteliali quiescenti e/o proliferanti. Le fosfolipasi A$_2$ possono essere potenzialmente coinvolte nella cascata del segnale per la quale le ECs promuovono la formazione e il mantenimento di complessi altamente organizzati, costituiti da endotelio, periciti cerebrali e astrocyti, sia di costituire cellule della microvascolarizzazione tumorale. Questa rassegna intende riassumere i recenti ritrovamenti sulla presenza e possibili ruolo attribuito alle fosfolipasi A$_2$ in una varietà di linee cellulari endoteliali, come pure di linee cellulari tumorali, mantenuta in coltura da sole, ove in sistemi di co-coltura nei quali la trasduzione del segnale e i meccanismi “cross-talk” tra cellule tumorali e ECs, largamente non identificati, cominciano ad essere svelati.

Parole chiave: Angiogenesi; Acido arachidonico; Aterosclerosi; Cellule endoteliali; MAP chinasi; Periciti; Fosfolipasi A$_2$; Prostaglandine; Cellule tumorali.

SUMMARY

Phospholipase A$_2$: new lessons from endothelial cells

The investigation of intracellular phospholipase A$_2$ (PLA$_2$) enzymes in endothelial cells (ECs) seems interesting because it may contribute to unveil the mechanisms of biological processes such as angiogenesis, adhesion and transmigration of inflammatory cells, atherogenesis, blood brain barrier and tumor progression. To date, limited information is available regarding the function and regulation of three well characterized phospholipases, Ca$^{2+}$-dependent cytosolic PLA$_2$ (cPLA$_2$), Ca$^{2+}$-independent PLA$_2$ (iPLA$_2$) and secretory PLA$_2$ (sPLA$_2$) along the intracellular signaling pathways in quiescent and proliferating ECs. PLA$_2$s could be potentially involved in signaling cascades by which ECs promote the highly organized multicellular complexes consisting of either an endothelium, brain pericytes and astrocytes, or cellular
constituents of the tumor microvasculature. This review will summarize recent findings on the presence and possible role attributed to PLA₂s in a variety of EC lines grown alone, as well as in isolated cancer cell lines, or in co-cultures in which signal transduction and cross-talk mechanisms between tumor cells and ECs, largely undefined, begin to be unravelled.

**Keywords:** Angiogenesis; Arachidonic acid; Atherosclerosis; Endothelial cells; MAP kinases; Pericytes; Phospholipase A₂; Prostaglandins; Tumor cells.

**Introduction**

Endothelial cells (ECs) form the innermost layer of blood vessels and play a major role in the active transport of molecules and hormones, degradation of lipoproteins, blood pressure regulation and fibrinolysis. Much progress has been made in elucidating the molecular mechanisms of such a role in physiological and pathological conditions, and rapid advances have been facilitated by the availability of culture and co-culture systems used to model the proliferation, motility and cellular interactions *in vitro*. An increased demand for representative models concerns the study of angiogenesis, blood coagulation, atherogenesis, adhesion and transmigration of inflammatory cells as well as intra- or intercellular signaling and transport routes across blood vessel walls. Structural and biochemical heterogeneity in ECs as a function of position along the vasculature, from artery to vein and microvasculature tree, is well recognized.

Among intracellular signaling pathways known so far, the involvement of phospholipase A₂ enzymes seems interesting as a key control point for mechanisms of the biological processes mentioned above. Few studies are focused on the role of endothelial phospholipases A₂ (PLA₂) as starring enzymatic molecules in processes associated with EC functions. Many EC activities, in fact, are dependent on the release of lipid mediators produced by these cells. The present review addresses recent findings on the role of PLA₂ in quiescent and stimulated cells isolated from the vascular system, particularly brain microvessel endothelial cells, and aims to elucidate regulatory pathways of signal transduction mediated by PLA₂ from membrane receptors to nucleus. Plenty of studies are devoted to PLA₂ activities and functions in leukocytes, macrophages, platelets, smooth muscle cells, myocytes, epithelial cells, preadipocytes, lymphocytes, mesangial cells, fibroblasts, CHO, COS-1 and MDCK cells, chondrocytes, astrocytes, neurons, and in a variety of other cells, but very few are focussed to ECs.

Three well characterized phospholipases, cytosolic PLA₂ (cPLA₂ or Group IV PLA₂), Ca²⁺-independent intracellular PLA₂ (iPLA₂ or Group VI PLA₂) and Ca²⁺-dependent secretory PLA₂ (sPLA₂ or Group II PLA₂) differ from each other in terms of substrate specificity, Ca²⁺ requirement and lipid modification. The main role they play is the liberation of arachidonic acid (AA) from membrane phospholipids (Burke and Dennis, 2009). All are known in several isoforms which show substantial differences in amino acid sequence and peptide motifs. Their subcellular localization and differential regulation in various cell types and under different physiological and pathological conditions still remain elusive.

Group VIA iPLA₂ activity, for which enzyme a housekeeping role in phospholipid homeostasis and remodeling was originally suggested, is involved in the regulated release of
AA in ECs (Rastogi and McHowat, 2009), and is linked to the regulation of endothelial cell proliferation (Herbert and Walker, 2006; Anfuso et al., 2009; Alberghina M., 2010). These data indicate that for iPLA₂ a signaling function is now an acquired concept.

RT-PCR and Western blot analyses demonstrated the presence of Group V sPLA₂, whereas group II sPLA₂ was undetected in quiescent ECs (Bernatchez et al., 2001). This last observation was confirmed by our own data in immortalized GP8.3 ECs from rat brain (Anfuso et al., 2009). However, sPLA₂-IIA gene expression and enzyme secretion are induced in rat ECs by cytokines (Schwemmer et al., 2001).

Today, it is necessary to identify upstream events that occur upon cell activation, particularly target protein, intracellular trafficking or metabolic pathways which account for the AA liberation, a required step for prostanoid and eicosanoid biosynthesis, in inflammation, tumor progression and angiogenesis. An understanding of the regulation of PLA₂ expression under these conditions would illuminate the cellular processes in which the enzymes participate and the possible therapeutic interventions.

**General properties of PLA₂ enzymes**

Several new phospholipases A₂, which differ drastically from the classic, low molecular weight digestive enzyme, have been identified and classified. Cytosolic PLA₂ and iPLA₂ are ubiquitously present and active in mammalian cells, whereas sPLA₂ may be silent or not expressed in quiescent cells. Even if PLA₂-s seem to catalyze an identical reaction on sn-2 position of phospholipid backbone, they are committed to different roles as mediators of cellular responses when activated, and their expression induced by a wide array of agonists, growth factors, prooxidants and apoptosis inducers. Extracellular stimuli, particularly oxidized LDL, β-amyloid peptides and growth factors alter the levels of PLA₂ mRNA and protein (Lupo et al., 2005).

By using specific inhibitors of kinases, the signaling cascade involved in the activation of cPLA₂ by mitogen activated protein kinases (MAPKs) has been well established. It has been thus demonstrated that p44/p42 MAPK phosphorylates cPLA₂ and increases its activity in cells and tissues. The phosphorylation of cPLA₂ at Ser-505 occurs concomitantly with the increase in intracellular Ca²⁺ that facilitates the binding of cPLA₂ lipid domain to phospholipids, promoting its translocation to cellular membranes (ER, Golgi and nuclear envelope) and AA release. Ceramide-1-phosphate (C1P) has been shown to be an allosteric activator of cPLA₂-α (Pettus et al., 2004). The interaction site for C1P has been localized to the cationic b-groove of the C2 domain of the enzyme (Arg57/Lys58/Arg59). Cross-talk between cPLA₂ and exogenous sPLA₂ seems a quite common phenomenon in stimulated cells, an event that deserves particular attention in view of the potential contribution of the latter to vascular diseases. To date, there is limited information available regarding the regulation of iPLA₂ and sPLA₂ along the intracellular signaling pathways.

By using mainly PKCα inhibitors, activation, and even phosphorylation of iPLA₂ by PKC, has been reported in several studies on macrophages and monocytes (Akiba and Sato, 2004; Tay and Melendez, 2004). Interactions between the two enzymes may be directed to several downstream targets. Whether PKC directly activated iPLA₂ by phosphorylation or indirectly activated iPLA₂ by phosphorylating a regulatory protein is not currently known (Meyer et al., 2005). In our studies with ECs we have also suggested that PKCα is a factor regulating iPLA₂ activation (Lupo et al., 2005), even if neither evidence for a direct interaction between the two proteins nor iPLA₂ phosphorylation was observed. More likely intermediate kinases or regulatory factors are required for optimal activity. PKCα in the membrane phosphorylates a number of proteins involved in various signal transduction processes,
including cPLA2 in activated human monocytes, at least in vitro (Li et al., 2007). Even if in in vitro assay, phosphorylation of cPLA2 by recombinant PKC has been demonstrated (Li et al., 2007), that this reaction occurs in in vivo conditions remains a controversial issue.

Free AA is oxidized to prostaglandins by cyclooxygenases, hydroxyeicosanoic acid by lipoxygenases, or epoxyeicosatrienoic acids by P450-dependent epoxygenase. Lysophospholipid, the other reaction product of PLA2 action, is in some settings the precursor of PAF which is another potent inflammatory mediator. Considering that all PLA2 enzymes sit at the beginning of the AA and lysophospholipid cascade, they have attracted attention as a target for controlling inflammation and cancer. In this context, the suppression of the production of phospholipid–derived lipid mediators has been long considered for therapeutic purposes. Several PLA2 enzyme inhibition therapy approaches (with small molecules) have shown antitumor efficacy in experimental studies; for instance the first clinical trials for treatment of malignant glioma were conducted in the 1990s. Given several drawbacks to inhibition of the eicosanoid production, the use of PLA2 inhibitors has not been conceived any longer as a useful strategy in the treatment of inflammation-related diseases and tissue injury. More attention has been paid to design molecules inhibiting receptors for lipid mediators downstream of liberated AA. It has also been proposed that early effects in ECs or tumor cells are mediated via cell surface receptors, whereas long-term responses are dependent upon intracellular receptor interactions (Norel, 2007; Wang and Klein, 2007; Kaneshiro et al., 2009).

Co-culture system mimicking blood-brain barrier (BBB)

The behavior of ECs strongly differs depending on their vascular origin. Endothelial cells present in microcapillaries of the brain, retina and nerves, contributing to counteract blood extravasation, and thus forming blood barriers, have at least three properties which distinguish them from their peripheral counterparts: continuous strands of tight junctions, low rate of endocytosis, and the presence of specific carriers and transport molecules. Most in vitro studies of capillary permeability focus on endothelial cell monolayers, and ignore the second cell type that forms the capillary wall: the microvascular pericyte. However, pericytes are perivascular cells with multifunctional activities which nowadays are being elucidated (Edelman et al., 2006).

To mimic atherosclerosis, inflammation, angiogenesis processes and blood-brain barrier function, binary co-cultures of EC monolayers with astrocytes, pericytes, smooth muscle cells, fibroblasts, T-lymphocytes, neutrophils, NK cells, 10T1/2 mesenchymal cells, retinal Müller cells were so far established. Very few of them have been undertaken aimed at evaluating the cell-cell and intracellular signaling mechanisms by which ECs promote the highly organized multicellular complex consisting of an endothelium, brain pericytes and astrocytes. The recent characterization of three-cell type in vitro models of BBB, i.e. rat brain ECs-astrocytes-neurons (Schiera et al., 2005) or rat brain ECs-astrocytes-pericytes (Nakagawa et al., 2009), paves the way to the novel evaluation of (i) functional relevance of interacting cells in a more realistic setting of BBB, and (ii) analyses of “in concert” biochemical events under strictly controlled conditions in the neurovascular unit of cerebral microvessels.

In a model of cell co-culture with retinal pericytes lasting for 24 h, rat brain ECs showed an increase in AA release and protein expression of cPLA2 and its phosphorylated form, as well as iPLA2 (Anfuso et al., 2007). No activation of the same enzymes was seen in companion pericytes. In ECs, the protein levels of PKCα and its phosphorylated form and phosphorylated extracellular signal-regulated kinase ERK1/2 were significantly enhanced. Inhibitors of upstream kinases (the PI3-kinase/Akt/PDK1 or MEK-1 pathways) markedly attenuated AA release and the expression of phosphorylated forms of endothelial cPLA2, PKCα,
and ERK1/2. By confocal microscopy, activation of PKCα in perinuclear regions of ECs grown in co-culture as well as strong activation of cPLA2 in ECs taken from a model of mixed culture was clearly observed. Thus, a sequential activation of PKCα contributes to endothelial ERK1/2 and cPLA2 phosphorylation induced by either soluble factors or direct cell-to-cell contact, and the PKCα-cPLA2 pathway appears to play a key role in the early phase of endothelial cell-pericyte interactions regulating blood retina or blood brain barrier maturation.

Expression of PLA2 in inflammation and atherosclerosis

ECs serve as a relevant model system for identifying lipid regulatory pathways in such inflammatory diseases as atherosclerosis, allergy, diabetes, multiple sclerosis or rheumatoid arthritis associated with neovascularization. Since all PLA2 enzymes hydrolyze phospholipids to produce inflammatory lipid mediators, it is generally accepted that the different PLA2 isoforms, and specifically the cPLA2 and sPLA2, take part in inducing vascular inflammatory processes. Many reviews describing the contribution of PLA2 enzymes to inflammation are available (Capper and Marshall, 2001; Oestvang and Johansen, 2006).

In considering the inflammatory role of PLA2s, it should be noted that these enzymes have long been assumed to be the major contributor of AA in monocyte/macrophages, neutrophils, eosinophils, mast cells and astrocytes. However, in ECs, apart from the numerous studies addressing the lipolytic activity triggered by inflammatory agents such as LPS, TNFα, IL-1, IL-6, IFNγ, phorbol esters, proteases and cyclic AMP-elevating agents, the association between endothelial PLA2s and the progression of atherosclerosis or acute inflammatory lung injury (Rastogi and McHowat, 2009) remains undetermined. More studies will be necessary to sort out the exact role of PLA2 family members in the production of important proinflammatory mediators after suitable EC stimulation, but neither the action of endogenous endothelial PLA2s in leukocyte adherence at the site of vascular injury nor the endothelial expression/production of proinflammatory serum lipoprotein-associated PLA2 (Lavi et al., 2007) has been studied up to now. In a solitary study, the bioactive lipid lysophosphatidic acid, acting through its receptors as an autocrine/paracrine factor and produced either in serum or in the cytosol by lysophospholipase D from lysophosphatidylcholine (deacylation end-product of PLA2 activities), has been proved to play a role on EC-leukocyte interactions (Rizza et al., 1999). A dose-dependent increase in the binding of monocytes, neutrophils, and HL60 to HAECs, facilitated by the increased endothelial cell surface expression of E-selectin and VCAM-1, was observed.

Among the mediators involved in inflammation and artery diseases are secretory PLA2s (Group II). Exogenous sPLA2 mediates endothelial cell proliferation, cell migration, hormone and growth factor release, eicosanoid and lysophosphatidylcholine production from phospholipids via its receptors in peripheral tissues. In human atherosclerotic plaques (the major cellular components of which are smooth muscle cells and macrophages), the presence and increased levels of secretory non pancreatic Group II and X PLA2, capable of hydrolyzing low-density lipoproteins and lipid accumulation in human monocyte-derived macrophages, have been detected (Oestvang and Johansen, 2006; Karabina et al., 2006).

Another class of lipid signals that may mediate MAPK activation in ECs in tandem with Group VI PLA2 is given by oxidized-low density lipoprotein (Ox-LDL). Ox-LDL results when reactive oxygen species oxidize the lipid constituents of LDL. Ox-LDL induces p42/p44 and p38 expression in vascular smooth muscle cells and macrophages using PKC and GPCR-mediated pathways. Few studies exist linking Group VI PLA2 to Ox-LDL. The one study that could be found suggested that Ox-LDL activates Group VI PLA2 and increases its expression in correlation with activation of MAPKs (Lupo et al., 2005).
Expression of PLA₂s in tumor cell lines

Cytosolic PLA₂α plays a role as important intracellular signal activated by inflammatory mediators in the pathogenesis of many human cancers (for a review, see Nakanishi and Rosenberg, 2006). C6 glioma cells (Qvist et al., 1995), human epatoma and neuroblastoma cell lines, 1321N1 astrocytoma cells, and myeloid lineages must be added to the list. Ovarian carcinomas express several PLA₂ isoforms (Gorovetz et al., 2006), including high amount of cPLA₂, but they do not contain type IIA 14 kDa sPLA₂. On the contrary, neoplastic prostatic tissue, prostatic and gastric adenocarcinomas showed positive immunostaining for secretory PLA₂ (Jiang et al., 2002). Expression of sPLA₂-III, with PGE₂-generating function, was associated with microvascular endotelium in human tissues with inflammation, ischemic injury as well as in various tumor cells (Murakami et al., 2005).

iPLA₂β participates in signal transduction in insulinoma (Bao et al., 2006), ovarian cancer (Zho et al., 2006), human hepatoma (Osada-Oka et al., 2006), and Caco-2 cells (Martin-Venegas et al., 2006), whereas the presence of endoplasmic reticulum iPLA₂γ was demonstrated in kidney carcinoma and glioblastoma cell lines (Kinsey et al., 2005).

We provide novel evidence that human melanoma cell lines expressed, at mRNA and protein levels, either iPLA₂ or cPLA₂ and its phosphorylated form (Scuderi et al., 2008). Normal human melanocytes contained the lowest levels of both PLA₂s. Cyclooxygenase-1 and -2 (COX-1 and COX-2) were also expressed in cultured tumor cells as measured by Western blots. Normal human melanocytes displayed no COX-2 expression. In SK-MEL28 and SK-MEL93 cells, we showed that iPLA₂, COX-1 and even cPLA₂ were equally located in the cytosol, membrane structures and perinuclear region while COX-2 was preferentially associated with the cytosol. These results, coupled with the inhibition of the cell proliferation by electroporation of melanoma cells with cPLA₂ or COX-2 antibodies, demonstrate that a possible correlation between PLA₂-COX expression and tumor cell proliferation in the melanocytic system does exist. In addition, the high expression level of both PLA₂s and COXs suggest that eicosanoids modulate cell proliferation and tumor invasiveness.

Thus the observed dysregulation of cPLA₂ and iPLA₂ activities plays a functional role in the pathogenesis of many types of human cancers. However, the effects exerted by diffusible eicosanoid metabolites and bioactive lipids, together with growth factors and cytokines, not only account for the high levels of cancer cell proliferation, but also for the migration of tumor cells towards ECs, the EC recruitment and sprouting of new microcapillaries, and the dysregulation of their barrier function (cross-talk between tumor cells and their microenvironment).

Role of PLA₂s in endothelial cells stimulated by tumor cells

Neovascularization and invasion are key features of malignant tumors, and are processes dependent on the surrounding ECs. Concomitantly, cancer cells during angiogenesis and cancer metastases, release growth factors that induce ECs to express specific ligands and their cognate receptors. The largely undefined signal transduction mechanisms and cross-talk between human melanoma cell lines and brain endothelial cells involved in tumor cell interaction and adhesion have been investigated. In immortalized rat brain GP8.3 EC cultures, conditioned media (CM) prepared from SK-MEL28 and OCM-1 melanoma cells significantly enhanced AA release, cPLA₂ and iPLA₂ specific activities, and cell growth (Anfuso et al., 2009). Inhibitors such as wortmannin and LY294002 (vs. PI3 kinase activity), AACOCF₃ (vs. cPLA₂ and iPLA₂), PD98059 (vs. ERK1/2 activity) and NS-398 (vs. COX-2 activity) were all able to block cell proliferation and motility determined using a scratch wound healing assay in
melanoma CMs-stimulated EC monolayers. Electroporation of anti-cPLA\(_2\) antibody into ECs markedly inhibited the EC proliferation in response to CMs. With both CMs, phosphorylation of cPLA\(_2\), PKC\(\alpha\), ERK1/2, protein and mRNA expression of cPLA\(_2\) and iPLA\(_2\), and COX-2 protein expression were significantly stimulated after 24 h coinoculation, and attenuated by specific inhibitors. Thus, MEK-PKC\(\alpha\)/ERK1/2 and PI3-K/Akt survival pathways are activated in EC cultures during the interaction with CM from both melanoma cell lines, providing new insight in understanding EC metabolism and signaling.

Glioma is characterized by an active production of proangiogenic molecules. We preliminarily observed that conditioned medium (CM) from C6 glioma significantly enhanced proliferation and migration of immortalized rat brain GP8.3 endothelial cells (J Lipid Res., 2010, in press). The glioma CM effect was significantly reduced by cPLA\(_2\) and iPLA\(_2\), COX-2 and protein kinase inhibitors. Endothelial cPLA\(_2\) and iPLA\(_2\) enzyme activities were markedly stimulated by glioma CM, but also depressed by PLA\(_2\) and protein kinase inhibitors. Electroporation of anti-iPLA\(_2\) and cPLA\(_2\) antibodies into CM-stimulated ECs and siRNAs directed against iPLA\(_2\) and cPLA\(_2\) significantly inhibited cell proliferation, and was able to reduce cell front migration using a wound healing assay. Vascular endothelial growth factor (VEGF)-A stimulated EC proliferation and migration. Incubation of CM- or VEGF peptide-stimulated ECs with antibodies against VEGF or VEGFR-1/-2 receptors strongly reduced mitotic rate, cell migration, and phospho-cPLA\(_2\) and iPLA\(_2\) protein levels. Our findings suggest that PLA\(_2\) activities, particularly iPLA\(_2\), are involved in stimulating EC migration and proliferation in the presence of C6 glioma CM, and that cPLA\(_2\) is positively regulated upstream by PI3-K, PKC\(\alpha\) and ERK1/2 signal cascades (Fig. 1). Our work provides new insights in understanding EC metabolism and signaling during tumor angiogenesis, which represent potential therapeutic targets to inhibit or enhance microvascular cell proliferation.

COX-1 and COX-2 catalyze the first committed step in prostaglandin synthesis. Several COX-2 inhibitors have been used for the treatment of different cancers, but many questions have arisen regarding their adverse cardiovascular effects. Various studies have shown the correlation between COX-2 overexpression and enhanced production of prostaglandin E\(_2\) (PGE\(_2\)) from AA by cancer cells. Thus, the concerns regarding the safety of COX-2 inhibitors, as well as the identification of the more effective therapeutic agents, prompted investigators to understand the downstream signaling events regulated by the production of PGE\(_2\) in ECs and tumor cells, which might help to develop new therapeutic approach in the treatment of various cancers.

PGE\(_2\) and PGF\(_{\alpha}\) production stimulates autocrine/paracrine EC growth. PGE\(_2\), as the more represented PG type, interacts with the E prostanooid (EP) family of G-protein coupled membrane receptors, which consist of four different subtypes (EP1-EP4), and at the cell nucleus activate gene transcription. The enhanced expression of EP2 and EP4 receptors has been shown in ECs and in several types of cancer cells including glioma (Hutterer et al., 2006). By autocrine mechanism in ECs, and by paracrine pathway in tumor cells, PGE\(_2\) stimulates VEGF production, principally through EP2 and EP4 receptors (Fig. 1). PGE\(_2\)-dependent signaling participates in the regulation of the invasive behavior of ovarian carcinoma cells by activating tumor-associated matrix metalloproteinases. Intracrine signaling for lipid mediators uncovers novel nuclear pathways to elicit their effects; accordingly, intracellular GPC-receptors constitute a distinctive mode of action for gene regulation (Bhattacharya et al., 1999; Zhu et al., 2006).

To model tumor/endothelial-cell interactions, binary in vitro co-cultures of ECs with cell lines from rat and human glioma, pancreatic tumors, melanomas, esophageal cancer, or with glioblastoma spheroids have been established. Surprisingly, in a co-culture system only one in vitro study has investigated the role of PLA\(_2\) by using a permeability assay and PLA\(_2\) inhibitors (Grabb and Gilbert, 1995), as proposed mechanism by which malignant gliomas cause brain
edema. The conclusion was that tumor or endothelial iPLA$_2$ activity was not responsible for glioma-induced increase in blood brain barrier (BBB) permeability. No further studies on the involvement of PLA$_2$ in tumor/ECs relationship are available in literature till now.

**Perspectives**

In the near future significant progress in the understanding of the BBB is expected, especially with respect to the molecular regulation of cell-to-cell interactions. Moreover, new insights will be gained concerning BBB functions, metastatic invasion through blood vessels, and tumor angiogenesis. In fact, the signaling pathways controlling those processes in ECs are far from being understood and governed by novel therapeutic approaches. As a next step, experimental models using co-cultures will be needed to elucidate the mechanisms and distribution of roles among ECs, pericytes, astrocytes and/or tumor cells, or ECs, epithelial cells and eosinophils (lung allergic inflammation), under non-pathological and pathological conditions.

Due to the large amounts of possible products derived from the PLA$_2$ reaction and to a multitude of diseases that seem to involve PLA$_2$ enzyme activities as an intermediate metabolic pivot, the role of individual phospholipases A$_2$ as regulators of cellular process deserves future studies.

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Fig. 1. A schematic model representing cPLA₂ and iPLA₂ pathways, and arachidonic acid metabolism in stimulated endothelial cells. Production of prostaglandins (PGs) and thromboxans (TXs) from arachidonic acid (AA), liberated by rapid activation of Ca²⁺-independent iPLA₂ and translocation to membranes of Ca²⁺-activated cPLA₂, induces EC proliferation, migration and vascular permeability by regulating growth factor responsive gene expression through downstream transcription factors. MAP kinase and cPLA₂ activation is mediated by PI3-K/PDK1 pathway.