Master Thesis

The role of lysophosphaditic acid-induced signalling in inflammation and tumourigenesis

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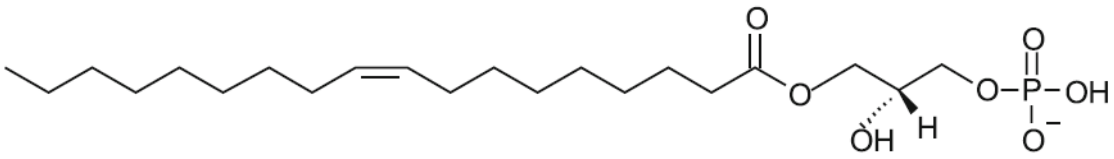

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Outline

1. Introduction

The role of the immune system is primarily to recognise and eradicate pathogens that invade our body. Human cells of the host itself can also be recognized and eradicated by the immune system. This process is essential for eradication of damaged cells to prevent them from developing tumours. On the other hand, self recognition can also contribute to overinflammation or even auto-immune diseases. The role of the immune system is therefore involved not only in to infectious diseases but is also in various types of diseases. Additionally, immune cells can have contradictional roles in diseases and even at the level of a single cell its role can be either beneficial or harmful.

Cancer is one of the major health threats in the western world and countries which share an advanced control of infectious diseases. In spite of all the manpower and financial resources which have been invested in cancer research, the mortality rates for the most frequent forms of cancer have not been reduced significantly. One of the targets to for cancer therapy is the immune system. Immune cells such as T-cells are capable to eradicate cancer cells like virus infected cells. This gives the opportunity to use the immune system to cure cancer. Immune therapies based on tumour antigens have found to be able to decrease the size of a tumour but only few patients have been cured. Additionally, not all immune cells affecting the tumour are able to eradicate tumour cells. As described further on, tumour associated macrophages can also stimulate growth and motility of cancer cells which makes these immune cells harmful.

The role of immune cells depends on communication between cells and tissues. Proteins such as cytokines are mostly described in immune cell activation and communication, but lipids are also found as important communicators for immune cells. Lysophosphaditic acid (LPA) is such a lipid. LPA stimulates proliferation, motility, survival and differentiation in both immune cells and cancer cells. This makes LPA an important signalling molecule to study the role of immune cells for tumourigenesis.

In this thesis, the role of LPA on both immune cells and cancer cells will be discussed to point out how it affects both cell types separately. Subsequently, the effects of LPA on immune cells will be integrated in tumourigenesis. The most important questions to be answered are how LPA affects immune and/or cancer cells, and what is the role of immune cells due to this signalling: friend or foe?

2. LPA synthesis and signalling

Phospholipids are major constituents of the plasma-membrane of all types of cells in all types of tissue. However, lysophospholipids are found in a relative smaller proportion in tissues and extracellular fluids. The prefix “lyso” is widely used in medical science to indicate an absence of one long chain fatty acid of the glycerol backbone. Lysophosphaditic acid, indicated as LPA, consists of phosphorylated glycerol containing just one long chain fatty acid. Concentrations of LPA in serum are low (<100 nM) but potentially increase to high concentrations (>1000 nM) during wound healing, inflammation and tumourigenesis. Most regulation mechanisms of LPA are not well understood, but some enzymes involved have been identified. LPA in plasma or extracellular fluid can be derived via at least two sources. First, LPA is synthesised extracellularly by one secreted enzyme called autotaxin. Second, it can be synthesised intracellularly and is subsequently secreted. LPA synthesis occurs in most cell types but is found to be high in activated platelets, adipocytes, inflammatory cells and cancer cells of various tumours.

2.1 LPA synthesis

Extracellular synthesis of LPA is not only found in serum but also in saliva, malignant effusions, follicular fluid and mildly oxidised low density lipoprotein (LDL). The main enzymes involved in extracellular LPA syntheses are secretory-PLA_2 ($sPLA_2$), lecithin: cholesterol acyl transferase (LCAT) and most important lyso-PLD which is also known as autotaxin (ATX).[1] The $sPLA_2$ and LCAT both catalyse cleavage of long fatty acid chain of phosphatidylcholine

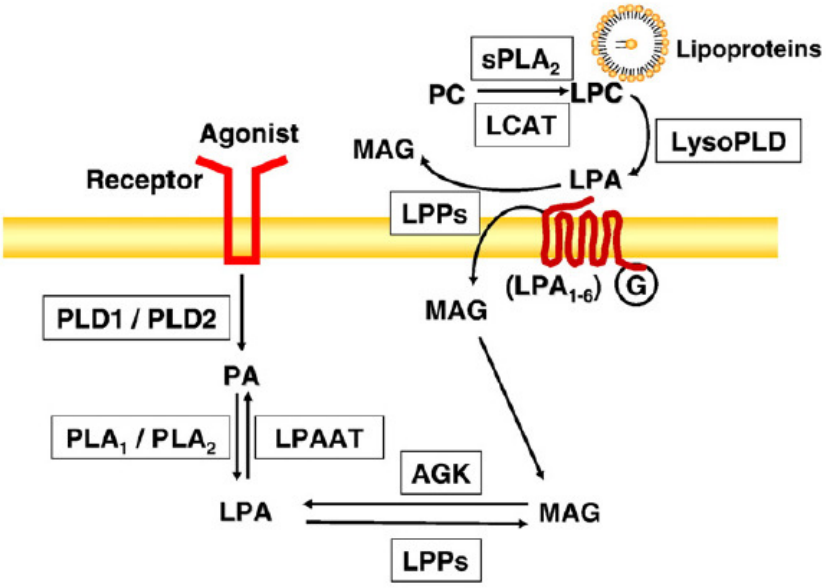

Figure 1. Intracellular and extracellular synthesis of LPA. Intracellular synthesis of LPA is showed here firstly with cleavage of one long chain fatty acid of PA catalysed by PLA1/2. This process can be reversed by LPAAT which synthesises PA from LPA again. Secondly, MAG can be process to LPA by phosphorylating its glycerol group. this process is catalysed by AGK and can be reversed by LPPs.
Extracellular synthesis of LPA occurs via cleavage the choline group of LPC which is catalysed by lysoPLD or ATX. LPC can be synthesised from PC via cleavage of one long chain fatty acid. Thid process can be catalysed by both LCAT and sPLA2.
Firgure from: **Y Zhao, V. Natarajan (2009) Cellular Signalling**

(PC) resulting in lyso-phosphatidylcholine (LPC). Thereafter, LPA is formed from LPC by ATX cleaving off the choline group from the phosphate group. The PC can be derived from high density lipoprotein (HDL) where mainly LCAT is responsible for LPC synthesis. Lysophosphatidylserine (LPS) is highly produced by platelets and also some other cell types. LPS can be synthesised to LPA via phosphatidyl (PS)-specific phospholipase A_1 (PLA_1) which cleaves off the serine group. The role of ATX is most important in LPA production and ATX expression is increased in platelets during wound healing, in various immune cells during inflammation processes and during tumourigenesis. The exact role of ATX in many inflammation processes like airway diseases is still unknown but its overexpression is associated with various cancers (Figure 1).

The intracellular production of LPA occurs via *de novo* synthesis and is regulated by two key enzymes: glycerophosphate acyl transferase and acly-glycerol kinase (AGK).[2] The function of glycerophosphate acyl transferase is catalysing the transfer of long fatty acid chain from an acyl-Co enzyme A complex to glycerol-3-phosphate resulting in LPA. This process can be reversed by lysophospholipases which cleave off the acyl group from LPA.[3] Mono-acyl-glycerol (MAG) already has one long fatty acid chain but needs to be phosphorylated to become an LPA molecule. The phosphorylation of MAG is catalysed by AGK, and can be reversed by lipid phosphate phosphatase (LPP) which catalyses cleavage of the phosphate group.[4] Di-acyl-glycerol (DAG) can also be processed to LPA via phosphorylation and lysis of one long chain fatty acid. Phosphorylation of DAG is catalysed by DAG-kinase or agonist stimulated phospholipase D (PLD) resulting in phosphatic acid (PA). Subsequently, cleavage of one fatty acid chain of PA is catalysed by phospholipase A_1 (PLA_1) or phospholipase A_2 (PLA_2) resulting in cleavage of the sn-1 or sn-2 positioned long chain fatty acid relatively.[5] This can be reversed by LPA acyl transferase (LPAAT) which adds an acyl group to LPA resulting in PA again (Figure 1).[6]

2.2 LPA G-coupled receptors and signalling

Discovery of LPA receptors in the plasma membrane of various cell types revealed the key investigating the mechanisms of LPA-induced intracellular signalling. LPA was first found to be a ligand for the ventricular zone-1 (vzg-1) receptor which is a member of the endothelial differentiation gene (EDG) family.[7] Until now, six LPA receptors (LPA_{1-6}) have been identified as G-protein coupled receptors (G-PCRs).[8] The best-characterised LPA receptors are LPA_{1-3} which belong to EDG-subfamily like EDG_2 (LPA_1), EDG_4 (LPA_2), and EDG_7 (LPA_3) and share about 50% sequence homology with each other.[9] EDG subfamily members such as $EDG_{1,3,5,6,8}$ are receptors for close-related bioactive lipid shingosine-1-phosphatse (S1P).[10] LPA_{4-6} share less than 40% sequence homology with LPA_{1-3} and are more distinct receptors

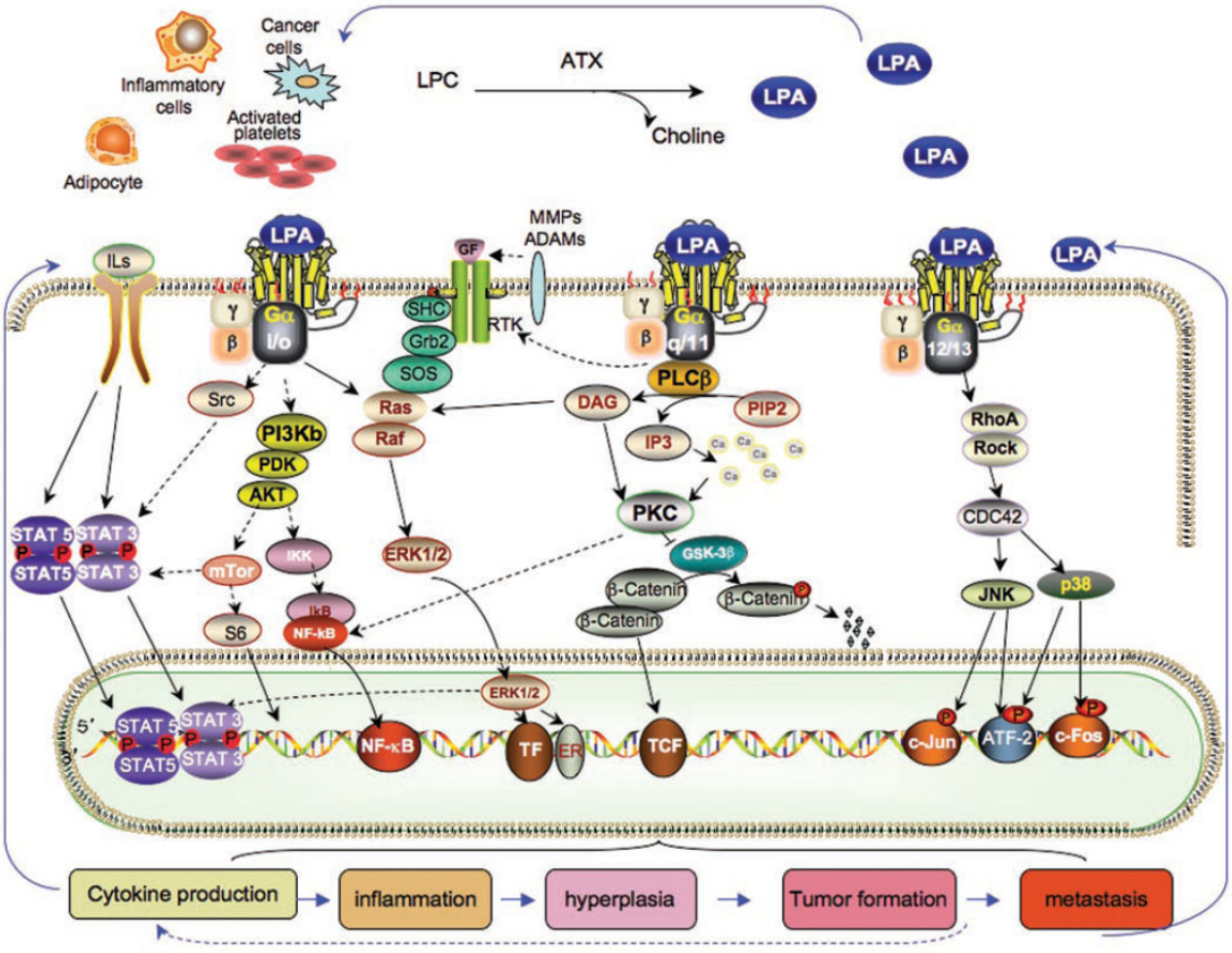

Figure 2. LPA_{1-3} mediated signalling via three different G-protein subunits. Firstly, the $G_{i/o}$ subunit is known to inhibit adenylate cyclase while it induces the Ras-ERK/MAPK and PI3K-PKB/Akt signalling pathways. This result in stimulation of proliferation and cell motility while apoptosis is suppressed. Secondly, the $G_{q/11}$ subunit does transiently increase the concentration of cytosolar Ca^{2+} ions via PLCβ activity. This signalling results in induction of PKC signalling which stimulates proliferation via β-catenin. Lastly, the $G_{12/13}$ subunit stimulates the RhoA family signal pathway which results in induced JNK/MAPK and p38/MAPK signalling and AP-1 transcription factor activity. As indicated low in the figure, the activated genes by these signalling pathways are associated with inflammation and tumourgenesis. Figure from: **S Lui, GB Mills *et al.* (2009) Cell Cycle**

like G-protein coupled receptor-23 (GPR23/LPA_4), GPR92 (LPA_5), and GPR87 (LPA_6).[11,12,13] LPA is also found to be a potent ligand for intracellular receptor PPARγ, which can be activated by a broader repertoire of ligands.[14]

Expression of LPA receptors is ubiquitously and variable in most cell types. There at least four subunits of G-proteins known which are associated with LPA_{1-6} associated signal transduction: $G_{i/o}$, G_s, $G_{12/13}$, and $G_{q/11}$.[15] EDG family receptors LPA_{1-2} are known to interact with $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$; while LPA_3 does solely interact with $G_{i/o}$ and $G_{q/11}$. LPA_4 can interact with all four G-proteins while LPA_5 cannot interact with $G_{i/o}$. Each G-protein subunit has its own pattern of downstream signal activation. The $G_{q/11}$ subunit transiently increases the free

Ca^{2+} concentration via phospholipase Cβ (PLCβ), which results in protein kinase C (PKC) activation. Activation of LPA receptors coupled to $G_{i/o}$ leads to inhibition of adenylate cyclase which suppresses synthesis of cAMP. Additionally, $G_{i/o}$ activates the Ras-ERK/MAPK pathway and PI3K-PKB/Akt signalling. This signalling is an important stimulation of cell motility and proliferation, and suppresses apoptosis. The family of guanine exchange factors (GEFs) and GTPases (GAPs) of RhoA and Rac are stimulated by $G_{12/13}$ activity, which results in p38/MAPK and JNK/MAPK signalling.[16] G_s activity is more associated with LPA_4 and LPA_5 which activate adenylate cyclase resulting in higher cAMP and free Ca^{2+} concentrations (Figure 2).

2.3 LPA-induced transcription factors

Two key transcription factors in immune response regulating are Nuclear Factor-κB (NF-κB) and activator protein 1 (AP-1) which are expressed in almost every mammalian cell type. NF-κB is a heterodimer of NF-κB-family subunits which consists of NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB and c-Rel (or Rel).[17] If inactive, a NF-κB heterodimer is bound to inhibitor of NF-κB (IκB). The NF-κB dimer can dissociate this complex when IκB becomes phosphorylated. The phosphor group on IκB forms a docking side for ubiquitinating enzymes, which will poly-K48 ubiquitinate it, leading to degradation by the 26S proteasome. The free NF-κB subunits can translocate from the cytosol into the nucleus where they can activate various genes.[18] LPA is found to activate NF-κB in human bronchial epithelial cells (HBEpCs) via p38/MAPK and PKCδ.[4] However, LPA activation of NF-κB can also occur via $G_{i/o}$ mediated PKB/Akt or Src and STAT activity, G_q mediated PKC activity or $G_{12/13}$ mediated activity of the RhoA-CDC42-p38 MAPK pathway.[19] Most genes activated by NF-κB are anti-apoptotic or pro-inflammatory.

The AP-1 transcriptoin factor is a dimer composed of Fos (c-Fos, Fos-B, Fra-1, Fra-2) and Jun (c-Jun, JunD, JunB) proteins. Whereas hetero- and homodimers of Jun proteins can bind DNA directly, Fos proteins require a Jun protein to form a AP-1 transcription factor.[20] LPA can induce AP-1 via MAPK-family proteins. The c-Jun protein is involved in regulation of inflammatory genes and can be activated by c-Jun NH2-terminal kinase (JNK), which belongs to the MAPK-family. However, the p38/MAPK can also activate c-Jun but this reaction is PKC dependent as well.[21] Both JNK and p38 can be activated via the $G_{12/13}$-protein signalling pathway as shown in Figure 2. The ERK/MAPK is also activated by LPA signalling and can activate AP-1. Activation of ERK can occur via $G_{q/11}$ or $G_{i/o}$ signalling, but also via cross talk of the LPA-receptor with the epidermalgrowth factor receptor (EGFR). The ERK/MAPK is an inducer of many Fos proteins, while it can inhibit c-Jun activity.[22] This is explained by the fact that ERK phosphorylates the C-terminal domain instead of the N-

terminal domain of c-Jun which makes it incapable of binding DNA. AP-1 activity is associated with inflammatory genes like cytokines, but also with genes affecting proliferation or differentiation.

Lastly, LPA can was also found to induce C/EBP transcription factors in HBEpCs. C/EBP is a family of six basic zipper transcription factors are involved in inflammatory genes and immune regulation. The C/EBPβ subunit is found to be regulating various cytokine genes and the cyclo-oxygenese 2 (COX-2) gene as well. COX-2 is an enzyme involved in prostaglandin synthesis which will be discussed further on. HBEpCs showed to induce COX-2 expression during LPA-induced signalling. COX-2 has a C/EBPβ binding element, and LPA-induced expression can be attenuated by down regulation of C/EBPβ as indicated by.[57]

In summary, LPA-induced signal transduction regulates transcription factors, such as NF-κB, AP-1 and C/EBPβ, which are associated with both inflammatory and survival/proliferative genes.

2.4 LPA receptor and ATX knockout mice

Further insight into the effects of EDG family members LPA_{1-3} has been acquired by LPA receptor gene (*lpa*) knockout mice. *lpa*$_1$*/edg2*$^{-/-}$ mice models showed approximately 50% neonatal lethality and craniofacial dysmorphism.[23] Models with both *lpa*$_1$*/edg2*$^{-/-}$ and *lpa*$_2$*/edg4*$^{-/-}$ did not show any additional phenotype compared to *lpa*$_2$*/edg4*$^{-/-}$ models, suggestion a similar role for LPA_1 and LPA_2 in developmental biology.[24] Models with an *lpa*$_3$*/edg7*$^{-/-}$ mutation showed to have a delayed implantation of the blastocyst in the uterus and a decrease in prostaglandin-induced uterine contraction.[25] These KO mice models revealed the important role of LPA signalling for embryonic development. Late disease onset was also studied but hindered by neonatal fatality rate in some models. However, a decrease of colitis induced tumours was found in *lpa*$_2$*/edg4*$^{-/-}$ models compared to mock mutation models.[26]

All these LPA receptor knockout models did not cover all LPA receptor types and therefore all effects of LPA signalling. Extracellular synthesis of LPA is essential to initiate LPA receptor activation and therefore ATX gene knockout mice (*Enpp2*$^{-/-}$) have been made. ATX-deficient mice die at day 9.5 of their embryonic development and show vascular defects in both the yolk sac and embryo.[27] Additionally, embryonic day 8.5 was already remarked by neural tube defects and asymmetric head-folds. Such severe effects were not found in any LPA_{1-3} deficient mice and suggest the role of other LPA receptors to be involved in vascular development.[28] However, LPA-induced signalling is essential for embryonic development and survival, and the differences between an ATX- and LPA receptor-deficient mouse indicate that more various receptors than just LPA_{1-3} are involved.

3. LPA signalling and inflammation

Most communication in the immune system occurs via proteins such as cytokines and chemokines. The role of lipids to regulate immunity is less understood and compared to proteins there are less lipids known to have such a function. LPA plays a role in immunity where it not only affects immune cells but also epithelium and endothelium. LPA as a new factor in various immune reactions gave more insight into some processes like lymphocyte migration and airway inflammation or asthma.

3.1 LPA and lymphocyte migration

Migration of lymphocytes from the blood stream into secondary lymph nodes is a rapid and coordinated process which is essential for lymphoid immune surveillance. Every second about 15,000 lymphocytes enter a single lymph node in sheep, which indicates how constantly active this process is.[29] Naïve lymphocytes which are on 'immune patrol' enter the lymph nodes and Peyer's patches to eventually encounter antigen offered by antigen presenting cells. The process of migration has always been attributed to the action of chemokines, selectins and integrins, which are certainly essential but not the only players. The effects of ATX and LPA mediated signalling have been shown to enhance migration of lymphocytes.[30] These findings introduced LPA as a new candidate to play a role in lymphocyte migration.

Migration of lymphocytes into secondary lymph nodes takes place at specialised post-capillary venules which are known as high endothelial venules (HEVs). These venules have cubic and plump shaped high endothelial cells (HECs) which are characterised with a well developed Golgi apparatus and rough endoplasmatic reticulum.[31] Endothelial cells in other tissues generally have a thin morphology and have more quiescent phenotypes. Migration of lymphocytes is known to occur in three steps: rolling over the endothelium, arrest, and migration through the endothelial layer.[32] It is known that the rolling occurs via interactions of the lymphocytes L-selectin and α4β7 integrin with the HEC its "sialyl-6-sulfo-Lewis X" carbohydrate and MAdCAM respectively.[33] The arrest step is initiated by chemokine CCL19 and CCL21 secretion of the HEC, which up regulates αLβ2 and α4β1 integrins on the lymphocyte.[34] The αLβ2 integrin can bind adhesion molecules ICAM-1 or ICAM-2 of the HEC which make the lymphocyte solid attached and arrests from rolling. Subsequently, the lymphocyte will transmigrate through the endothelial layer which is a poor understood process (Figure 3).

HECs from human lymphoid organs have been investigated by gene-profiling analysis to get more insight into HEV functions.[35] A human tonsillar HEC gene profiling study revealed 5% of

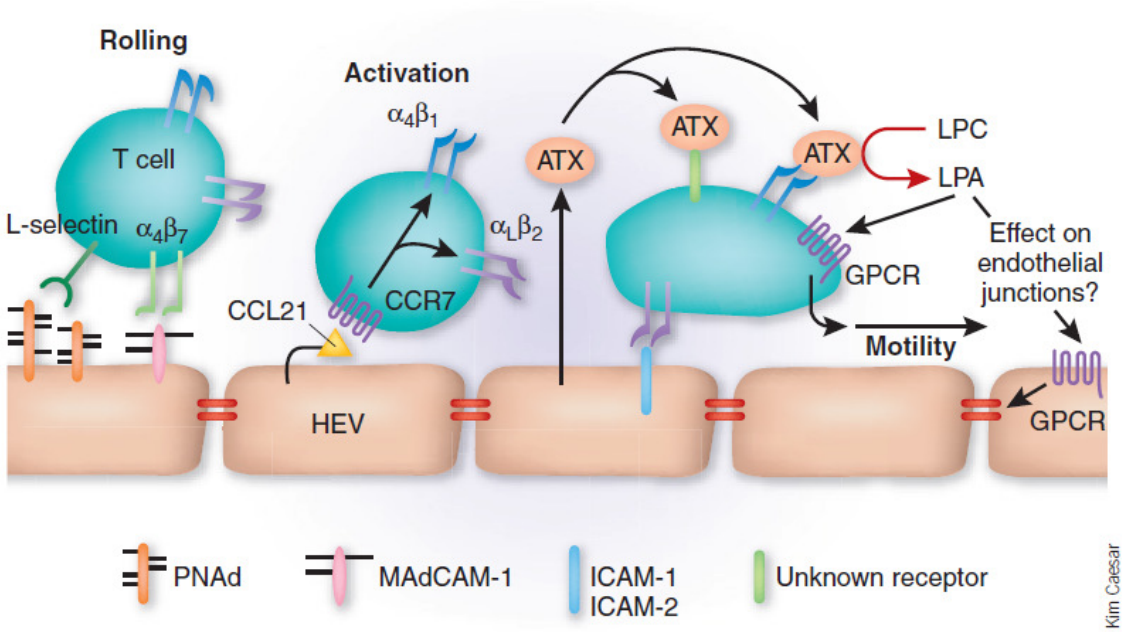

Figure 3. The role of ATX in lymphocyte migration. Rolling is initiated by the interactions of L-slecetin and α4β7 integrin with PNAd and MAdCAM-1 respectively, of the HEV endothelial cell surface. The HEV endothelial cell will secrete a CCL21 chemokine which activates αLβ2 and α4β1 integrin up regulation in the lymphocyte. The αLβ2 integrin will solid attach to ICAM-1/2 molecules which make the lymphocyte arrest. Autotaxin (ATX) is secreted by the endothelium and will bind the lymphocyte ate the α4β1 integrin via an unknown receptor. Here, ATX will convert LPC into LPA, which will active G-protein coupled receptrs (GPCRs) on both the lymphocyte and endothelium. GPCR activity will induce motility and cytoskeletal remodelling in the lymphocyte which enhances transmigration through the endothelial layer. Figure from: **D Vestweber, MK Wild (2008) Nature Immunology**

the transcripts to be coding for ATX.[36] These findings indicated that LPA, and therefore LPA signalling, must have a role in lymphocyte migration. It was first found by rtPCR that secondary lymph nodes had the highest expression of ATX in mice.[37] Second, they were able to isolate ATX from purified HECs and found HECs to secrete ATX on their apical side (into the blood stream). It was demonstrated that ATX bound lymphocytes at the α4β1 integrin, which is upregulated by the CCL21 chemokine. LPC is abundant in blood plasma, so the presence of ATX on the surface of lymphocytes will locally deliver LPA. T-cell lymphoma cell lines have been found to rearrange their actin cytoskeleton due LPA-induced signalling.[38] Similar results were obtained for primary lymphocytes, which were even found to induce motility due to LPA mediated $G_{i/o}$-coupled receptor activity.[37] Lymphocytes are found to express mainly LPA_1, LPA_2 and LPA_5 receptor which use a variety of G-proteins for transduction.[39] In spite of this fact, it was shown that specific inhibition of $G_{i/o}$ by pertussis toxin (PTx) was sufficient to block lymphocyte chemokinesis.[37] However, these results were not obtained by in lymphoma cells where PTx had no effect on lymphoma invasiveness.[38] This process is probably more linked to G_q and $G_{12/13}$ G-protein families instead of $G_{i/o}$. The fact that LPC cannot have a certain effect is proven in an assay where only LPA, and not LPC, was capable to induce lymphocyte migration and cytoskeleton remodelling in ATX-depleted cells.[40]

All these data demonstrate the principle that HECs enhance lymphocytes to migrate via a paracrine route of ATX secretion and LPA synthesis. In addition, HECs themselves have LPA receptors which suggest an autocrine function for ATX as well.

3.2 LPA-induced platelet activation

Platelet activation is an important process for blood clotting during tissue damage in blood vessels. Platelets are responsible for stopping bleeding from the vessel by changing their shape and to aggregate. Fibrinogen fibres are present in the serum to provide a matrix for platelets to adhere together during aggregation. Injury can initiate platelet activation by exposing the serum to collagen of the endothelial matrix. Collagen will activate serum factors to produce trombin, which can activate a platelet. The trombin-associated signalling activates phospholipase C (PLC) in the cytosol of the platelet. PLC will catalyse hydrolysis of phosphoinositides into diacylglycerol (DAG), which is subsequently phosphorylated into phosphatic acid. As described earlier, PA can be converted into LPA by $PLA_{1/2}$.enzymes. LPA is a potent activator of platelets to induce shape changes and aggregation. Another source of LPA in the blood are lipoproteins such as HDL and mildly oxidised-LDL (moxLDL).[41] The moxLDL exposes LPA on the outside of the lipoprotein which can directly activate present LPA receptors on platelets or other cell types. It does together with HDL also release phosphatidylcholine (PC), which is converted into LPC by secreted PLA_2 and ATX will catalyse this into LPA. The secretion of enzymes such as $sPLA_2$ and ATX can be carried out by activeted platelets, but also by activated immune cells in the blood which are triggered by injury-derived cytokines.
Low concentrations of LPA and moxLDL stimulate two G-protein derived signal pathways in platelets, which are Ca^{2+}-independent. One of the pathways proceeds through $G_{12/13}$ activation, resulting in Rho and Rho-kinase activity and subsequently in phosphorylation of myosin light chain (MLC).[42] This pathway is responsible for changes in the actin cytoskeleton, which makes the platelet able to change its shape. Second, LPA and moxLDL activate Src-family tyrosine kinase, which occurs via G_i-protein activity. Src-tyrosine kinase will activate the Syk tyrosine kinase and both will contribute to phosphorylation of tyrosine residues of various proteins. This tyrosine kinase pathway will mediate expression of fibrinogen-binding $\alpha_{2b}\beta_3$ integrins, making platelets able to aggregate while they are changing their shape. Aggregation can also be induced by Ca^{2+}-dependent signalling in platelets, which requires high LPA or moxLDL concentrations. Here, an induced Ca^{2+} concentration in the cytosol is responsible for the expression of $\alpha_{2b}\beta_3$ integrins. These free Ca^{2+} ions are mobilised not from intracellular stores but from entry of extracellular Ca^{2+} ions.[43]

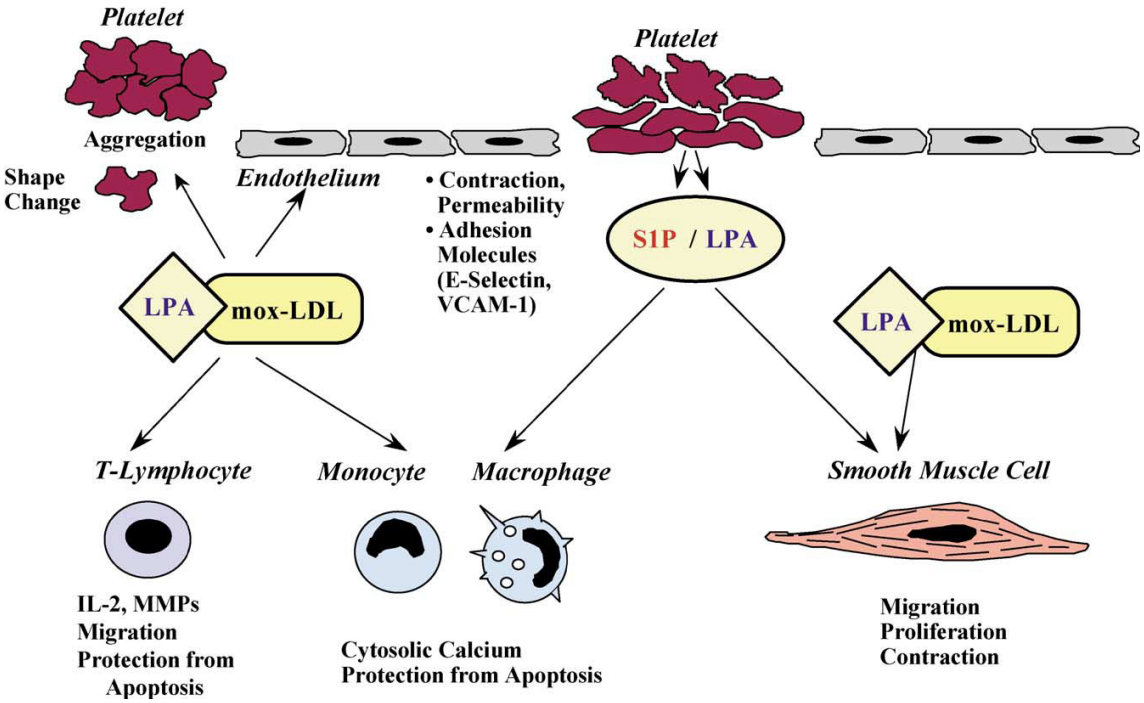

Figure 4. LPA activation of platelets, immune cells, endothelium and smooth muscles. Serum LPA is produced by platelets, together with its related sphingosine-1-phosphate (S1P), which can stimulate activation of various cell types related to injury mediated inflammation. Platelets them self are activated which results in shape changes and aggregation, while survival factors, migration and inflammation are stimulated in macrophages, monocytes and lymphocytes. Endothelium will be activated to express adhesion molecules and to prepare itself for migration of immune cells while smooth muscle cells migrate to the location of injury for wound healing processes. Figure from: **W Siess (2002) Biochimica et Biophisica Acta**

RT-PCR analysis has revealed that LPA_{1-3} receptors are present on platelets, but it is still unsure which receptor is most responsible for shape change or aggregation. However, an inhibitor of LPA_1 and LPA_3 receptors, diacyl-(8:0)-glyceropyrophosphate, can inhibit LPA-induced shape change and Ca^{2+} influx. This suggests that LPA_2 receptor has a main role in platelets. Other findings support the idea that platelets carry unidentified LPA receptors that lack molecular similarity with the traditional LPA receptors. One example comes from the fact that platelet aggregation was enhanced by an LPA analog that has no affinity for LPA_{1-3} receptors. Although LPA has been shown to affect platelet shape change and aggregation, more research will be needed to establish which receptors on platelets are responsible (Figure 4).

3.3 Macrophage and Monocyte activation by LPA

LPA can activate immune cells to induce immunological reactions or increase cell proliferation. Macrophages are phagocytes that are important for innate immunity and they respond to LPA. Micromolar concentrations of LPA are enough to induce cell proliferation,

production and release of reactive oxygen intermediate (ROI), prostaglandins, leukotrines and arachidonic acid in THP-1 cells (macrophage/monocyte precursor and myeloid leukaemia cell line).[44] An inhibitor of LPA_1 and LPA_3 receptor, Kil6425, has been demonstrated to abolish LPA mediated activation of macrophages. This suggests that LPA_1 and LPA_3 receptors are mainly responsible for LPA signalling in macrophages. However, only LPA_3 expression increases significantly during activation, but LPA_1 still can have a certain role. LPA receptor signalling increases Ca^{2+} concentrations in monocytes and macrophages, which results in enhanced motility and migration. LPA also induces survival via PI3K-mediated activation of PKB/Akt to promote anti-apoptotic signalling and gene activation (Figure 4).

Macrophages are also a potential source of LPA. Activation of macrophages by bacterial lipopolysaccharides (LPS) activates the JNK and p38/MAPK pathway. These pathways are potentential inducers of ATX expression and LPA production[45]

3.4 The role of LPA in airway inflammation

LPA is present in significant amounts in serum, saliva and also in bronchoalveolar lavage fluid (BALF). The amounts of LPA BALF have been determined by tandem mass spectrometry and were found to increase after segmental allergen challenges.[46] These data suggest a role for LPA and LPA signalling in airway diseases and allergic reactions. Expression of LPA receptors was studied in an mRNA profile of human bronchial epithelial cells (HBEpCs) which revealed that LPA_1, LPA_3 and LPA_2 showed highest expression, while LPA_4 was barely expressed and LPA_5 was undetectable.[47] Additionally, LPA_1 and LPA_2 receptors have been found by in lung epithelial cells.[46] The presence of ATX was not investigated in this study but the fact that LPA and LPA receptors are involved has been enough evidence for further research of LPA signalling in HBEpCs and airway diseases.

Infiltration of immune cells in airway epithelium is an important mediator for asthma and airway inflammation. It was demonstrated that the production of IL-8, an important chemoattractant for neutrophils, is effectively stimulated by LPA in HBEpCs and other airway epithelium cell types.[48] The $G_{i/o}$ and $G_{12/13}$ coupled LPA_{1-3} receptors are responsible for IL-8 gene activation. Additionally, LPA inhalation induced eosinophil and neutrophil numbers in guinea pig BALF.[49] The motility of HBEpCs is regulated by cMet and E-cadherin, and can be regulated by LPA-induced PKCδ and ζ.[50] In this way, infiltration of immune cells can be enhanced by LPA via reduced epithelial adhesion. These data suggest that LPA has a certain pro-inflammatory role and will contribute to the progression of airway diseases (Figure5).

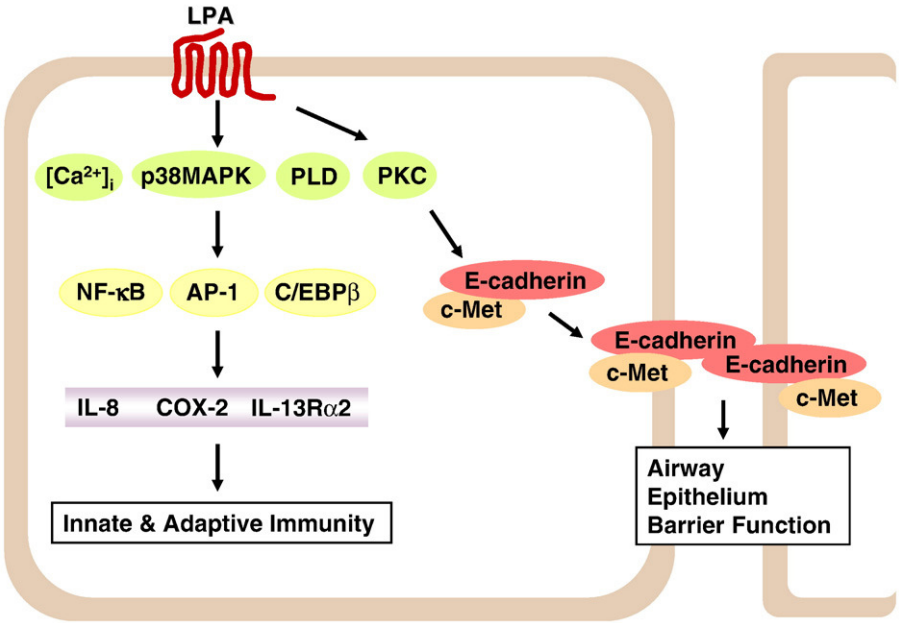

Figure 5. LPA-induced signalling in HBEpCs airway epithelial cells. Activation of the LPA receptors in airway epithelium results in elevation of the free Ca^{2+} concentration, p38/MAPK signalling, PLD and PKC activity. Transcription factors, such as NF-κB, AP-1 and C/EBPβ, regulate genes as IL-8, COX-2 and IL-13Rα2. IL-8 is a pro-inflammatory chemoatrractant while the role of COX-2 (producing PGE2) and IL-13Rα2 are associated with reduction of inflammation in airway epithelium. PKC isoforms δ and ζ affect epithelial cell adhesion and integrity by regulating cMet and E-cadhering. this makes airway epithelium more accessible for infiltrationg immune cells such as eosinophils and neutrophils. Figure from: **Y Zhao, V. Natarajan (2009) Cellular Signalling**

T-helper cells type 2 (Th2)-type cytokines play an important role in allergic reactions and associated diseases. For example, interleukin-13 (IL-13) is increased in BALF of asthma patients.[51] Activation of the IL13 receptor α1 (Il-13Rα1) will initiate a signal via Janus kinases (JAKs) resulting in activation of signal transducer and activator of transcription-6 (STAT-6).[52] Many pro-inflammatory genes in epithelium cells are activated by STAT-6 and this mechanism is known to be involved in bronchial asthma pathogenesis. The function of IL-13Rα1 can be inhibited by overexpression of IL-13 decoy receptor, or IL-13Rα2, which can bind IL-13 by a 10-fold higher affinity.[53] LPA has been found to increase IL-13 secretion in T-cells., In HBEpCs it upregulates IL-13Rα2 via $G_{i/o}$-coupled receptor activity.[54] IL-13Rα1 expression is not altered by LPA, but IL-13Rα2 activity inhibits STAT-6 phosphorylation. These data suggest that LPA is a protective and anti-inflammatory compound in airway inflammation and remodelling. Such a role has also been attributed to other kinds of cytokines, like prostaglandins. Prostaglandin E2 (PGE2) has a protective role against innate immunity reactions and tissue remodelling in airway inflammation.[55] The enzyme cyclo-oxygenase-2 (COX-2) is responsible for the synthesis of PGE2 from arachidonic acid.[56] The exact regulation of COX-2 and the effects of PGE2 on epithelium are not well understood, but it is known that COX-2 expression and PGE2 synthesis are increased in LPA challenged HBEpCs.[57] The LPA-mediated expression of COX-2 is induced by various transcription factors like NF-κB, c-Jun and C/EBPβ. LPA-induced COX-2 expression can be blocked by

pertussis toxin (PTx), suggesting an important role for $G_{i/o}$-coupled receptors. Additionally, a *Schistosoma mansoni* egg sensitised mouse model for airway allergy with an LPA_1 or LPA_2 haplotype revealed a relatively lower COX-2 expression and PGE2 in BALF.[58] This showed the importance of LPA_1 or LPA_2 expression for COX-2 expression in inflamed airway conditions.

The effects of LPA, or LPA-induced signalling, in HBEpCs can be both pro- and anti-inflammatory.[59] The role of LPA depends on the physiological context and cannot be considered as an inducing or reducing factor in immune reactions in general. Future studies related to LPA and its effects on airway epithelium will increase our understanding of how LPA affects the immune system and airway epithelium during inflammation.

4. The role of LPA in tumourigenesis

The growth and development of a tumour is a process that requires dynamic changes in the genome. These changes include n various mutations that produce oncogenes with dominant gain of function and tumour suppressor genes with recessive loss of function. These mutations eventually lead to a malignant phenotype l. There are more than 100 distinct types of cancer, with possible subtypes within each type as well. In spite of this complexity and variation, the malignancy in each type of cancer is dictated by six essential properties known as the hallmarks of cancer: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replication potential, sustained angiogenesis, and tissue invasion and metastasis.[60] As far as is known, LPA does not induce mutations in the genome, but it can alter the signal transduction of a cell. Altered growth factor signalling is one of the hallmarks of cancer. As a growth factor, LPA is a likely player in tumourigenesis.

4.1 LPA signal transduction in cancer cells

The LPA signal transduction pathways have been discussed in an immunologic context, but many of these pathways have relevance for tumourigenesis as well. For example, migration and proliferation of immune cells can be translated into tumour growth and metastasis. The effects of the G_q-protein pathway involve Ca^{2+} release and enhanced PKC activity. This can also activate β-catenin, which plays a role in tumour growth in various cancers particularly colon cancer. The $G_{i/o}$-protein pathway is an important inducer of PI3K-PKB/Akt and RAS-ERK/MAPK signalling. The PI3K and PKB route deliver an important survival signal, rendering cancer cell less sensitive to apoptosis, while PI3K also activates the Rac

GTPase which induces metastasis. RAS, a well-known proto-oncogene, and ERK stimulate the cell cycle which leads to activation of the growth-promoting transcription factor c-Myc.[61] Lastly, the $G_{12/13}$-protein pathway activates RhoA, which is an important player together with RAC in cytoskeletal remodelling, cell migration and metastasis. RAC is responsible for actin-depedent cell flattening, while RhoA remodels the actin filaments to contract and round up the cell body. The RhoA protein activates p38 and JNK MAPKs, which are important stimulators of NF-κB. This results in transcription of anti-apoptotic genes leading to enhanced tumour cell survival. (Figure 2)

The LPA receptors can stimulate growth and motility by themselves, but they also can transactivate various receptor tyrosine kinases (RTKs) and thereby enhance signal strength. The human EGF receptor (EGFR) can be activated by the cleavage of membrane-bound heparin-binding EGF (HB-EGF), which is a ligand for EGFR. LPA signalling activates various proteinases, such as metallo-proteinases (MMPs), which can cleave HB-EGF causing autocrine or paracrine EGFR (trans-)activation. The EGFR is an important inducer of the RAS-ERK/MAPK pathway to induce proliferation. Other important LPA trans-activated receptors for proliferation are platelet-derived growth factor-receptor β (PDGF-Rβ) and cMet. The PDGF-Rβ binds a platelet secreted ligand, while the cMet ligand is secreted by hepatocytes. The transactivation mechanisms of PDGF-Rβ and cMet occur intracellularly and are still less understood than EGFR transactivation. The fact that cancer cells often bear mutations in their growth factor receptors and other signalling molecules can also affect the communication between GPCRs and RTKs. Many cancer cells share a high expression of LPA receptors, making them capable to use LPA signalling and RTK transactivation to develop into a full blown tumour.

4.2 The role of LPA in ovarian cancer and breast cancer

The first suggestions for LPA as protumourgenic factor was based on an ovarian cancer patient in 1964, who had increased serum levels of LPC, the main LPA precursor. Later on, ATX was identified as a motility-stimulating factor for cancer cells, while its function as a lysoPLD remained l unknown until 2002.[62] Research on the role of LPA as a mediator in gynaecological cancers has expanded since it was discovered to induce proliferation of ovarian cancer cells (OCCs).[63] Some cancer types, including breast and ovarian cancer, show elevated levels of LPA near the tumour or in serum, probably as a result of ATX overexpression.[64] Breakdown of LPA is suppressed by reduced expression of a lipid phosphate phosphatase (LPP), which results in elevated LPA levels.[65] Additionally, cancer cells and tumour-associated cells, like endothelial and stroma cells, have an increased expression of LPA receptors.[66] In ovarian cancer patients, LPA levels were found to be

increased in ascitic fluids. Here, both mesenchymal cells and cancer cells showed increased ATX expression, which means that both malignant and benign cells contribute to LPA synthesis in ascites.[67] Furthermore, the expression of LPA_2 and LPA_3 receptor is increased by 15% to 49% in benign tumours. The tumourigenesis of ovarian cancer shows adherence of cancer cells to lining cells and invasion into the underlying stroma. Transcription patterns of LPA exposed human epithelial ovarian cancer cells revealed regulation of migration and adhesion associated genes, consistent with the idea that LPA is an inducer of ovarian cancer cell invasion and metastasis.[68]

Breast cancer cells have an increased expression of ATX. The expression of LPA receptors is also increased in invasive mammary ductal carcinoma cells, which have an increased LPA_2 expression.[69] However, breast cancer metastasis can be inhibited by the tumour suppressor Nm23-H1 which acts like an LPA_1 antagonist.[70] The metastatic capability of breast cancer cells is also correlated with the ATX expression, which indicated the importance of LPA and LPA_1 and LPA_2 mediated signal transduction for metastasising in breast cancer. Many breast cancer cases are associated with increased expression of an EGFR-like receptor, known as HER2/neu. HER2 is an important growth signal producing receptor in breast cancer and can also be trans-activated by LPA signalling. Thanks to innovative medication, HER2 overexpression can be treated with a monoclonal antibody known as trastuzumab (Herceptin™). This gives breast cancer patients a better prognosis for treatment.

SKOV-3 cells revealed *in vitro* that over expression of only one of the EDG family LPA receptors was enough to induce proliferation and invasiveness.[71] Secondly, *in vivo* studies in mice showed that transgenic overexpressing ATX, or any of the EDG-related LPA receptors (LPA_{1-3}) was sufficient for the development of metastatic breast cancer.[72] All these findings have made LPA a serious player and drug target in breast and ovarian cancer.

4.3 LPA and Wnt signalling in colon cancer

Colon cancer is the third most common cancer in the world and is ranking second in cancer-related death. Malignant tissue in the colon can be surgically removed but, as in most forms of cancer, death is always a result of metastatic disease. Approximately 60% of the colo-rectal cancer patients develop liver metastasis.[73] LPA induces motility and migration in immune cells, here it is an important mediator to induce colon cancer metastasising. One of the important effects of LPA on various colon cancer cell lines is its ability to induce factors of the Wnt/Frizzled receptor pathway.[74] The Wnt-signalling pathway was first discovered as and important regulator differentiation in the developmental biology in fruit flies.[75] Later on in

research, it was revealed to play in important role proliferation and migration and deregulation is associated with tumour development.[76]

The Frizzled receptor is a 7-transmembrane segmented receptor that is G-protein coupled as well. Its ligand, Wnt, is a secreted glycoprotein which binds the Frizzled receptor extracellular and makes it active. The active Frizzled receptor activates an intracellular G_i or G_o-proteins.[77] The G_o-protein is responsible for the 'cannonical' pathway via activation of a phosphoprotein called Dishevelled (Dvl), the exact mechanism for this is still unknown. It is suggested that Dsv can interact with the Frizzled-1 C-terminus its PDZ ligand because Dvl has a PDZ domain.[78] However, Dsv can inhibit the glycogen synthase kinase 3β (GSK3β) which is part of a protein complex that phosphorylates β-catenin. The phosphorylation of β-catenin promotes it for ubiquitination and degradation, but this is prevented by Dvl activity to inhibit GSK3β. Subsequently, β-catenin can migrate into the nucleus and activate various genes affecting motility, differentiation or proliferation (Figure 6).

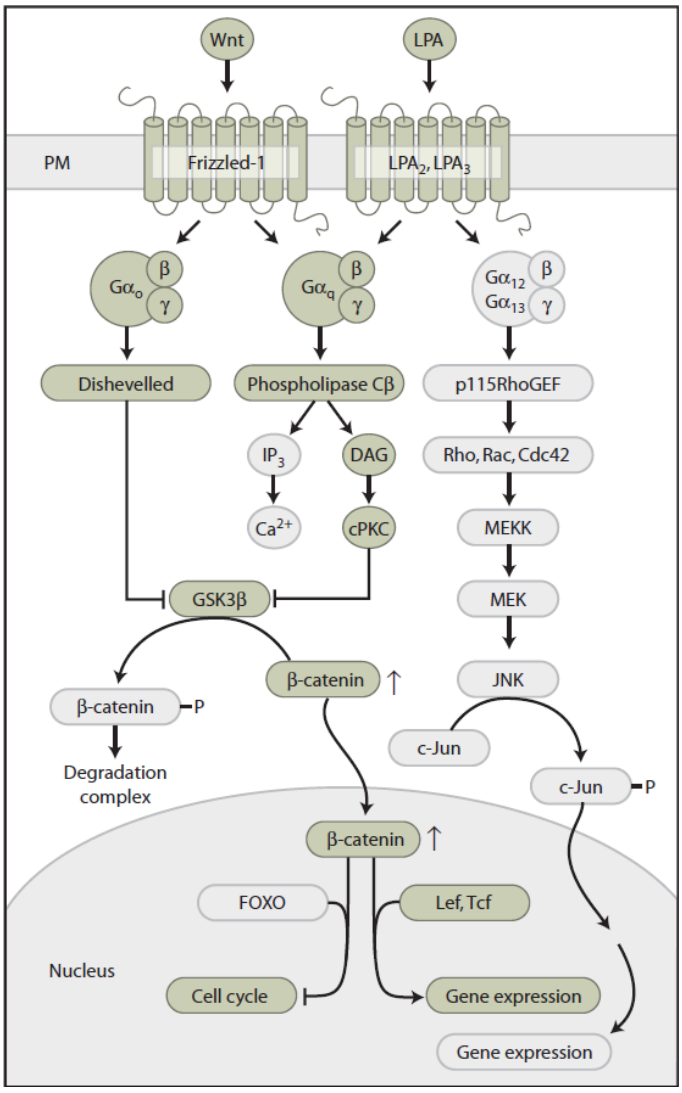

Figure 6. $LPA_{2/3}$ and Wnt signalling and both integrated in β-catenin activation. The cononical Wnt/Frizzlded-1 signalling pathway occurs through G_o which activates Dishevelled (Dvl). GSK3β can be inactivated by Dvl, stopping β-catenin degradation. $LPA_{2/3}$ and Frizzled-1 can both activate G_q, which can activate PLC to induce Ca^{2+} via PI3 and to synthesize diacylglycerol (DAG). Both Dag and Ca^{2+} can activate PKC to inhibit GSK3β. $LPA_{2/3}$ also activates $G_{12/13}$ which is able to induce RhoA and JNK/MAPK activity. JNK can phosphorylate c-JUN which can regulate various genes. Both β-catenin and c-JUN are responsible for cancer associated gene activity in colon cancer.
Figure from: **CC Malbon (2005) STKE**

LPA_2 and LPA_3 receptors, as indicated earlier, are also signalling via G-proteins and are highly expressed in colon cancer cells. Suppression of LPA_2 and LPA_3 receptors in HTC116 xenograft nude mice attenuated tumour growth.[79] *In vitro* research using the SW480 colorectal cancer cell line pointed to $G_{12/13}$ activation of RhoA as an inducer of metastasis.[80] The implication based on these data is that $G_{12/13}$ and $G_{q/11}$-proteins are mostly responsible for the LPA-induced signalling effects in colon cancer cells. Compared to

the Wnt signalling pathway, both LPA and Wnt signalling can activate G_q, which is thought to allow LPA to bypass Wnt in β-catenin activation. This mechanism provides a new explanation of how the β-catenin can be induced in colon cancer cells.[81] Activated G_q induces cytosolic PLC, resulting in DAG and IP3 production. IP3 increases the Ca^{2+} concentration in the cytosol. Both Ca^{2+} and DAG activate PKC, which can phosphorylate the Ser9 fragment at GSK3β making it inactive. As stated before, inactive GSK3β prevents β-catenin from degradation, resulting in β-catenin-induced gene transcription (Figure 2, 6).

In human colon and gastic cancer, the LPA receptor can also transactivate the cMet receptor. The natural ligand for cMet is secreted by hepatocytes and is available for liver cells to metastasise. The mechanism of transactivation is not well understood but is thought to occur via PKCδ or other subtypes. The signal transduction of cMet is associated with cell adhesion, migration and proliferation, and can be connected with β-catenin regulation. In the context of colon cancer, cMet might induce metastasisis. More research will establish its true mechanism, but it is sure that cMet can be trans-activated by LPA in colon and gastic cancer cells.

Activation of β-catenin leads to activation of lymphoid enhancing factor/T-cell factor family (Lcf/Tcf)-sensitive genes, and interaction with FOXO transcription factors.[82,83] The FOXO transcription factors respond to oxidative stress and inhibit the cell cycle while Lcf/Tcf-sensitive genes induce proliferation. These paradoxical findings make β-catenin regulated genes complex; the functions of β-catenin in colon cancer are still not well understood. However, β-catenin activation in colon cancer is associated with increased proliferation and metastasis. It is no longer just a matter of Frizzled receptor activity, but can also be linked to LPA signalling.

5. The role of the immune system in tumourigenesis

The role of our immune system in preventing cancer is recognition and destruction of malignant cells. Various anti-cancer therapies are focussed on this role of the immune system, like immune therapy. Some proteins expressed by cancer cell are not or less expressed by other tissues. Such proteins can be used for vaccination to induce immune reactions against cancer cells. Especially virus-induced tumours, like HPV (human pappiloma virus) induced cervical cancer, are suitable for such an approach. Other immune therapies are applied by injection of prepared antibodies, directed against a tumour associated protein, like trastuzumab for breast cancer. The same approach is also under investigation by using prepared T-cells to a comparative treatment. The application of the immune system for therapy suggests that the immune system is a reliable ally in the fight against cancer. Anyway, scientific research revealed also another side of the immune

system. Chronic inflammation, which causes tissue damage and long-term cytokine exposure to surrounding tissues, is known to cause and progress tumours. Additionally, immune cells present in the tumour stroma do not attack the cancer cells but participate in secretion of growth factors and growth-associated cytokines. Here, the immune system can initiate and induce tumourigenesis. With the immune system helping tumours, it should be targeted by drugs itself instead of stimulating it for immunotherapy. These contradictions show that the interaction between the immune system and cancer is complex and cannot easily be simplified. LPA has been described as both a mediator in immunity and in cancer. The role of LPA in the interaction between cancer and immune system is not obvious but it can be an important link in tumourigenesis.

5.1 Inflammation-induced tumourigenesis

It was already stated over 150 years ago by Virchow that inflammatory cells are present within tumours and that tumours can arise from sites of chronic inflammation. Current epidemiology points out that approximately 25% of all cancer cases are caused by chronic inflammation.[84] The meaning of chronic inflammation is most often translated into bacterial or viral infections; but allergy, toxicology, auto-immune diseases and even obesity can be sources of chronic inflammation as well. The number of diseases related to cancer is increasing, while the association of diseases with inflammation is also increasing. Most mechanisms of inflammation causing cancer are based on the chronic and constant disruption made by the immune reactions of surrounding tissue. In contrast, a well-regulated and acute immune response, to a virus for example, can be even beneficial for clearance of imbalanced or malignant cells.[85] This becomes different when inflammation gets out of control and becomes chronic and de-regulated, which frequently predisposes cells for oncogenic transformations.[86]

The basic mechanism for a cell to become a cancer cell is based on genetic mutations. Specific genes have to be mutated, enhanced in expression or destroyed to provide a cell the 'hallmarks of cancer'. These genetic alterations do induce expression of many inflammatory genes, such as cytokines, which attracts immune cells to the tumour. One example is the *RAS* proto-oncogene. When dominantly mutated it induces proliferation and inflammation. RAS signalling is discussed above. It is dominant active in approximately 25% of all malignancies, known as the KRAS mutation.[87] This explains the full name of RAS, namely 'rat sarcoma oncogene'. The effects of permanently active KRAS are not restricting to the cell carrying the mutation. KRAS signalling activates pro-inflammatory genes of IL-1β, IL-6 and IL-8 creating a pro-inflammatory and pro-tumourgenic micro-environment around the cell.[88] Surrounding tissue is stimulated to induce proliferation, angiogenesis and

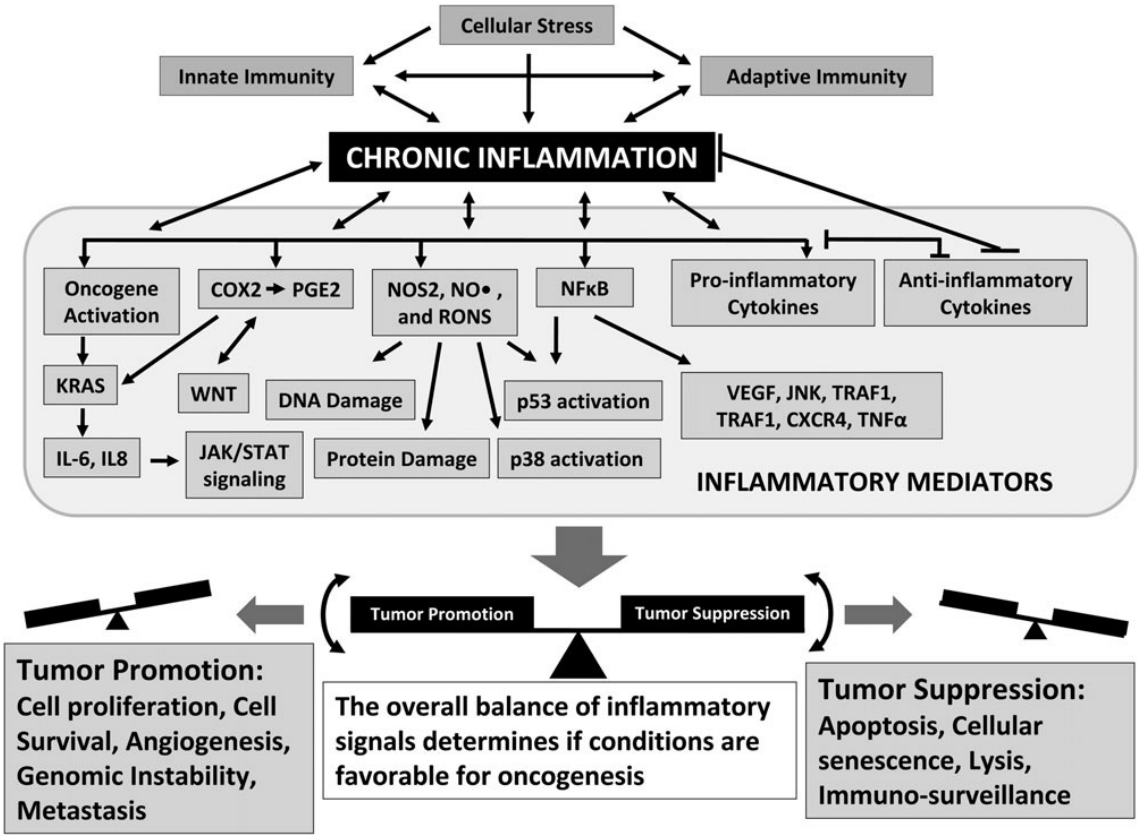

Figure 7. The effects of chronic inflammation-induced signal pathways to suppress or promote tumourigenesis. Many factors of chronic inflammation, such as growth factors or cytokines, are potent activators of signal transduction. Gene activation and cellular response is depending on physiologic background, cell type and combination of active signal pathways. It is necessary that immune responses are capable enough to destroy al malignant and damaged cells which are a treat. In this way tumourigenesis can be suppressed. Otherwise, malignant cells survive and damaged cells mutate, and then tumourigenesis will be promoted by the mutations and al the survival and growth factors secreted by immune cells.
Figure from: **Schetter AJ *et al.* (2010) Carcinogenesis**

inflammation resulting in recruitment of immune cells and growth factor synthesis and secretion. This response of surrounding tissue generates a positive feedback loop. Another example of such a mutation is inactivation of the tumour suppressor gene PTEN. When both alleles are inactive, it results in the loss of function of feedback on PI3K signalling. This results in transcription of anti-apoptotic genes en inflammation as well. Many of these carcinogenic signalling pathways end up in activation of transcription factors such as NF-κB. The NF-κB dimers can regulate many genes, directly or indirectly, involved in proliferation, survival and inflammation. Survival is induced by many anti-apoptotic genes such an inhibitor of apoptosis (IAP) or cellular FLEECE inhibitory protein (cFLIP), which inhibit caspase-8 cleavage.[89] The caspase signalling route is essential to activate apoptosis and induces anti-tumour genes such as *p53*. NF-κB is also capable to induce metastasis by activating and inducing chemokine receptor-4 expression. As stated before, NF-κB regulates many

inflammatory genes such as cytokines and enzymes like COX-2 and nitric oxide synthase-2 (NOS-2). (Figure 7)

Most discussed effects of inflammation contribute to tumour progression by stimulating cell proliferation, survival and further inflammation. However, inflammation can also be the cause of cancer by initiating mutations in healthy cells. Various factors, produced by active inflammatory cells, can damage DNA. Most DNA-damaged cells do not further divide and end up in apoptosis or necrosis. Some mutations can lead to dominant oncogenes or to loss of function of tumour suppressor genes, introducing malignancies.[90] The rate of malignant mutations is low but will increase when the exposure period to DNA damaging factors increases, as occurs during chronic inflammation. Two important inflammatory mediated DNA damaging factors are reactive oxygen and reactive nitrogen specious (RONS).[91] As the full name of RONS indicates, these are highly reactive radicals that can induce damage by reacting with many molecules. This results in the transfer of an electron of this molecule to the radical, neutralising the radical while the affected molecule becomes the new radical. When this reaction reaches the DNA of the cell it can cause single or double strand breaks, or cross-linking of nucleotides.[92] This DNA damage can be the basis of genomic instability or mutations. One of the mechanisms of reaching the DNA by RONS is lipid peroxidation of membrane lipids. These oxidised lipids can form malondialdehyde or 4-hydroxynonenal, which can form DNA adducts and random point mutations in the genome. The RONS induce also oxidative stress in cells resulting in inflammation and production of RONS by the cells them self, especially when oncogenes are activated. For example, RONS can be induced by inflammation via NF-κB mediated transcription of the NOS-2 enzyme, which produces reactive nitrogen specious.[93] The mutagenesis activity of RONS is associated with inactivation of the Rb and p53 genes, and induction of dominant RAS mutations. (Figure 7)

Many therapies are still based on induction of inflammation, although this can lead to unbeneficial results. Immune therapies aim to create a controlled and acute immune response against the tumour. On the other hand, control of the present chronic inflammation reactions in tumours is needed. Therefore, the search for the ideal approach of the immune system to strike cancer effectively is not completed. This requires more knowledge of the role of the immune system in cancer.

5.2 The role of LPA in inflammation induced cancer

LPA has been discussed as an inducer of tumourigenesis and inflammation. Subsequently, inflammation was discussed to induce tumourigenesis as well. A logic hypothesis is that LPA also plays a role in tumour associate chronic inflammation, which would make LPA a foe in

the fight against cancer. Some cancer induced inflammation is LPA mediated, like gastric and colon cancer types.

Gastric cancer causes ulcers which are associated with bleeding in the stomach. This bleeding introduced platelets to the tumour. Activated platelets express ATX and synthesise LPA. Many gastric cell lines express identified LPA receptors: differentiated cells preferentially express LPA_2 while more aggressive undifferentiated cells express more LPA_1.[94] Gastric tumours were not driven to metastasise by LPA_2 signalling. Metastasis is induced, which is caused by transactivation with hepatocyte growth factor receptor c-Met. Furthermore, LPA_2 signalling also transactivates the EGF receptor in gastric cancer, which contributes to the expression of sphingosine kinase-1 (SphK1).[95] This enzyme is associated with carcinogenesis in gastric cancers, but its exact role and mechanism is not well understood.

Colon cancer can be induced by inflammatory bowel disease (IBD), which causes chronic inflammation in the colon.[96] Additionally, IBD worsens the prognosis in colon cancer which is probably due to enhanced metastasising by the inflammation. Mouse models with colitis induced colon cancer by azoxymethane and dextran sulphate sodium, revealed less carcinogenesis in LPA_2 knockouts.[97] Control mice had a higher tumour incidence, epithelial proliferation and infiltration of immune cells. In addition, these LPA_2 knockouts also showed less inflammation like macrophage infiltration, COX-2 secretion and cytokines. The signal transduction of β-catenin was also attenuated in colon cancer cells of LPA_2 knockouts. As stated before, β-catenin signalling is an important mediator for the progression and metastasising of colon cancer. The fact that only a LPA_2 receptor knockout can have such an effect shows its importance and relevance as driver of tumourigenesis.

Both gastric and colon cancer demonstrate that LPA-induced signalling is associated with inflammation and tumourigenesis. Inflammation is regulated by many factors that share relevance for tumourigenesis as well, just like LPA does. Here, more studies showed that LPA signalling alone already has an effect on both inflammation and tumourigenesis, which are tightly connected to each other.

6. Conclusion

LPA has been discussed in the context of tumourigenesis and inflammation, and how both processes can affect each other. It has been demonstrated by various studies that relevant signalling pathways can be affected by LPA. The fact that LPA is a simple molecule and can be synthesised from basic membrane phospholipids make it a quick and effective inducer during inflammation and tumourigenesis. Expression of ATX alone is enough to increase plasma LPA levels because of the presence of plasma serum LPC. Many inflammation associated diseases, like auto-immune diseases or even atherosclerosis, are associated with LPA. As mainly discussed in this thesis, cancer and cancer-induced chronic inflammation can also be associated with LPA. This makes ATX, LPA receptors and associated signal transduction pathways ideal targets for various drug therapies in a large range of pathologies. For cancer and inflammation, a better insight into how inflammation interacts with tumours and how this can be regulated will form an important challenge for future research. LPA is only one of the many players involved in inflammation and cancer, but it can possibly act as a first step in the regulation of the immune system to fight tumour progression.

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