# 中文摘要

水解磷酸脂 Lysophosphotidic Acid (LPA)與 Sphingosine 1-Phsophate (S1P)均為低分子量之水解磷酸脂(Lysophospholipids)，藉由活化Edg (endothelial differentiation gene)族受器進而調控各種細胞生理活性，其中包括發炎反應的調控。於本研究第一部份中發現S1P或LPA於內皮細胞中各別調控之ICAM-1 mRNA的表現，與之後的單核球與內皮細胞層黏著現象，分別經由$S1P_1$或$LPA_1$受器作用。另一方面，S1P或LPA於內皮細胞中調控之IL-8 與MCP-1 的mRNA表現，與之後的單核球趨化至內皮細胞層之現象，是經由活化$S1P_1$、$S1P_3$或$LPA_1$、$LPA_3$受器。此外，LPA與S1P同時能夠刺激IL-1β於內皮細胞中的表現，並且有著時間依賴性的現象。藉由前處理IL-1 受器抑制劑或是IL-1β中和抗體均能夠明顯的抑制LPA與S1P於內皮細胞中對IL-8 與MCP-1 的提昇效果。這些結果證實了LPA與S1P於內皮細胞中對於IL-8 與MCP-1 mRNA表現的提昇效果至少必須仲介IL-1 的表現。此外，在我們先前的研究中發現到$LPA_1$基因剔除斑馬魚胚胎中，淋巴系統之生成受到明顯的影響，進一步的推測LPA很有可能是一種淋巴血管形成因子。我們在本研究中發現到LPA是經由COX-2 的活化提昇內皮細胞表現VEGF-C，進而調控在體內或體外形成的管狀構造。此外，這些管狀構造物呈現出淋巴管特異抗原表達。而且，這些淋巴管構造之形成是經由$LPA_1$與$LPA_3$受器活化所達成。結果亦顯示此一調控現象是經由EGFR-transactivation機制所仲介。此外，本研究中也發現S1P能夠提昇TIMP-2 及TIMP-3 mRNA之表達而LPA與S1P同時能夠提昇MT1-MMP蛋白質在內皮細胞中的表現量與活性之上升。總而言之，第一部分的研究成果能夠提供我們許多針對發炎反應與動脈硬化症治療新藥的開發，第二部份的成果則是首次提出LPA可能是淋巴血管增生因子，期待做為未來之抗癌症轉移治療提供相關之基礎知識。

關鍵詞：水解磷酸脂、內皮細胞、發炎反應、血管新生、淋巴血管形成

## Abstract

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are both low-molecular-weight lysophospholipid (LPL) ligands which are recognized by the endothelial differentiation gene (Edg) family of G protein-coupled receptors. In this study, I demonstrated that the enhancement effects of S1P on intercellular adhesion molecule (ICAM)-1 mRNA, total protein and cell surface expression in human umbilical vein endothelial cells (HUVECs) are mediated through the activation of $S1P_1$. On the other hand, the S1P-dependnet increase in increased interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 mRNA expression in HUVECs which were mediated by both $S1P_1$ and $S1P_3$. Moreover, $S1P_1$ expressed on endothelial cells mediates the positive regulation of monocytes adhesion and chemotaxis toward the endothelium, whereas $S1P_3$ is only critical the chemoattraction of monocytes by endothelial cells. These findings suggest that $S1P_1$ and $S1P_3$ might be essential receptors for S1P in modulating monocyte-endothelial cell interactions. Furthermore, $LPA_1$ might mediate the enhancement effects of LPA on ICAM-1 mRNA expression and subsequent monocyte/endothelium adhesion. In addition, both $LPA_1$ and $LPA_3$ mediated LPA-enhanced IL-8 and MCP-1 mRNA expression and the subsequent chemoattraction of monocytes toward the endothelium. My study also demonstrated that LPLs increase IL-1 mRNA expression in HUVECs, and the enhancement effects of LPLs on IL-8 and MCP-1 mRNA expressions were at least partially mediated by IL-1. We observed that the knocking-down of $LPA_1$ in zebrafish embyo profoundly affected the lymphatic vessel formation. In the third part of the thesis, results implied that LPA might regulate the lymphangiogenesis process. My data demonstrated that LPA might participate in the regulation of the lymphangiogenesis process. I demonstrated that LPA enhanced vascular endothelial

growth factor (VEGF)-C mRNA expression through a cyclooxygenase (COX)-2-dependent mechanism, thereby inducing the endothelial cell tube formation both *in vitro* and *in vivo*. This induction of tube formation was possibly stained with the lymphatic vessel marker, prospero-related homeobox gene (prox)-1. $LPA_1$ and $LPA_3$ are required for LPA-induced HUVECs tube formation *in vitro*, whereas $LPA_3$ mediated mouse endothelial cells tube formation *in vivo*. These enhancement effects were EGFR transactivation-dependent. Finally, S1P upregulated tissue inhibitor of metalloproteinase (TIMP)-2 and TIMP-3 mRNA expression and both LPA and S1P stimulated elevations of mt-1-matrix metalloproteinase (MT1-MMP) protein expression and activity elevation in HUVECs in dose- and time-dependent manners, which modulate leukocytes-endothelial cell's extracellular matrix attachment and subsequent angiogenesis processes. The first half of this thesis provides valid information for possibly developing new therapeutic drugs against LPLs receptors to control the inflammation process and therefore atherosclerosis formation. The second half of study first reports that LPA might be a pro-lymphangiogenic factor, which may provide information for therapeutics against tumor metastasis.

Keywords: LPA, S1P, endothelial cells, inflammation, angiogenesis, lymphangiogenesis