京都大学	博士（　医　学　）	氏名	LIU LIN（　柳　林　）
論文題目	Discovery of lipid profiles in plasma-derived extracellular vesicles as biomarkers for breast cancer diagnosis （血漿由来細胞外小胞内の脂質プロファイルに注目した乳癌診断バイオマーカーの発見）		

（論文内容の要旨）

According to the latest Global Cancer Statistics in 2020, breast cancer (BC) ranked first in both incidence and mortality among female cancers. Early diagnosis can improve BC patients' overall survival. While mammography and ultrasonography are commonly used for early BC detection, their accessibility and accuracy can vary across populations. Liquid biopsy is gaining interest because it can facilitate non-invasive and real-time diagnosis of BC. Extracellular vesicles (EVs), also referred to exosomes, are 30 to 150 nm vesicles released from cells into body fluids which can carry the information of parental cells and regulate distant cell activities. Although research has shown that proteins and miRNAs within blood-derived EVs have potential as biomarkers for BC, it remains unclear whether lipid profiles of blood-derived EVs can also serve as diagnostic or predictive biomarkers for BC. In the previous study using imaging mass spectrometry, BC tissues within the human breast exhibit distinct lipid profiles in accordance with their progression.

Therefore, this study aimed to investigate whether the alterations of lipids in BC tissues can be reflected in those of circulating EVs, and whether these alterations can be used as biomarker for BC or its stages and subtypes.

Since there was no reliable and established method of EV isolation for lipidomic analysis, this study initially conducted a comparison among the ultracentrifugation, sucrose cushion ultracentrifugation (CUC), and size exclusion chromatography methods for isolating plasma-derived EVs. Based on the assessment of EV yield and purity using transmission electron microscopy, nanoparticle tracking analysis, and western blotting, the CUC method proved to be superior in isolating plasma-derived EVs for lipidomics since it could minimize lipoprotein contamination with excellent efficacy of isolation. Then, the lipid profiles of plasma-derived EVs, which can be used as biomarkers for BC diagnosis, were explored by targeted lipidomic analysis covering 380 lipid species. A total of 159 blood samples from two independent cohorts were used, comprising 126 cases in cohort 1 and 33 cases in cohort 2, including BC patients (n = 105), benign breast tumor patients (n = 11), and healthy individuals (n = 43). Finally, a BC diagnostic model based on three specific lipids, PS_C36:1, THC_16:0, and PC_C36:3, was established. The model exhibited favorable performance with an area under the receiver operating characteristic curves of 0.759, 0.743, and 0.804 in the training, internal validation, and external test sets, respectively. In addition, the distinct lipid signatures that could discriminate tumor subtypes and stages were also identified.

In conclusion, this study established a robust workflow for targeted lipidomic analysis using plasma-derived EVs. By using this workflow, specific lipid profiles were identified that can diagnose BC and predict its stage and subtype. Importantly, this study shows that the lipid profiles of plasma-derived EVs can generate potential biomarkers for BC, suggesting a new way for liquid biopsy of BC. Further investigations with larger cohorts, broader coverage of lipid species, and more cancer-oriented EV isolation methods are warranted to optimize the model's efficacy and to gain deeper insights into tumor lipid metabolism.

（論文審査の結果の要旨）

乳がん組織内の脂質組成は疾患進行に応じて変化することが示されている。血液中細胞外小胞（EV）の分子プロファイリングによる乳がんバイオマーカー開発が近年注目を集めているが、EV 中の脂質を対象とした研究は少ない。本研究では、ヒト血漿中 EV の脂質プロファイリングによって乳がん診断モデルが構築可能かを検討した。

まず、EV の収量と純度、食事による影響を異なる EV 精製法間で比較評価し、スクロースクッション遠心法が血漿中 EV の脂質分析に最も適することを確認した。評価には、透過型電子顕微鏡、ナノ粒子トラッキング分析、ウエスタンブロッティング等を用いた。次に、乳がん、乳腺良性疾患、健常者からなる独立した 2 コホート（計 159 検体）を用いて血漿中 EV の脂質分析を行った。分析は計 380 種の脂質を標的に、LC-MS の手法で行った。結果、PS_C36:1、THC_16:0、PC_C36:3 の 3 脂質の違いによって乳がんの有無を診断できるモデルを構築した。同モデルは訓練データ：ROC=0.759、検証データ：ROC=0.743、テストデータ：ROC=0.804 と良好な性能を示した。探索的解析で、腫瘍のサブタイプとステージも脂質組成の違いによって区別できる可能性が示された。

本研究で構築した血漿中 EV 脂質組成解析フローは測定信頼性が高く、乳がんの有無の診断だけでなく、ステージやサブタイプの診断にも有用な可能性が示された。また、乳がんの病態に応じた脂質のダイナミックな変化が示唆され、がんの脂質代謝機構を用いた診断技術の開発に寄与するものと考えられた。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　5　年　11　月　13　日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降