Lipid signaling and inflammation: metabolomics for better diagnosis and treatment strategy

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Appendix

Summary

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Curriculum Vitae

List of Publications

Acknowledgements

Summary

Inflammation is a part of the body's defense and is triggered by many factors such as injuries, pathogens and effects of chemicals and radiation. It is a protective response aiming for eliminating deleterious stimulus, promoting homeostatic restoration and in the case of infection, establishing memory to achieve a faster action upon future exposure. As a biologically dynamic process, inflammatory response is characterized by a chain of cellular activities and molecular regulations. For instance, lipids, during inflammation, are released from cell membranes and modified to generate a variety of lipid mediators that regulate the initiation, progression and resolution of inflammation. Proper regulation of lipid signaling is crucial for maintaining immune homeostasis and physiological functions and dysregulation of lipid signaling has been increasingly recognized as a key player in the development of various diseases. The realization of biological roles and biomedical values of signaling lipids has greatly motivated the development of analytical approaches for signaling lipid profiling. However, currently existing methods are either not covering a wide range of signaling lipids or not sensitive enough to probe critical molecules in biological samples. In addition, the exact roles of lipid molecules in inflammation are complex and dependent on specific lipid species as well as biological context. The intricate interplay between classes of lipid molecules has not yet been fully elucidated. Therefore, the scope of this thesis is to firstly develop a better method for signaling lipid profiling and secondly to explore the biology of these molecules in various inflammatory related conditions to provide further knowledge into the roles of signaling lipids in health and disease.

Chapter I introduced metabolomics and lipidomics as well as classes of signaling lipids studied in this thesis. This chapter also described the scope of the whole thesis by giving an overview of the following research chapters.

Chapter II described the development and validation of a method targeting a variety of signaling lipids using LC-MS/MS. This metabolomics platform enables the comprehensive profiling of different signaling lipid classes including lysophospholipids, sphingoid bases, platelet activating factors, fatty acids, oxylipins, endocannabinoids, bile acids and several resolvins. The method performance was characterized by a series of validation parameters including linearity, limit of detection, limit of quantitation, precision, recovery and matrix

effect. The method was then utilized to quantitate signaling lipids in NIST SRM 1950 plasma and pooled human plasma, which demonstrated it was comprehensive and robust with higher sensitivity compared to published methods especially in profiling prostaglandins, isoprostanes and specialized lipid mediators. Quantitative data demonstrated comparable metabolite concentrations to reported values and provided for the first time absolute concentrations of 37 signaling lipids in NIST 1950 plasma.

The second part of the thesis presented applications of our analytical method in clinical metabolomics studies. **Chapter III** described a metabolomics study in patients from two cohorts with mild cognitive impairment (MCI). MCI is a prodromal stage of Alzheimer's disease (AD) and may precede the onset of clinical AD. Emerging research has suggested that lipid signaling pathways are dysregulated in AD and MCI, and these lipid alterations can contribute to the pathogenesis of these conditions. The apolipoprotein E (APOE) gene is recognized as a well-established risk factor for AD, but how lipid profiles are regulated by APOE is not fully understood. Focusing on 16 isoprostanes and 12 prostaglandins, we performed a metabolomics study using patient plasma and CSF and evaluated the associations of these lipid molecules with CSF levels of ATN biomarkers (Aβ-42, p-tau, and t-tau) in the overall sample and stratified by the APOE genotype. The results demonstrated CSF levels of two isoprostanes (5-iPF2α VI; 8,12-iso-iPF2α VI) and 1 prostaglandin (PGF2α) were positively associated with p-tau and t-tau. In APOE stratified analysis, 8,12-iso-iPF2α VI showed a positive association with both p-tau and t-tau in APOE ε33 carriers and a positive association with t-tau in APOE ε3 carriers. PGF2α and 5-iPF2α VI were positively associated with p-tau and t-tau in only APOE ε4 carriers and in only APOE ε33 carries respectively. The roles of these metabolites in MCI to AD progression were further investigated, but none of the metabolites showed evidence of association. In conclusion, we demonstrated APOE specific correlations between studied lipid mediators, and clinically validated markers of AD. Therefore, our research contributed to a deeper understanding of APOE related pathology in AD and unraveled the metabolic characteristics of inflammatory lipid mediators associated with the APOE genotype.

In **Chapter IV**, we explored the role of signaling lipids in the development of an autoimmune disease ocular mucous membrane pemphigoid (MMP). Ocular MMP is characterized by the formation of blisters and erosions on the affected mucous membrane

of the eyes, causing chronic inflammation and damage to the conjunctiva. In this study, we profiled conjunctival impression cytology derived samples, aiming to identify potential biomarkers for better diagnosis of ocular MMP and providing promising drug development targets. This proof-of-concept metabolomics study assessed volume limited cytology samples and demonstrated the metabolic changes of signaling lipids upon disease onset and progression. By comparing healthy controls and patients, we identified a panel of oxylipins, lysophospholipids, fatty acids and endocannabinoids which showed significantly different levels in conjunctival biopsies of ocular MMP patients. Increasing levels of oxylipins and lysophospholipids were observed along with disease progression. Based on these, underlying enzymatic mechanisms were also hypothesized and discussed in this chapter.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation and joint damage. Early diagnosis and treatment initiation are crucial in RA to achieve optimal outcomes. However, the response to RA treatment can vary widely among individuals which leads to challenges in choosing the most effective clinical therapy. **Chapter V** studied the roles of baseline metabolites prior to drug treatment in predicting the response to tocilizumab (TCZ) based and methotrexate (MTX) based treatment in early rheumatoid arthritis (RA) patients. By performing metabolomics profiling of signaling lipids and related metabolites from patient serum, we successfully identified baseline metabolic profiles predictive of good response to specific treatment, i.e., monotherapy of TCZ, monotherapy of MTX and combined therapy of TCZ and MTX. Pathway visualization was presented by including identified metabolites in each strategy arm. The results demonstrated that relevant metabolic pathways indicative of achieving sDFR were therapy arm specific. In TCZ plus MTX arm, “histidine metabolism”, “sphingolipid metabolism” and “arachidonic acid and linoleic acid metabolism” were most relevant. In TCZ arm, “arachidonic acid metabolism”, “lysine degradation” and “cysteine and methionine metabolism” were most relevant. In MTX arm, most metabolites were associated with the “arginine and proline”- and “histidine metabolism”- pathway. In addition, network analysis integrating metabolites, proteins and transcripts provided treatment arm specific correlations between transcripts and proteins as well as between proteins and metabolites, Signature biomarkers were successfully identified which could potentially serve as prognostic factors for developing personalized care in early RA.

Lipid signaling also plays a pivotal role in exercise physiology which may potentially involve inflammation and oxidative stress. Understanding signaling lipids responsive to exercise will provide guidance for optimizing exercise performance and improving overall health outcomes related to exercise training. **Chapter VI** investigated the role of different chronic exercise training methods on regulating signaling lipids, focusing on oxylipins and endocannabinoids. The research was based on a randomized controlled trial studying the effects of a 12-week chronic exercise intervention on middle-aged sedentary adults. Plasma metabolomics profiling was performed to compare oxylipin and endocannabinoid profiles in four exercise groups: no exercise (control group), concurrent training based on international physical activity recommendations (PAR group), high-intensity interval training (HIIT group) and HIIT with whole-body electromyostimulation (HIIT+EMS group). It was found that 12 weeks of PAR, HIIT, and HIIT +EMS showed a decreasing trend in plasma levels of oxylipins while the different exercise interventions did not alter the plasma levels of endocannabinoids. On the other hand, metabolite changes in each exercise group were compared to the changes in control group, no statistical significance was identified between exercise groups. Therefore, we concluded that upon different chronic exercise training modalities, changes of oxylipin and endocannabinoid profiles were not significantly different between different types of chronic training methods in middle-aged sedentary adults.

The conclusions from the individual chapters were presented in **Chapter VII**. This chapter also discussed chapter specific perspectives as well as overall envisions for future studies on signaling lipids.