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Keio University

Thesis Abstract

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Registration Number	□ “KOU” □ “OTSU” No. *Office use only	Name	Ryosuke Hayasaka

Thesis Title

Development of the metabolomic analysis method for extracellular vesicles and its application to cancer cell profile analysis

Thesis Summary

Small extracellular vesicles (sEVs), including exosomes released by cancer cells, play an important role in cancer progression via angiogenesis and the formation of the premetastatic niche. sEVs are lipid bilayer-bound vesicles that contain a variety of molecules, including proteins, lipids, and nucleic acids such as deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), and micro ribonucleic acid (miRNA). Although the relationship between sEVs and cancer has been analyzed extensively, the hydrophilic metabolites contained in sEVs remain unknown. In this study, I constructed a metabolome analysis system for sEVs released by cultured cancer cells to elucidate whether the cancer microenvironment or genetic mutations alter the metabolite content in sEVs. Metabolomic analysis of sEVs recovered from the human pancreatic cancer cell line PANC-1 by ultracentrifugation (UC) revealed that the sEVs contained 140 hydrophilic metabolites and 485 lipids (Chapter 2). The metabolomic profile of the cells differed from that of the sEVs. Hypoxic stress under 1% O2 altered the metabolite profile of sEVs and increased the loading of angiogenesis-associated metabolites (Chapter 2). However, UC requires a large amount of medium and is unsuitable for the pretreatment of multiple samples. Therefore, I established a semi-automated preparative method based on size-exclusion chromatography (SEC) to improve the recovery of sEVs (Chapter 3). This SEC method, followed by UC, was used to recover sEVs from the human colon cancer cell line HT29 cells. I found that differences in the recovery method resulted in variations, mainly in purine-pyrimidine metabolism, in the sEVs. Furthermore, sEVs derived from mutant strains of isocitrate dehydrogenase 1 (IDH1) contained large amounts of 2-hydroxyglutaric acid (2-HG), an oncometabolite involved in carcinogenesis and cancer progression, suggesting that sEVs may contain oncometabolites (Chapter 4). These results indicate that the metabolite profile of cells and sEVs differ and that the metabolite profile of sEVs is altered by genetic mutations and microenvironments, such as hypoxia. This study contributes to a better understanding of the mechanism of sEV-mediated oncogenic transformation.

Keywords: Cancer, Small extracellular vesicles, Metabolomic analysis, Lipidomic analysis, Hypoxic stress, Isocitrate dehydrogenase 1 mutation