School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

The Role of Extracellular Vesicles in the Transfer of Oncogenes and Retrotransposons in Cancer

（細胞間の遺伝情報伝達における細胞外小胞の役割）

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Abstract

Introduction

Extracellular vesicles (EVs) represent a heterogeneous population of cell-derived vesicles that contain cellular components such as proteins, lipids, metabolites, and nucleic acids. Upon their release in the extracellular environment, they can be delivered locally and systemically to interact with other cells in in the body. EVs are capable of affecting the phenotype of recipient cells, by ligand-receptor interactions with the target cell membrane or through the release of their molecular constituents upon uptake at recipient cells. They have been recognized as important mediators of intercellular communication, both in physiological and pathological states. EVs are found in most human biological fluids, including blood, urine, breast milk, cerebrospinal fluid, saliva, and sweat. A growing body of functional studies has provided strong evidence that disease-specific markers can be identified from EVs well before the onset of symptoms, making them promising targets for diagnostic and monitoring applications for a variety of diseases. As these entities can act as paracrine mediators, a close examination of their molecular composition and functions in recipient cells could be beneficial in obtaining a more comprehensive picture of molecular processes involved in the spread of disease-derived EVs in the human body, which may help to identify new pathways involved in various pathological processes. Therefore, the overall aim of this PhD work was to assess the genetic composition and function of EVs from cancer cells, and to assess their activity in the propagation of cancer-associated genes and in recipient cells.

The main section of this thesis explores the roles of extracellular vesicles in the transfer of oncogenic DNA

sequences and retrotransposon RNA in recipient cells, and has been discussed in detail in chapters 2 and 3. The final section of this thesis examines the clinical relevance of extracellular vesicles in the detection of cancer-associated genes using patient-derived blood samples, and explores how these findings can be applied in the development of liquid biopsy platforms. These findings have been discussed in detail in chapter 4. Below is a general summary of these chapters.

EVs contain RNA but DNA is found on the outer membrane of EVs

Chapter 2 discusses the presence of EV-associated DNA from cancer cell-derived EVs. In this study, it was found that DNA is predominantly found on the outer surface of EVs isolated from cancer cells. The presence of DNA-associated with circulating EVs (termed EV-associated DNA) has potential applications in detecting genetic mutations or cell-specific molecular signatures. EV-associated DNA and DNA extracted from HCT116 cells were analyzed and it was found that the heterozygous KRAS mutation is present in EV-associated DNA from HCT116 cells. To assess the ability of EVs to deliver oncogenic DNA to recipient cells, HCT116 EVs or the culture supernatant of HCT116 cells were applied to NIH 3T3 cells. NIH 3T3 cells incubated with HCT116 EVs or the culture supernatant of HCT116 cells underwent profound morphological change, involving the formation of foci. In contrast to this, only a small number of spontaneous foci appeared in culture of NIH 3T3 cells when HCT116 EVs were pretreated with DNase I.

EVs mediate the horizontal transfer of an active L1 retrotransposon

In Chapter 3 it was demonstrated that RNA transcripts derived from an active human L1 retrotransposon are packaged in EVs and can initiate retrotransposition in EV-recipient cells. This study provides evidence that an active L1 retrotransposon can be transmitted to neighboring cells without direct cell-to-cell contact mediated by EVs secreted by the cell. Additionally, it was shown that EV transfer can influence transcriptional and post-transcriptional regulatory mechanisms in recipient cells. L1-derived RNA transcripts and translated proteins are targeted by intrinsic host factors such as APOBEC3 family members, potentially restricting L1 activity to ensure genome stability in the cell.

L1 transcripts are elevated in EVs from CRC patients

Chapter 4 introduces the clinical relevance of L1 retrotransposons and their activities in the context of cancer development. In this study, protein and RNA expressions of L1 in colorectal tissue were assessed using in silico methods. It was found that L1 retrotransposons are significantly overexpressed in colorectal cancer tissues compared to normal colorectal tissues. L1 expression was also found to be associated with precancerous conditions preceding CRC development, such as colorectal adenoma, Crohn's disease and ulcerative colitis. These findings suggest that increased L1 activity and subsequent L1 insertions could be involved in early stages preceding CRC development. To examine whether L1 RNA could be detected from circulating EVs, serum samples from CRC patients and healthy controls were processed by ultracentrifugation to purify EVs. Total RNA was extracted from EVs and analyzed for L1 RNA transcript

levels. It was found that EVs from CRC patients have significantly elevated levels of L1 ORF1 and ORF2 RNA transcripts compared to EVs from healthy controls. These results could be reflective of the hypomethylation status and provides additional support for the clinical importance of EVs as disease biomarkers.

Conclusion

The findings presented in this thesis suggest an EV-mediated mechanism of intercellular communication and horizontal gene transfer in the cancer microenvironment. These findings represent additional functions of EVs in cell-to-cell communication, and may have important implications for the intercellular regulation of gene expression and gene function. EVs are packaged with abundant cellular information that has been shown to reflect the status of the cell, however, the mechanisms that regulate EV cargo selection is still largely unknown. Further investigation into the biogenesis and cargo selection of EVs may elucidate many of the biological processes that are regulated by EVs. Despite their widespread attention in the scientific field, the detection of target EVs from the total EV population from biological samples remains a challenge. The use of EVs as biomarkers for disease in liquid biopsy is a promising field, and overcoming the technical difficulties involved in the isolation and detection of EVs from biological samples would be beneficial in monitoring health and detecting diseases at an early stage.