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学位論文題目	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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論文の要旨
Abstract of thesis

In this doctoral dissertation, Yumi Kawamura describes the role of extracellular vesiclesin the transfer of oncogenes and retrotransposon in cancer. The summary is as follows:

Purpose

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 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 work by the

applicant 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.

Materials and Methods

To explore the roles of extracellular vesicles in the transfer of oncogenic DNA sequences and retrotransposon RNA in recipient cells, the applicant isolated the EV from HCT116 cells andtransfected into NIH 3T3 cells and investigated the transformation. The applicant also isolated the EV from human cancer cells with an expression construct containing a retrotransposition-competent human L1 tagged with a reporter gene, and transfected into recipient cells and examined thehorizontal transfer of an active L1 retrotransposon. To examine the clinical relevance of extracellular vesicles in the detection of cancer-associated genes using patient-derived blood samples and to explore how these findings can be applied in the development of liquid biopsy platforms, endogenous L1 retrotransposons RNA transcript abundance in EVs isolated from cancer cells and cancer patient serum was evaluated as an alternative method to detect the transcriptional activation of L1 in colorectal cancer.

Results

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

In this study, the applicant 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. The applicant analyzed EV-associated DNA and DNA extracted from HCT116 cells and 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.

2. EVs mediate the horizontal transfer of an active L1 retrotransposon

The applicant also 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.

3. L1 transcripts are elevated in EVs from colorectal cancer (CRC) patients

The applicant further assessed protein and RNA expressions of L1 in colorectal tissue using in silico methods. She found that L1 retrotransposons are significantly overexpressed in CRC 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. She 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 cells. 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.

審査の要旨
Abstract of assessment result

【批評 Review】

EVs contain cellular components such as proteins, lipids, metabolites, and nucleic acids and are recognized as important mediators of intercellular communication, both in physiological and pathological states. In this thesis, the applicant has discovered the roles of EVs in the transfer of oncogenic DNA sequences and retrotransposon RNA in recipient cells. Moreover, the applicant also showed the clinical relevance of EVs in the detection of cancer-associated genes. The findings presented in this thesis suggest novel modalities of intercellular communication and horizontal gene transfer in the cancer microenvironment mediated by cancer cell-derived EVs.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 20August, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.