論文 / 著書情報
Article / Book Information

題目(和文)	天然化合物による細胞内microRNAとAnnexin A2による細胞外microRNA調節機構の解明
Title(English)	Regulation of intracellular microRNA by natural products and extracellular microRNA by Annexin A2
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出典(和文)	学位:博士（理学）, 学位授与機関:東京工業大学, 報告番号:甲第9956号, 授与年月日:2015年9月25日, 学位の種別:課程博士, 審査員:黒川　顕,徳永　万喜洋,川上　厚志,立花　和則,中島　信孝
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第9956号, Conferred date:2015/9/25, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	生命情報　専攻	申請学位（専攻分野）： Academic Degree Requested	博士　（　理学　） Doctor of
学生氏名： Student's Name	萩原　啓太郎	指導教員（主）： Academic Advisor(main)	黒川　顕
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Therapeutic strategies are essential for curing human cancer. Therefore, a continuous search for new approaches to tackle human diseases is important. Indeed, I previously describe a novel and important function of RPN2-mediated CD63 glycosylation, which regulates MDR1 localization and cancer malignancy, including drug resistance and invasion. The discovery of RNA interference (RNAi), which is mainly performed microRNAs (miRNAs) that attenuate translation, for target-specific gene silencing has rapidly created a powerful tool for the exploration of pathogenesis of human cancer. The inhibition of the miRNA biogenesis pathway results in severe developmental defects and lethality in many organisms. Deregulation of miRNAs leads to the development of several types of diseases. In fact, more than 70 diseases have been reported to be associated with miRNA deregulation. The identification of these remarkable molecular pathways has manifested a new field of gene therapy. An increasing number of experimental studies have shown that the knock-down or the re-expression of specific miRNAs could induce drug sensitivity, inhibit the proliferation of cancer cells, and suppress cancer cell invasion and metastasis For instance, I previously reported key miRNAs that were deregulated in a highly malignant CD133high population and found that miR-133a regulated the cell invasion that characterizes a lethal tumor phenotype. Silencing of miR-133a with locked nucleic acid (LNA) reduced cell invasion of this cell population, and systemic administration of LNA along with chemotherapy suppressed lung metastasis and prolonged the survival of osteosarcoma-bearing mice. In addition, I also identified a miR-197/CKS1B/STAT3-mediated PD-L1 network in chemo-resistant non-small-cell lung cancer (NSCLC), independent of immune-inhibitory signals. In addition, miR-197 is downregulated in platinum-resistant NSCLC specimens, resulting in the promotion of chemo-resistance, tumorigenicity, and pulmonary metastasis *in vitro* and *in vivo.* Moreover, two human clinical trials have assessed directed miRNA-targeting as therapeutics, according to Clinical Trials.gov (http://clinicaltrials.gov). One study is anti-miR-122 therapy against chronic hepatitis C and another trial is miR-34 mimics as a therapeutic against primary and metastatic liver cancer. However, the clinical use of miRNA entails at least two critical steps: delivery of miRNA to the appropriate tissues and subsequent maintenance and expression. A key goal of target-specific RNAi-delivery technology for cancer is the development of delivery systems directed at the target tissues only. Currently, there are many types of drug delivery systems. However, the clinical use of miRNA has several limitations such as the lack of delivery systems that are safe, efficient, tissue specific and that do not cause immune and inflammatory responses when they are used *in vivo*. In this thesis, I reported two approach to resolve these problems; miRNA-upregulation by using natural products in chapter 1, miRNA-based screening in chapter 2, and the mechanism of the packaging process of miRNAs into extracellular vesicles (EVs) regulated by Annexin A2 (ANXA2) in chapter 3. My study will have an important impact for future development of miRNA therapy. Diet is one of the most important modifiable cancer risk determinants. Dietary components have been implicated in many pathways involved in carcinogenesis. In

addition, carcinogenic processes are associated with the altered expression of several miRNAs. In chapter 1, we hypothesised that the dietary intake of natural products maintains tumour-suppressive miRNA expression in cancer cells, leading to the prevention of carcinogenesis. We demonstrated that resveratrol suppresses cancer cell malignancy *in vitro* and *in vivo* through the transcriptional activation of tumour-suppressive miRNAs and Argonaute2 (Ago2). Furthermore, we provided evidence that Ago2 over-expression enhances the RNAi activity. These findings provide evidence that dietary intake of natural agents can have beneficial effects on human physiology and survival by modulating miRNA biogenesis. However, it is still necessary to identify potent natural substances that activate tumor-suppressor miRNAs. In chapter 2, we have described a relevant experimental approach for the screening of natural products with the ability to induce tumor-suppressor miRNAs. Recently, miRNAs have been found in human body fluids despite the abundant presence of ribonucleases. This finding has led to the proposal of a scenario in which miRNAs could be packaged in certain RNase-resistant containers when they are secreted out of cells. Indeed, it has been reported that miRNAs are present in EVs. However, the exact molecular mechanisms of the recruitment of miRNAs in EVs are not well characterized. Based on proteomic analysis, we identified that silencing of ANXA2 significantly decreased the amount of miRNAs in EVs in chapter 3. My goal is preemptive medicine that is new medical paradigm that identifies an individual's susceptibility to a disease at the earliest possible stage by using predictive diagnosis and precise medicine. It is well known that miRNAs as biomarkers are one of the basis for preemptive medicine and available for early diagnosis of several diseases. My study will have an important impact for future development of miRNA therapy for preemptive medicine.