論 文 の 内 容 の 要 旨

論文題目　The function of nuclear RNA decay in the regulation of immune response gene expression and cancer cell survival

（免疫応答遺伝子発現と癌細胞増殖制御における核内RNA分解制御機構）

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Summary

Regulated RNA degradation has drawn attention to explain more about gene expression regulation and shows the link with disease conditions. Dysregulation of RNA decay results in altered gene expression regulation found in several diseases includes infectious disease as well as cancer. Therefore, studying the underlying mechanism of diverse RNA species degradation and more about the degradation component dysregulation is necessary to understand RNA degradation mediated regulation of disease condition.

In this thesis, I will present two pieces of research revolving around RNA degradation-mediated gene expression regulation from two different focus points. Here we mainly discussed nuclear RNA degradation regulation. In our previous studies, we showed that nuclear decay factors MTR4 and RRP6 are abolished under bacterial infection, and many short short-lived noncoding RNAs including long noncoding RNA (lncRNA) are accumulated due to skipping RNA degradation. Among lncRNAs, NEAT1v2 was also found to be accumulated. These accumulated non-coding RNAs are found to induce

antibacterial immune responsive genes. As NEAT1 is one of the functional lncRNAs has diverse gene regulatory roles in infectious as well as non-infectious diseases, knowledge of NEAT1v2 degradation-pathway might be essential to understand its gene regulatory functions. Therefore, we were interested to reveal the degradation pathway of NEAT1v2.

On the other, upregulation of RNA decay factors is reported to involve in several cancers condition and linked with cancer progression. How these upregulated decay factors impact the RNA degradation system in cancer is quite unknown. In this study, we focused on an RNA decay component EXOSC4 which was found to be overexpressed in pancreatic cancer. Here, we aim to investigate, the impacts of overexpressed EXOSC4 on its target RNA expression. Thus, the thesis is divided into four chapters.

In chapter 1, I reviewed the general theme of nuclear RNA degradation and the factors involved in this degradation process. Generally, RNA degradation is carried out by RNA-exosome both in the nucleus and cytoplasm. Although the cytoplasmic RNA decay system is mainly involved in mRNA quality control. Thereafter, nuclear RNA decay shows a more complex feature where several RNA binding proteins (RBPs) are involved in substrates recognition and differentiate functional mRNA species for cytoplasmic transportation and RNA degradation. Nuclear RNA decay systems ensure the processing and degradation of all RNA species including non-coding RNAs. Moreover, the selection of specific substrate RNA is mainly offered by a variety of RNA binding proteins (RBPs) and RBPs interact and present RNA substrates to the core decay system. Therefore, studying the interaction of different RBPs with substrate RNAs and degradation components is key to understanding how diverse RNA species are degraded in a regulated way. Additionally, I discussed RNA degradation mediated gene expression regulation their importance in disease progression.

In chapter 2, I report the nuclear degradation pathway of a well-known lncRNA NEAT1v2. We decided, two criteria to search target protein factor possibly involved in NEAT1v2 degradation. Firstly, we consider NEAT1v2 localized proteins, because lncRNA regulation and functions are largely

determined by its associated proteins and we pick up previously reported paraspeckle proteins, as they are localized to NEAT1v2. Secondly, the association of MTR4 helicase with NEATv2-colocalized proteins is necessary to carry out nuclear RNA exosome-mediated degradation as we reported previously. For that reason, we took previously published MTR4 associated proteins and search for common proteins among NEAT1v2 colocalized proteins. Four target proteins are identified as overlapped proteins between NEAT1v2 colocalized and MTR4 associated proteins. The knockdown experiment reveals only HNRNPH1 knockdown induces NEAT1v2 expression level, therefore considered as a possible candidate for NEAT1v2 degradation. Moreover, NEAT1v2 lncRNA stabilization and no induction of NEAT1v2 promoter in HNRNPH1 knockdown indicate HNRNPH1 regulates NEATV2 degradation post-transcriptionally. RNA immunoprecipitation with HNRNPH1 shows a significant enrichment of NEAT1v2 whereas co-immunoprecipitation with MTR4 and HNRNPH1 confirms their association with each other in RNA independent manner. All of these results indicate that HNRNPH1-MTR4 regulates NEAT1v2 degradation. Moreover, depletion of HNRNPH1 impacts NEAT1v2 stabilization mediated expression of a cytokine, *IL8* mRNA suggests that RNA degradation remains to be worth considering in the understanding of gene expression regulation.

In chapter 3, I report the role of an RNA decay factor EXOSC4 in the regulation of pancreatic cancer cell proliferation. Gene expression database GEPIA2 revealed that EXOSC4, an exosome component is overexpressed among pancreatic cancer patients. Therefore, we were interested to investigate whether overexpressed EXOSC4 drives pancreatic cancer progression and identified target genes essentially regulated by EXOSC4. Pancreatic cancer is enigmatic and is one of the most lethal human cancers that has a worse prognosis and higher metastatic properties. Gene expression profiling interactive analysis based on TCGA database reveals overexpression of exosome component 4 (EXOSC4) gene in several cancers including pancreatic cancer. EXOSC4 a core component of RNA exosome provides 3´-5´exoribonuclease activity and is involved in rRNA processing RNA degradation

systems both in the nucleus and cytoplasm. In this study, we showed target genes of overexpressed-EXOSC4 and how EXOSC4 knockdown affects nuclear RNA decay factors in pancreatic cancer. In this study, we showed that EXOSC4 is necessary for pancreatic cancer cell survival. Depletion of EXOSC4 interferes with the cell cycle and reduces pancreatic cancer cell growth. We perform RNA sequencing to find out the target genes of EXOSC4 and identified a group of negative regulatory genes of cell growth is induced in EXOSC4 knockdown, includes BCL-2 interacting killer (BIK) and Sestrin-2 (SESN2). Moreover, SESN2 is accumulated and stabilize under EXOSC4 depletion. Additionally, we showed that SESN2 degradation is aided by nuclear exonuclease RRP6 and DIS3. Knockdown of EXOSC4 reduces RRP6 and DIS3 at their protein level which may result in SESN2 stabilization. These findings indicate the importance of EXOSC4 in RNA degradation regulation in pancreatic cancer which is critical for pancreatic cancer cell survival by regulating many of its target genes and might be a therapeutic target for pancreatic cancer treatment.

In chapter 4, I concluded all of my research works from chapter 2, chapter 3, and discuss future aspects of this work. Observation from chapter 2 clearly showed the HNRNPH1 dependent degradation pathway of NEAT1v2 and the importance of this degradation pathway in IL8 gene expression regulation. Furthermore, these studies provide evidence that specific degradation of diverse RNA species may depend on different RNA binding proteins which remain unexplored. Chapter 3, discussed the necessity of RNA decay component EXOSC4 in pancreatic cancer cell proliferation through regulating a group of cell cycle regulatory genes. EXOSC4 knockdown mediated reduction of nuclear decay enzyme, which importantly indicates that not only nuclear but also cytoplasmic RNA decay may be regulated by EXOSC4 overexpression. Overall findings of this study suggest that RNA degradation regulation is beneficial to understand the molecular mechanism of gene expression which may have the advantage to control disease conditions.