博士論文（要約）

MicroRNA-124a Inhibits Endoderm Lineage Commitment in Mouse Embryonic Stem Cells

（マイクロ RNA-124a はマウス胚性幹細胞の内胚葉分化抑制に寄与する）

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論文の内容の要旨

論文題目 MicroRNA-124a Inhibits Endoderm Lineage Commitment in Mouse Embryonic Stem Cells
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Despite the current advances in stem cell research, the application of stem cell in cell replacement therapy for human diseases, such as macular degeneration, spinal cord injury, heart disease, diabetes, chronic liver diseases and so forth, was hampered by the scarce of a method that could successfully generate fully functional mature cell types *in vitro*. Understanding the molecular mechanism in cell fate decision that involves stem cell differentiation into a desired cellular identity should, therefore, be the central focus of research in order to ensure success in regenerative medicine.

MicroRNA are known to regulate gene expression and are important regulators of fundamental cellular processes. Growing evidence has shown that miRNA plays a critical role in fine-tuning the expressions of genes involved in stem cell self-renewal and differentiation. Although much has been known about the roles of miRNAs in directing ectoderm and mesoderm differentiation from pluripotent stem cells, their roles during endoderm developmental processes are yet to be explored. Since endoderm progenitors are important intermediates that give rise to the fully functional hepatic and pancreatic cells, considerable weight should be given to the study of the basic molecular mechanism that governs the formation of endoderm progenitors. Thus, the present work aimed to study the miRNAs profile during the course of differentiation from mouse embryonic stem cells (mESCs) to definitive endoderm (DE) and to investigate the role of miRNAs involved in endoderm development.

Mouse mESCs were first induced into DE and the miRNA profiles between mESC and differentiated endodermal cells at different stages were then investigated via miRNA array analysis. Studies have shown that Sox17 is indispensable for the proper formation and maintenance of the endoderm germ layer. Using in silico target prediction, a list of 6 miRNAs that were predicted to target Sox17 and down-regulated during endoderm differentiation was identified and validated through qPCR. To determine whether the selected miRNAs were involved in regulating Sox17 expression, transfection of miRNA mimics into embryoid bodies (EBs) was performed. In particular, transfection of miR-

124a mimics showed remarkable inhibition of Sox17 expression in Day 10 EBs (81.74 ± 18.25%; $p < 0.01$; t-test).

Next, the functional relevance of miR-124a expression in the differentiation of the 3 germ layers was tested through examination of lineage-specific gene expressions in miR-124a-overexpressed EBs. Over-expression of miR-124a also significantly inhibited Gata6 expression (64.25 ± 29.75%; $p < 0.05$; t-test), another important transcription factor for endoderm development. In line with the gene expression data, similar inhibition of both Sox17 and Gata6 were also observed at the protein level in miR-124a-overexpressed EBs. Gene expression of other lineages such as ectodermal- (Nestin and Pax6) and mesodermal-related genes (Actc1), were not affected. Using 3'- untranslated region (UTR) luciferase assay, Sox17 and Gata6 were identified as direct targets of miR-124a, demonstrated by the significant downregulation of the luciferase activities. The effect of miR-124a on downstream targets of Sox17 and Gata6 were further examined. Interestingly, over-expression of miR-124a significantly suppressed Hnf1ß (68.08 ± 23.12%; $p < 0.05$; t-test) and Hnf4α (62.94 ± 30.34%; $p<0.05$; t-test), 2 of the master regulators in the cascade of hepatic differentiation. Taken together, these findings revealed a regulatory network of miR-124a in the development of endoderm lineage, in which miR-124a repressed endoderm lineage differentiation in EBs by inhibiting the expression of Sox17 and Gata6, leading to the downregulation of Hnf1ß and Hnf4α expressions, thereby possibly affecting hepatic specification.

Lastly, the upstream regulator of miR-124a expression was also examined. In mouse genomes, miR-124a was encoded by 3 different genes, miR-124-1 (also known as Retinal Noncoding RNA 3, Rncr3), miR-124-2, and miR-124-3. In this preliminary study, it was found that only primary (pri)-miR-124-1 was expressed in mESCs and was down-regulated in induced-endoderm. The expression levels of pri-miR-124-1 and its host gene, Rncr3, were showed to be positively correlated, suggesting that miR-124a expression during endoderm development is likely to be controlled by Rncr3.