［課程－２］

審査の結果の要旨

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Stem cells hold great promise for regenerative medicine, owing to their extraordinary capacity to differentiate into all cell types. Understanding the molecular mechanism that governs the differentiation of stem cell into a desired cellular identity could provide insight into stem cell biology and contribute to the development of effective regenerative therapy. Growing evidence has shown that miRNAs are important modulators in cell fate determination. Although much have been known about the roles of miRNAs in ectoderm and mesoderm differentiation, their roles in endoderm lineage commitment are yet to be explored. Thus, the current work aimed to study the miRNA profile during endoderm differentiation in mouse embryonic stem cells (mESCs), and to investigate their roles in endoderm development. To do this, mouse mESCs were first induced into endoderm and the miRNA profiles between mESC and differentiated endodermal cells at different stages were then investigated via miRNA array analysis. The summary of the findings in this study are as follow:

1. Previous studies have shown that SRY-related HMG-box (SOX) 17 is indispensable for the proper formation and maintenance of the endoderm germ layer. Using in silico target prediction, miRNA candidates that were predicted to target Sox17 was identified. In particular, a negative correlation between the expression of miR-124a and Sox17 during endoderm differentiation was observed.

2. The functional role of miR-124a in early differentiation was tested by transfecting miR-124a mimic during embryoid bodies formation. Over-expression of miR-124a suppressed endoderm differentiation through directly targeting the 3'-untranslated region (3'-UTR) of Sox17 and GATA-binding protein (Gata) 6, the two important transcription factors for endoderm development. Gene expression of other lineages such as ectodermal- (Nestin and Pax6) and mesodermal-related genes (Actc1), were not affected.

3. The effect of miR-124a on downstream targets of Sox17 and Gata6 were further examined. Over-expression of miR-124a significantly suppressed hepatocyte

nuclear factor (Hnf)1β and Hnf4α, two of the master regulators in the cascade of hepatic differentiation. These findings revealed a regulatory network of miR-124a in the development of endoderm lineage, in which miR-124a repressed endoderm lineage differentiation in embryoid bodies (EBs) by inhibiting the expression of Sox17 and Gata6, leading to the downregulation of Hnf1β and Hnf4α expressions, thereby possibly affecting hepatic specification.

4. The upstream regulator of miR-124a expression was also examined. 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, Retinal Noncoding RNA 3 (Rncr3), were showed to be positively correlated, suggesting that miR-124a expression during endoderm development is likely to be Rncr3-dependent.

In conclusion, the role of miR-124a in the early development of mESCs was discovered and miR-124a expression during endoderm development is likely to be Rncr3-dependent. Along with this, the existence of a possible interplay between miR-124a and Sox17/Gata6 transcription factors in hepato-specific gene regulation was revealed, which could be considered as a new clue to be added into the transcription machinery. Overall, these findings provide interesting mechanistic insights of miRNAs in endoderm development, therefore deserves the award of Ph.D. degree.