Functional analysis of microRNAs by the using of gene disruption

F. Harada, S. Ishii and Y. Kido

MicroRNAs (miRNAs) are small (approximately 22 nucleotides) RNAs that negatively regulate gene expression through interactions with 3'-untranslated regions of their target mRNAs, which leads to mRNA cleavage or translational repression. To date, 326 miRNA genes have been identified from human genome, the precise functions of these non-coding RNAs remains largely obscure. Interestingly, many miRNAs are found in close proximity to other miRNAs, and these clustered miRNAs are transcribed from a single polycistronic transcription unit by RNA polymerase II. Therefore clustered miRNAs might have very important roles in maintenance of the specific cell lineage. Human chromosome 13 has a one of the clustered miRNA gene, which encodes 6 miRNAs (miR-17-5P, 18a, 19a, 20a, 19b-1 and 92-1) in 800bp. To clarify the function of these miRNAs, we tried to obtain homologue of this clustered gene from chicken B cell line DT40 and disrupt it by targeted integration.

First of all, we obtained a chicken DNA fragment containing miRNA gene cluster homologous to human chromosome 13 by PCR amplification using human miR-17-5P and miR 92-1 sequences as primers. Screening of DT40 genomic library with this DNA fragment as a probe, we obtained several chicken genomic clones. These clones contained 6 miRNA genes in the same order as the human gene and the nucleotide sequence of each miRNA was completely identical with that of human. Therefore these clustered miRNAs are conserved between human and chicken cells and might have essential roles in these animals. To disrupt the clustered miRNA gene in DT40 cells, the targeting vectors containing blasticidin S and puromycin resistance genes were constructed and then introduced into DT40 cells by sequential homologous recombination. Three knockout cell lines on this clustered miRNA gene were obtained. The growth rate of these mutant cells was slightly slower than that of wild-type DT40 cells. The expression of each miRNA was analyzed by Northern hybridization of total RNA isolated from mutant and wild-type cells. In the mutant cells, miR-20a was undetectable while other five miRNAs still expressed about 50% of wild-type cells. In human genome, paralogous cluster in X chromosome, which encodes miR-106a, 18b, 20b, 19b-2 and 92-2 has been reported. Diminished expression of 5 miRNAs in mutant cells must be attributable to this miRNA cluster. We prepared DT40 genomic clone containing new miRNA cluster and construction of double knockout cells on miRNA clusters is now in progress.