Reverse Genetic Studies on Mitochondrial tRNA-related Disorders

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Mitochondria contain multiple copies of mitochondrial DNA (mtDNA) as their own genome inside the mitochondrial inner membrane. Mammalian mtDNA encodes 13 structural genes that code for complexes I, III, IV, and V, 22 tRNA genes, and two rRNA genes that are necessary for oxidative phosphorylation. It has been reported that accumulation of pathogenic mutant mtDNAs results in abnormalities of oxidative phosphorylation and induces mitochondria-related disorders, such as mitochondrial diseases, diabetes, cancer, neurodegenerative diseases, and infertility, and as well as aging. It has been considered that multiple clinical phenotypes are dependent on types of mutations, proportion of mutant mtDNA, and tissue distribution. Nevertheless, there is no evidence to explain how the accumulation of these pathogenic mutant mtDNAs is responsible for the expressions of various clinical phenotypes yet. To elucidate this problem, generation and analysis of disease mouse models is a crucial tool for elucidating disease pathogenesis but mouse models with specific mutations in mtDNA are notoriously difficult to generate. In this study, I focused on $tRNA^{Leu(UUR)}$ gene encoded by mtDNA since this gene is known as a hotspot of human pathogenic mtDNA mutations. Point mutations in the mitochondrial $tRNA^{Leu(UUR)}$ gene are implicated in a wide variety of diseases, including mitochondrial and neurodegenerative diseases as well as diabetes; however, whether or not these point mutations can induce several disease phenotypes remains unelucidated. Here, I succeeded in establishing *trans*-mitochondrial mice carrying both WT mtDNA and pathogenic mutant mtDNA with the point mutation in the $tRNA^{Leu(UUR)}$ gene. This mutation is orthologous to the pathogenic mutation in humans, where carriers of this mutant mtDNA exhibit mitochondrial diseases. Although maternal transmission of this mutation to offspring decreased with maternal age and it was difficult to obtain sufficient model mice for a comprehensive pathological analysis, I nevertheless

uncovered several interesting findings, as well as questions. The percentage of randomly inherited mutant mtDNA showed a uniform distribution in different organs and did not change with age in individual mice up to 10 months of age. Young mice (3-month-old) with a high percentage of mutant mtDNA exhibited hyperglycemia but no clinical signs of mitochondrial diseases. This progressed to diabetic phenotypes, hepatic dysfunction, and mitochondrial diseases in older mice (10-month-old) that showed abnormalities of oxidative phosphorylation. In contrast, the phenotypes of mice with a low percentage of mutant mtDNA were similar to those with WT mtDNA. I believe that this research would make a significant contribution to the study of mitochondria-related disorders because here I established the first disease model mice with a pathogenic point mutation in the $tRNA^{Leu(UUR)}$ gene. Moreover, I demonstrated that accumulation of the mutant mtDNA can result in diabetes, hepatic dysfunction, and mitochondrial diseases in mice.