Department of Laboratory medicine

Generation and transmission of mtDNA mutations and their effect on aging

AKADEMISK AVHANDLING
Som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i konferensrum Tellus, Retziuslaboratoriet, Scheeles väg 1, Karolinska Institutet
Fredagen den 24 Januari, 2014, kl 09.00

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Stockholm 2014

ABSTRACT

Mutations in mtDNA are known to cause neuromuscular diseases and have been associated with several common age-associated diseases as well as being a contributor to the aging process. Despite their importance there are still important aspects of mtDNA inheritance and mtDNA mutations that remain unknown and need to be addressed. The aim of this thesis is to examine how mtDNA mutations behave in in vivo systems with regard to inheritance and distribution. A further aim is to investigate the phenotypic consequences of mtDNA mutations, specifically in the aging process. For this purpose we used mouse models and different approaches for DNA sequence analysis.

Our group previously demonstrated that increased somatic mtDNA mutagenesis can cause premature aging in the mouse, thus providing the first experimental data for the involvement of mtDNA mutations in aging. This work was based on analysis of the mtDNA mutator mouse, which is a knock-in mouse model with increased mtDNA mutation load due to a proofreading deficiency of mitochondrial DNA polymerase (Pol□). The work presented in this thesis demonstrates that a significant proportion of mtDNA mutations are passed on via the maternal germline and expand clonally in the offspring to contribute significantly to the observed phenotypes. By performing a variety of mouse crosses to generate mice with different combinations of maternally inherited and somatic mtDNA mutations we found that even low levels of inherited mtDNA mutations can lead to premature aging phenotypes. These phenotypes could be rescued by reintroduction of wild-type mtDNA. Our findings show that clonal expansion of maternally transmitted mtDNA mutations can be an important factor in the aging process. This finding is very intriguing in light of recent findings that inherited heteroplasmy is common in humans. Furthermore, a combination of inherited and somatic mtDNA mutations can disturb development to cause stochastic brain malformations.

There is a lack of understanding how pathogenic mtDNA mutations are inherited and studies in the area have been hampered by the lack of appropriate animal models. By backcrossing mtDNA mutator females we were able to isolate a mouse line carrying a single base pair deletion in the mitochondrial $tRNA^{Met}$ gene. Although this mutation was under selection in living offspring, we failed to observe any clear selection in germ cells and primary oocytes. The selection is instead occurring after fertilization as the embryo develops.

Finally, we investigated the clonality of mtDNA inheritance. Mutations in mtDNA are the most commonly used marker in population genetics studies, and much of this popularity is based on the assumed lack of recombination in mtDNA. This assumption has now been questioned in a number of studies that report frequent recombination of mtDNA. In this thesis, I developed a novel direct cloning protocol to trap single mtDNA molecules, allowing for DNA sequence analysis without prior amplification by PCR. Applying this technique, there was no evidence for germline recombination of mtDNA in mice that has been heteroplasmic for >50 generations.

ISBN 978-91-7549-422-7