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Mitochondrial DNA mutations. Brain developmental and ageing consequences, and possible treatments

AKADEMISK AVHANDLING
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ABSTRACT

Ageing is a complex process that involves cellular senescence, a gradual loss of tissue homeostasis, and decline in organ function. Abundant evidence implicates mitochondria in ageing suggesting: (i) accumulation of mitochondrial DNA (mtDNA) damage, (ii) progressive respiratory chain dysfunction, and (iii) increased reactive oxygen species production. The "Mitochondrial Theory of Aging", first proposed by Denham Harman in 1972, suggests that damage to mtDNA slowly accumulates with time and causes ageing phenotypes by interfering with bioenergetic homeostasis and/or by loss of cells because of apoptosis and/or cellular senescence. This theory is supported by a wealth of correlative data, but has remained controversial in the absence of experimental proof.

In this thesis, the first mouse model to experimentally address the "Mitochondrial Theory of Aging", the mtDNA mutator mouse, was used to study how elevated somatic mtDNA mutations might translate to age-related functional changes in the central nervous system. The mtDNA mutator mouse is a homozygous knock-in transgenic mouse model that expresses a proof-reading deficient version of the nucleus-encoded catalytic subunit of mtDNA polymerase-γ (PolgA). The model demonstrates a cause and effect relationship between slowly increasing somatic mtDNA mutation levels and several human-like phenotypes associated with ageing that are manifested much earlier in life.

There are few means to track symptomatic stages of brain ageing. Using both prematurely ageing mtDNA mutator mice and normally ageing mice, a molecular link was established between mitochondrial dysfunction and abnormal metabolism in the ageing process, resulting in marked increases in brain lactate levels, even before the appearance of overt ageing phenotypes. The lactate dehydrogenase (LDH) genes responsible for the interconversion of pyruvate-to-lactate, LDH-A and LDH-B, which generate the H and M subunits of the 5 different tetrameric LDH isoenzymes, were analyzed and the isoenzyme composition and activities were found to have changed in favor of pyruvate-to-lactate conversion (Paper I).

To correlate the striking increase in lactate with tissue histopathology, the activities of cytochrome *c* oxidase and succinate dehydrogenase were investigated. Failing respiratory chain function was found in key brain areas of mtDNA mutator mice, and, later in life, in normal mice (Papers I and II).

Endurance exercise has been recently found to counteract progeroid ageing in prematurely ageing mice similar to the mtDNA mutator mice. Thus, the effect of diet supplementation with natural ingredients, vitamins, and antioxidants was investigated as an alternative strategy. The lifespan of mtDNA mutator mice receiving the dietary supplement NT-020-BV was increased by approximately 12%. The onset of the progeroid ageing phenotypes, such as canities, alopecia, kyphosis, elongation of ears, reduced body size, and weight loss was also delayed, and the enlargement of organs and sarcopenia were ameliorated. Moreover, locomotion and gait, as well as motor programming were also improved in mtDNA mutator mice receiving an NT-020-BV-enriched diet (Paper IV).

In addition to developing a premature ageing syndrome, approximately 30% of mtDNA mutator mice also exhibit stochastic brain malformations, ranging from major local perturbations of brain organization to symmetrical hippocampal and cortical migration disturbances. However, such brain malformations were only seen if heterozygous ($PolgA^{mut/WT}$) females had been maintained as maternal lineages for several generations. Instead, when wild-type mtDNA was re-introduced by crossing heterozygotes with C57Bl/6 females, the resulting mtDNA mutator mice had no brain malformations. Furthermore, the reintroduction of wild-type mtDNA also improved fertility and viability of offspring, and also delayed the onset of ageing phenotypes. This suggests that the extent of mtDNA mutation load during embryogenesis may also predict the rate of ageing *per se* in mtDNA mutator mice (Paper III).

In summary, these results support a role for mtDNA mutation load and mitochondrial dysfunction in ageing as well as in neurodevelopment, and show that intervention with diet might combat such mitochondrial deficiencies in ageing.

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