Microphotometric analysis of NADH-tetrazolium reductase deficiency in fibroblasts of patients with Leber hereditary optic neuropathy

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Summary: We employed a microphotometric approach to examine whether a defect in the mitochondrial respiratory complex I expected in Leber hereditary optic neuropathy (LHON) as the consequence of a mtDNA (11778G>A) mutation in the ND4 gene coding for a subunit of the respiratory complex I can be detected at the single-cell level. Genetically stable fibroblast cell lines were established from skin biopsies of two members of a Chinese Indonesian family with LHON. The fibroblasts were homoplasmic for the 11778G>A mutation. The activity of the respiratory complex I was examined histochemically by staining for NADH-tetrazolium reductase. The histochemical staining showed a typical pattern with an apparent concentration of the activity around the nucleus, suggested as the reflection of the gradient in the thickness of the unsectioned fibroblast cells. Microphotometric quantification of the staining intensity showed that the activity is linear for at least 60 min. The activity shows a discontinuity in its Arrhenius kinetics with a break point at 13.0–13.5°C (activation energy at 50–58 J/mol and 209–238 J/mol above and below the break temperature, respectively), indicating the membrane association of the NADH-tetrazolium reductase activity. Both patients showed lower fibroblast NADH-tetrazolium reductase activity, with a reduction of ~30%. Our results demonstrate the utility of microphotometric analysis in the study of biochemical defects associated with mutations in the mtDNA.

Mutations in the mitochondrial DNA (mtDNA) have been shown to be the underlying molecular defects in a number of adult-onset neuromuscular and infantile
multisystems disorders. Many of these mutations have now been identified, but the molecular mechanisms responsible for the disease process have not in general been elucidated. A typical example is Leber hereditary optic neuropathy (LHON), which is an inherited degenerative disorder characterized by visual failure resulting from a bilateral optic atrophy, with multiple organ involvement in certain families. Around 50–70% of LHON pedigrees carry a mutation at 11778G>A in the ND4 gene of the mtDNA, assumed to affect the function of this subunit of the respiratory complex I (see Riordan-Eva and Harding 1995). The relationship between this mutation, the resulting biochemical defects and its clinical manifestation, however, is not well understood and appears to be complex.

Enzymatic biochemical disorders consequential upon mtDNA mutation and their clinical manifestations appear to be the outcome of an interplay between the causal mutation and other sequence variants of the mtDNA, and between the nuclear and the mitochondrial genetic systems (see Hanna and Nelson 1999; Wallace 1999; Zeviani and Antozzi 1997 for recent reviews). The expression of pathological mtDNA mutations is determined and modulated also by apparently random cellular events during development. mtDNA carrying a pathological mutation often coexists in cells with the normal mtDNA in a heteroplasmic manner, and the two populations of mtDNA segregate randomly during cell division, resulting in varying degrees of heteroplasmicity in different cells. The age-related somatic accumulation of mtDNA mutations, proposed to be an important factor in the ageing process (Linnane et al 1989; Trounce et al 1991), is also a random cellular event. The adult-onset manifestation of most pathological mtDNA mutations has been suggested to be the result of the expression of these mutations against a background of an age-related decline in the tissue capacity for oxidative metabolism. Thus, the respiratory enzyme deficiencies observed in tissues as the consequence of pathological mtDNA mutations (see Collins et al 1991 and Keightley et al 1996 for examples) and that observed in ageing tissues (see Muller Hocker 1990) are in general of a mosaic nature. Important information regarding the phenotypic expression of mtDNA mutations, therefore, could be gained from the ability to analyse biochemical and molecular defects at the single-cell level.

Histochemical staining is commonly used to assess respiratory enzyme deficiency associated with mtDNA mutations in various neuromuscular disorders at the single-cell and tissue levels (Collins et al 1991; Dubowitz and Brooke 1973), but it has not been possible in the past to obtain quantitative data from such studies. The introduction of microphotometric facilities in recent years, however, has provided a means for the measurement of cellular enzyme activities microscopically. In the present study, we have assessed the utility of the microphotometric approach to the single-cell analysis of the respiratory enzyme deficiency, and have employed this approach to examine whether the expected functional defect in the mitochondrial respiratory complex I can be detected in Leber hereditary optic neuropathy.