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Immunolocalisation pattern of complex I–V in ageing human retina: Correlation with mitochondrial ultrastructure

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ABSTRACT

Earlier studies reported accumulation of mitochondrial DNA mutations in ageing and age-related macular degeneration. To know about the mitochondrial status with age, we examined immunoreactivity (IR) to markers of mitochondria (anti-mitochondrial antibody and voltage-dependent anion channel-1) and complex I–V (that mediate oxidative phosphorylation, OXPHOS) in donor human retinas (age: 19–94 years; $N = 26$; right eyes). In all samples, at all ages, IR to anti-mitochondrial antibody and voltage-dependent anion channel-1 was prominent in photoreceptor cells. Between second and seventh decade of life, strong IR to complex I–V was present in photoreceptors over macular to peripheral retina. With progressive ageing, the photoreceptors showed a decrease in complex I-IR (subunit NDUFB4) at eighth decade, and a weak or absence of IR in 10 retinas between ninth and tenth decade. Patchy IR to complex III and complex IV was detected at different ages. IR to ND1 (complex I) and complex II and V remained unaltered with ageing. Nitrosative stress (evaluated by IR to a nitro-tyrosine antibody) was found in photoreceptors. Superoxide dismutase-2 was found upregulated in photoreceptors with ageing. Mitochondrial ultrastructure was examined in two young retinas with intact complex IR and six aged retinas whose counterparts showed weak to absence of IR. Observations revealed irregular, photoreceptor inner segment mitochondria in aged maculae and mid-peripheral retina between eighth and ninth decade; many cones possessed autophagosomes with damaged mitochondria, indicating age-related alterations. A trend in age-dependent reduction of complex I-IR was evident in aged photoreceptors, whereas patchy complex IV-IR (subunits I and II) was age-independent, suggesting that the former is prone to damage with ageing perhaps due to oxidative stress. These changes in OXPHOS system may influence the energy budget of human photoreceptors, affecting their viability.

1. Introduction

Mitochondria are involved in oxidative phosphorylation (OXPHOS), by means of which they conserve energy in the form of ATP released during electron transfer in the electron transport chain (ETC; Winkler, 1981; Scheffler, 1999). In OXPHOS, they significantly generate reactive oxygen species (ROS) and free radicals (Scheffler, 1999; Jarrett et al., 2008). In the absence of substantial levels of mitochondrial antioxidants, the accumulated ROS may cause oxidative stress (OS) and ultimately damage to the mitochondrial DNA (mtDNA) that encodes the OXPHOS enzymes. OS is central to ageing (Finkel and Holbrook, 2000; Navarro, 2004) and the mtDNA damage is an initial signal towards pathogenesis of a number of neurological and retinal disorders (Miquel et al., 1980; Isashiki et al., 1991; Huoponen, 2001; Triepels et al., 2001; Navarro, 2004; Jarrett et al., 2008; Kenney et al., 2010; Kong et al., 2011).

Biochemically, five mitochondrial inner membrane-bound enzyme complexes are involved in catalysing OXPHOS. These are: NADH-ubiquinol oxidoreductase (complex I), succinate-ubiquinol oxidoreductase (complex II), ubiquinol-cytochrome *C* oxidoreductase (complex III), cytochrome *C* oxidase (complex IV) and ATP synthase (complex V).

During ageing, the human retina shows qualitative as well as numerical changes in its neuronal populations, especially of ganglion, bipolar and photoreceptor cells (Marshall et al., 1979; Gao and Hollyfield, 1992; Harman et al., 2000; Curcio et al., 1993; Jackson et al., 2002; Nag et al., 2006; Aggarwal et al., 2007; Pow and Sullivan, 2007; Shelley et al., 2009; Nag and Wadhwa, 2012). Photoreceptors have a high requirement for ATP (Futterman, 1975), which is synthesized in the mitochondria that are densely packed in their inner segments (Hoang et al., 2002; Perkins et al., 2003; Stone et al., 2008; Wong-Riley, 2010). It is known that 90–95% of the photoreceptor ATP demand is achieved via OXPHOS (Winkler, 1981). With normal ageing of the human macula, and in age-related macular degeneration (AMD), cone photoreceptor mitochondria undergo various degenerative structural, neurochemical and genetic changes (mtDNA deletions) (Barreau et al., 1996; Barron et

Abbreviations: OXPHOS, oxidative phosphorylation; mtDNA, mitochondrial DNA; OS, oxidative stress; ETC, electron transport chain; RPE, retinal pigment epithelium; NFL, nerve fibre layer; IR, immunoreactivity; TEM, transmission electron microscopy; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, OPL, inner and outer plexiform layers; ONL, outer nuclear layer; AMD, age-related macular degeneration; P, photoreceptors.

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al., 2001; Nag et al., 2006). AMD subjects at autopsy were reported to contain high levels of mtDNA damage in their retina and retinal pigment epithelium (RPE; Jarrett et al., 2008; Karunadharma et al., 2010; Kenney et al., 2010). The reasons behind those mitochondrial changes are at present unclear; one study reported that human RPE cells in vitro exposed to oxidising or alkylating agents develop mtDNA damage (Jarrett et al., 2008), suggesting that OS is a culprit in it. Clinically, retinal manifestations of mitochondrial diseases are associated with mtDNA mutation, especially in complex I (Isashiki et al., 1991; Triepels et al., 2001). For example, mutations in the mtDNA that encodes different subunits of complex I cause a severe thinning of the nerve fibre layer (NFL) in Leber's hereditary optic neuropathy (Carelli et al., 2004; Yu-Wai-Man et al., 2011). Cooper et al. (2002) reported abnormal scotopic ERG responses in patients with mitochondrial disorders involving OXPHOS system.

Earlier studies demonstrated OXPHOS enzymes in the retina by histochemical reactions (Wong-Riley, 1979; Kageyama and Wong-Riley, 1984; Chen et al., 1989; Andrews et al., 1999; Barron et al., 2001). Andrews et al. (1999) reported high enzyme activities for succinate dehydrogenase (complex II) and cytochrome *C*-oxidase (complex IV) in human optic nerve and retina. Barron et al. (2001) showed age-related decreases in the number of cytochrome *C* oxidase-positive macular photoreceptors in aged individuals due to acquired mtDNA mutations. In rodent retina, it is expressed more prominent in cone inner segments than in rod counterparts (Perkins et al., 2003; Stone et al., 2008). Wang et al. (2010) reported increased mtDNA damage in aged rodent photoreceptor and ganglion cells, compared to that in young animals. They found that increases in mtDNA damage are due to reduced repair capacity in aged retinas, and suggested this to be responsible for age-related retinal diseases. Given that ageing is apparently linked with OS, it is important to know age-related expressions of complex I to V in human retina and their variations, if any.

The aim of this study was to examine the immunoreactivity (IR) to OXPHOS enzymes (complex I–V) in ageing human retinas by immunohistochemistry (IHC). The mitochondrial status was examined by IHC to anti-mitochondrial antibody (that recognizes a 60 kDa protein component of mitochondria) and voltage-dependent anion channel-1 (VDAC-1, a protein present in mitochondrial outer membrane), two established markers for mitochondria. OS, if any, was examined by IHC for nitro-tyrosine (marker of protein oxidation) and OS response, with an antibody to superoxide dismutase-2 (SOD-2; a mitochondrial antioxidant enzyme), in the retinal samples employed. We demonstrate a significantly decreased expression of complex I-IR, and complex III and IV in select retinas of aged human retina. Additionally, this study examined the photoreceptor mitochondrial ultrastructure in select, aged retinas (left eyes) whose counterparts (right eyes) showed weak or no IR to complex I and complex IV, to find a basis for downregulation and loss of certain OXPHOS enzymes.

2. Materials and methods

2.1. Human eyes

Human eyes from donors ($N = 26$; age range 19–94 years) with no history of ocular diseases were procured from the National Eye Bank, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi. The details regarding their age, sex, cause of death and postmortem delay in tissue fixation are summarized in Table 1. The eyes chosen and used in this study had a postmortem delay in fixation by 1–5 h; preliminary investigations of eyes

Table 1
Information about the eye donors and status of IR to complex I–V, and mitochondrial ultrastructure.

Case number	Age*/sex	Cause of death	Delay in fixation (hours)	IR status***	Mitochondrial features in photoreceptor cells
1	19M	Asthma	2		
2	35M	Heart attack	2		
3	50M**	Haemorrhage	2		Normal shape with well-ordered cristae[a,b]
4	54F	Road-traffic accident	1	Complex IV–II (−)	
5	56M**	Myocardial infarction	2		Normal shape with well-ordered cristae[a,b]
6	62M	Cardiac arrest	5		
7	63F	Retro-pharyngeal abscess	3	Complex IV–II (−)	
8	64M	Cardiac arrest	3		
9	67M**	Heart attack	1	Complex IV–II (−)	
10	70M	Myocardial infarction	2	Complex IV–II (−)	
11	72F	Heart attack	4		
12	74M	Heart attack	2	Complex IV–I (−)	
13	75M**	Heart attack	2		
14	78F	Heart attack	2		
15	80M	Cataract	4	Complex IV–II (−)	
16	81M**	Heart attack	2		
17	83M	Heart attack	3	Complex I (±)[b]	
18	85M	Myocardial infarction	4	Complex I (±)	Tapered[a]
19	86M	Myocardial infarction	3	Complex I (±)	
20	87M	Myocardial infarction	1	Complex I (±)	Tapered[a]
21	89M	Cardiac arrest	4	Complex I (±), complex IV–IV (±)	Bent, dumb-bell shaped[b], autophagosomes with mitochondria-like organelles[a]
22	90M	Heart attack	3	Complex I (±)	Loss of mitochondria
23	90M	Heart attack	5	Complex I (±), complex III (±)	Peculiar mitochondrial inclusions[a]
24	91M	Heart attack	3	Complex I (±)	Dense matrix[a]
25	91F**	Cardio-respiratory attack	1	Complex I (±), complex III (±)	Dense matrix[a]
26	94M	Cardio-respiratory arrest	1	Complex I (±)	Dumb-bell shaped[b], autophagosomes with mitochondria-like organelles[a], fused[b]

M, male; F, female; ± indicates reduced or almost absence of IR.
[a] Denotes macular part.
[b] Denotes mid-peripheral part.
* In years.
** Retinal sections used earlier in a study (Nag et al., 2011).
*** Unless otherwise stated, complex I to V - IR is prominent in this retina; complex IV–I (−) indicates complex IV-subunit I negative [and likewise other complexes].