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Prognostic significance of mitochondrial oxidative phosphorylation complexes: Therapeutic target in the treatment of retinoblastoma

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ABSTRACT

Purpose: Altered energy metabolism plays an important role in the development and progression of cancer. The objective of this study was to elucidate the role of mitochondrial oxidative phosphorylation complexes and their prognostic significance in retinoblastoma (Rb).
Methods: Immunohistochemistry was performed on 109 primary enucleated retinoblastoma tissues for mitochondrial OXPHOS complexes and their expression was confirmed by western blotting.
Results: Histopathological high risk factors (HRFs) were identified in 42.2% cases. Mitochondrial OXPHOS complexes III, IV and V were expressed in more than 50% of primary retinoblastoma cases each whereas mitochondrial complex I was expressed in only 29/109 (26.60%) cases by immunohistochemistry. Loss of mitochondrial complex I correlated well with poor tumor differentiation and tumor invasion ($p < 0.05$) whereas expression of mitochondrial complexes III, IV and V was associated with better survival (Kaplan–Meier method).
Conclusions: This was the first study predicting a relevant role of mitochondrial OXPHOS complexes and highlights the prognostic significance with patient outcome in retinoblastoma. Loss of mitochondrial complex I immunoexpression could prove to be a useful independent prognostic biomarker to identify high risk retinoblastoma patients. Differential expression of these mitochondrial complexes is a novel finding and may be used as an attractive future anticancer target in primary retinoblastoma tumors.
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1. Background

Retinoblastoma is the most common intraocular childhood malignant cancer. It is caused by the RB1 gene mutation which was the first described tumor-suppressor gene. Incidence of retinoblastoma is constant worldwide at one case per 15,000–20,000 live births, which corresponds to about 9000 new cases every year Dimaras et al., 2012. Leukocoria is the most common presenting symptom of retinoblastoma.

Mitochondria are essential subcellular organelles and important key regulators of metabolism Brenner and Grimm, 2006; Knudson and Brown, 2008; Rasola and Bernardi, 2007. Mammalian mitochondria contain their own DNA (mtDNA). Human mtDNA is remarkably small (16,569 bp) compared to nuclear DNA and encodes only a few, but important proteins Higuchi, 2012. It encodes 13 polypeptides of oxidative phosphorylation complexes, 12S and 16S rRNAs, and 22 tRNAs required for mitochondrial function DiMauro, 2004. They are involved in a series of cellular processes including cellular differentiation and proliferation, cell signaling, programmed cell death, the control of the cell cycle and cell growth. Besides these, mitochondria are also called cellular power house for generating most of the cellular chemical energy known as adenosine triphosphate (ATP) for cell use which occurs through oxidative phosphorylation (OXPHOS) system.

The mitochondrial OXPHOS system is controlled at the genetic level by two distinct genomes: the circular mitochondrial genome (mtDNA) and the nuclear genome. In the Krebs or OXPHOS cycle, sequential oxidation and reduction reactions take place upon a chain of five multiprotein complexes called complex I (NADH dehydrogenase or NADH: ubiquinone oxidoreductase), complex II (succinate dehydrogenase or succinate: ubiquinone oxidoreductase), complex III

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Table 1
Clinical and histopathological features of primary enucleated retinoblastoma patients.

Parameters	No. of patients (N = 109) N (%)
Sex	
Male	61 (55.9%)
Female	48 (44.0%)
Age	
<2 years	49 (44.9%)
≥2 years	60 (55.0%)
Laterality	
Unilateral	84 (77.0%)
Bilateral	25 (22.9%)
Grouping	
Group E	100 (91.7%)
Group D–A	9 (8.3%)
pTNM staging	
$T1N_oM_o$–$T2_bN_oM_o$	65 (59.6%)
$T3_aN_oM_o$–$T4_bN_oM_o$	44 (40.3%)
Tumor differentiation	
PDRB	82 (75.2%)
WDRB	27 (24.7%)
Necrosis	
Presence	65 (49.6%)
Absence	44 (40.3%)
Calcification	
Presence	34 (31.2%)
Absence	75 (68.8%)
Invasion of choroid	
Massive	31 (28.4%)
Focal	27 (24.7%)
Invasion of anterior chamber	5 (4.6%)
Invasion of sclera	9 (8.25%)
Invasion of iris & ciliary body	10 (9.2%)
Invasion of optic nerve	
Pre-lamina	28 (25.7%)
Retrolaminar and cut end	29 (26.6%)
Invasion	
Invasive tumor	46 (42.2%)
Non-invasive tumor	63 (57.8%)

(the bc1 complex or ubiquinone: cytochrome c oxidoreductase), complex IV (cytochrome c oxidase, cyclooxygenase or reduced cytochrome c:oxygen oxidoreductase), and complex V (ATP synthase); which are localized on the inner mitochondrial membrane Poyton and McEwen, 1996.

Cancer cells are characterized by uncontrolled proliferation due to gain-of-function of oncogenes and loss-of-function of tumor suppressor genes DeBerardinis et al., 2008. Uncontrolled cell growth and altered energy metabolism are two essential properties of tumor. Mitochondria play a fundamentally important role in energy metabolism and programmed cell death, suggesting that mitochondria might serve as the key switch for carcinogenesis. Among the various recognized hallmarks of cancer, aerobic glycolysis or the Warburg effect is also a robust metabolic hallmark of most tumors Seyfried and Shelton, 2010. Otto Warburg hypothesized that the increased rate of glycolysis observed in tumor cells, might be due to their suppressed aerobic respiration Warburg, 1956.

Cancer cells are characterized in general by a decrease in mitochondrial respiration and oxidative phosphorylation, together with an enhancement of glycolysis Warburg, 1956. Although the decrease in mitochondrial oxidative phosphorylation is considered by many investigators as a universal feature of neoplastic cells, studies have reported that cancer cells also show normal or even high respiratory activity Zu and Guppy, 2004; Weinberg and Chandel, 2009.

Since oxidative phosphorylation plays an important role in causing cancer, therefore, we aimed to determine the expression of mitochondrial complexes I, III, IV and V in retinoblastoma tumor samples and examine their correlation with clinicopathological parameters and patient outcome.

2. Material & methods

2.1. Study design

The study was approved by ethics committee of All India Institute of Medical Sciences and the committee deemed that it conformed to the generally accepted principles of research, in accordance with the Helsinki Declaration. Informed consent was obtained from all patients prior to enrollment in the prospective study. A total of 109 patients of

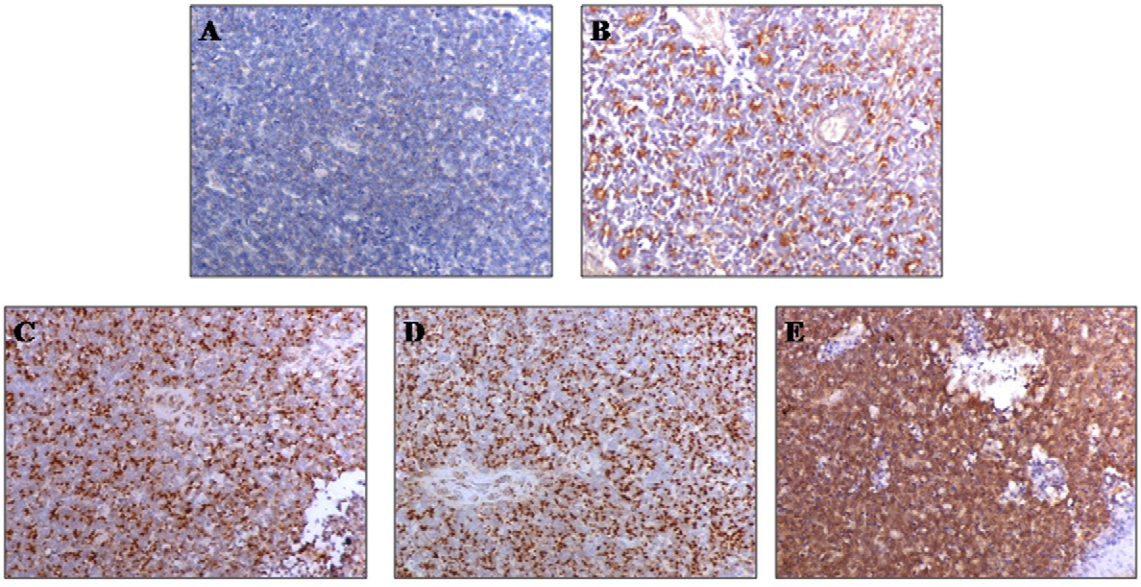

Fig. 1. (A–E): Cytoplasmic expression of mitochondrial OXPHOS complexes in human retinoblastoma tissues using immunohistochemistry (×200). a) Loss of mitochondrial complex I in poorly differentiated retinoblastoma (PDRB). b) Expression of mitochondrial complex I in well differentiated retinoblastoma (WDRB) using anti-mouse mitochondrial complex I antibody. c) Expression of mitochondrial complex III using anti-mitochondrial complex III antibody. d) Cytoplasmic expression of mitochondrial complex IV using anti-mitochondrial complex IV antibody. e) Expression of mitochondrial complex V using anti-mitochondrial complex V antibody.