Mitochondrial Cristae Remodeling in HeLa Cervical Carcinoma Cells Under Hypoxia-Induced OPA1 Oligomerization: A Quantitative Ultrastructural Analysis

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Abstract. Mitochondrial cristae architecture is a critical determinant of oxidative phosphorylation efficiency and apoptotic regulation. This study examines hypoxia-driven OPA1 oligomerization and its downstream effects on cristae junction width, respiratory supercomplex assembly, and cytochrome c sequestration in HeLa cervical carcinoma cells. Using a combination of focused ion beam scanning electron microscopy (FIB-SEM), proximity ligation assay (PLA), and Blue Native PAGE, we quantified ultrastructural remodeling events under 1% O_2 conditions over 6–48 hours. Our data demonstrate that sustained hypoxia induces a statistically significant narrowing of cristae junctions ($p < 0.001$) concurrent with elevated OPA1 high-molecular-weight oligomer formation and enhanced OXPHOS supercomplex I+III_2+IV stoichiometry. These findings establish a mechanistic link between inner mitochondrial membrane fusion dynamics and metabolic adaptation in hypoxic tumor microenvironments, with implications for mitochondria-targeted cancer therapeutics.

Keywords: mitochondrial cristae remodeling, OPA1 oligomerization, hypoxia-induced metabolic adaptation, inner mitochondrial membrane dynamics, respiratory supercomplex assembly, cytochrome c sequestration, FIB-SEM ultrastructural analysis, tumor microenvironment bioenergetics, oxidative phosphorylation regulation

Introduction

Mitochondria are highly dynamic organelles whose structural plasticity is inextricably coupled to their bioenergetic and signaling functions. The inner mitochondrial membrane (IMM), characterized by its deep invaginations known as cristae, provides the scaffolding upon which the electron transport chain (ETC) complexes and ATP synthase dimers are

organized into functional respiratory supercomplexes. The architecture of cristae—particularly the geometry of cristae junctions (CJs), the narrow tubular neck-like structures connecting the cristae membrane to the inner boundary membrane—has emerged as a pivotal regulatory node controlling both oxidative phosphorylation (OXPHOS) efficiency and the retention of pro-apoptotic intermembrane space proteins, most notably cytochrome c. Disruption of this architecture is now recognized as a fundamental event in cancer cell metabolic reprogramming, particularly under conditions of chronic hypoxia characteristic of solid tumor microenvironments. Hypoxia, defined operationally as tissue oxygen tension below 2%, is a pervasive feature of advanced cervical, breast, and colorectal carcinomas, and drives transcriptional and post-translational cascades that fundamentally reconfigure mitochondrial identity. Among the key molecular effectors mediating IMM remodeling is Optic Atrophy Protein 1 (OPA1), a dynamin-like GTPase anchored to the IMM whose proteolytic processing generates distinct long (L-OPA1) and short (S-OPA1) isoforms. L-OPA1 oligomers have been demonstrated to directly tighten CJ apertures, thereby restricting cytochrome c diffusion into the intermembrane space and conferring resistance to intrinsic apoptotic stimuli. However, the precise quantitative relationship between hypoxia duration, OPA1 oligomerization state, CJ remodeling, and supercomplex stoichiometry remains insufficiently characterized at the ultrastructural level in cancerous epithelial cell backgrounds. Previous studies have employed transmission electron microscopy (TEM) to describe hypoxia-associated mitochondrial elongation and cristae densification; however, the volumetric and nanoscale precision required to resolve CJ width distributions across whole-cell mitochondrial networks has historically been beyond the reach of conventional two-dimensional imaging modalities. The advent of focused ion beam scanning electron microscopy (FIB-SEM) with isotropic voxel resolutions approaching 4–8 nm now permits three-dimensional reconstructions of entire mitochondrial populations within single cells, enabling statistically robust morphometric analyses previously inaccessible to the field. The present study addresses this gap by providing a rigorous, quantitative ultrastructural characterization of OPA1-mediated cristae remodeling in HeLa cervical carcinoma cells subjected to graded hypoxic exposure. The paper is organized as follows: Section 2 details the cell culture, hypoxic treatment protocols, FIB-SEM sample preparation, Blue Native PAGE conditions, and proximity ligation assay methodology; Section 3 presents morphometric measurements of CJ widths, mitochondrial network topology parameters, and supercomplex abundance data; Section 4 discusses the mechanistic implications of our findings in the context of mitochondria-targeted therapeutic vulnerabilities; and Section 5 summarizes the principal conclusions and proposes directions for future in vivo validation.

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