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Comparative survey of mitochondrial ultrastructure in *IDH1*-mutant astrocytoma and *IDH1*-wildtype glioblastoma (GBM)

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Abstract

Gliomas are the most common malignant brain tumors with poor prognosis. The WHO's classification recognizes isocitrate dehydrogenase 1 (*IDH1*) mutant astrocytoma and *IDH1*-wildtype glioblastoma (GBM). The *IDH1* mutation confers a survival advantage over the wildtype. There are several explanations for the metabolic advantage of the *IDH1* mutation, some involve mitochondrial implications. Since an ultrastructural comparison of both tumor genotypes is still lacking, we surveyed the ultrastructural effects of the *IDH1* mutation on the mitochondria of the *IDH1*-mutant astrocytoma (n = 15) and *IDH1*-wildtype glioblastoma (n = 15) tumors. Our results show that both *IDH1* genotypes have degenerate and uncoupled mitochondria; this has not been reported before. The presence of ample lipid inclusions and lipid droplets in the cytoplasm of both genotypes support our conclusion of dysfunctional uncoupled mitochondria. Thus, the *IDH1* mutation may have no ultrastructural consequences on the mitochondria, and the aberrant mitochondria in both genotypes may be the result of other unknown mutations. The status of the mitochondria in these genotypes portends a clinical challenge since tumor cells with uncoupled mitochondria are more primitive, aggressive, and considerably treatment resistant.

Keywords

Glioblastoma; mitochondria; isocitrate dehydrogenase; TCA cycle; beta-oxidation

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Experimental implementation/Data acquisition: MAA

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Introduction

Since the discovery of isocitrate dehydrogenase (IDH) mutation in gliomas, multiple studies have evaluated its effect on tumor progression and response to treatment, and proposed that *IDH*-mutant astrocytoma grade 4, referred to previously as "secondary glioblastoma," most likely evolve from lower grade gliomas that were initially undetected.[1] Meanwhile, some adult low-grade gliomas that lack the mutant *IDH* are considered "pre-glioblastoma." The mutations of IDH1 affect the active site arginine residue that recognizes isocitrate, IDH1 R132; these point mutations result in the replacement of arginine with histidine (H, majority of cases), lysine (K), or cysteine (C).[2]

Mutations affecting the tricarboxylic acid cycle (TCA) metabolites are known to generate structural aberrations within the mitochondria as well as its vesicle and microvesicle formation and trafficking.[3–5] Since the IDH1 enzyme affects the mitochondrial functions, the effect(s) of its mutation ought to be detected inside the mitochondria.[6] To date, there is very little known about the ultrastructural differences between IDH mutant tumor cells in comparison to IDH wildtype tumor cells. More specifically, there are no reports on the mutation's effects on the ultrastructure of the mitochondria, for example, it is unknown whether it affects the coupling status of the mitochondrial membranes. Therefore, examining the ultrastructural effect of the *IDH1* mutation on the ultrastructural morphology of the mitochondria may bring about an insight into the differential survival of the patients' two groups. Additionally, the mitochondrial ultrastructural morphology of the *IDH1*-wt GBM has not been surveyed before. For all these reasons, we embarked on an ultrastructural survey of both tumor genotypes.

IDH1 catalyzes the cytoplasmic oxidative decarboxylation of isocitrate to produce alpha-ketoglutarate (AKG, also known as 2-oxoglutarate) and carbon dioxide.[7] AKG is a critical component of the TCA cycle affecting the cycle's overall rate[8]; it is a nitrogen scavenger as well as a generator of glutamate and glutamine, thus, it promotes protein synthesis and prevents protein breakdown.[8] Furthermore, AKG is an essential metabolite that engages in a variety of biological functions such as anti-oxidative defense, energy generation, signaling modules, and genetic modifications; it is also utilized as a nutritional supplement. As a consequence, manufacturing AKG is a key survival issue and highlights the importance of IDH in humans.[9]

The mutant *IDH1* is disadvantageous to tumor cells as the redox state of the cell is altered. Not only NADPH generation is reduced (isocitrate + $NADP^+$ → ketoglutarate [KG] + NADPH), but NADPH is also consumed as a proton donor in the conversion of KG to d-2-hydroxyglutarate (d-2-HG) (according to this equation: KG + NADPH → d-2-HG + $NADP^+$). As a result, the antioxidative and detoxifying protection provided by NADPH is reduced, which is eventually harmful to the cell in general.[10] Moreover, changes in the endoplasmic reticulum (ER) and Golgi apparatus caused by the IDH1 mutated enzyme, and mediated by d-2-HG, induced upregulation of stearoyl-CoA desaturase (the rate-limiting enzyme in monounsaturated fatty acid [MUFA] biosynthesis).[11] These biochemical alterations are imitated in patient tumor tissue and point out the tumor

cell vulnerabilities. The accumulation of d-2-HG compromises the TCA cycle functions and causes the accumulation of acetyl-CoA in the cytoplasm, which gets diverted to lipogenesis.[6,12]

The biochemical effect of the *IDH1* mutation is obviously deleterious to the tumor cell and this may explain the differential in survival period; however, the ultrastructural implication has not been explored yet. Our research is addressing this deficiency.

Materials & methods

Tissue specimens

All 30 brain tumor tissue samples were obtained from patients with histopathological confirmed high-grade gliomas. The study protocol was approved by the Institutional Review Board of The University of California Davis. The study utilized specimens of formalin-fixed paraffin-embedded tumors. The specimens represented 15 samples of *IDH1*-mt astrocytoma tumors and 15 samples of *IDH1*-wt GBMs. The tissues were dug out of the paraffin blocks after examining their H&E slides and determining the areas of the tumor viable cells.

Tissue preparation

The specimens were deparaffinized in xylene overnight followed by one more change of xylene for 20 min, two changes of absolute ethanol (5 min each), one change of 70% ethanol (5 min), and three changes of phosphate buffered saline (PBS, 5 min each), then processed for transmission electron microscopy (TEM) according to Abu-Asab.[13] Briefly, specimens were washed in PBS, post-fixed in 0.5% osmium tetroxide (OsO_4), rinsed, dehydrated, then embedded in Spurr's epoxy resin. Blocks were sectioned at ~90 nm thickness on a Leica EM UC6 ultramicrotome (Leica, Austria), double-stained with uranyl acetate and lead citrate, and imaged with JEOL JEM-1010 electron microscope (JEOL, Japan).

Results

The mitochondria of the IDH1-wildtype GBMs:

Out of the 15 wildtype GBM tumors that we surveyed none have shown normal mitochondria (Figure 1). Mitochondria were degenerate with intact outer membrane but disintegrated inner membrane (Figure 1: b3 & c3); others without outer membrane and with inner membranes forming lattice structure without cristae (Figure 1: a3); some mitochondria contained lipid inclusions (Figure 1: a2 & a3). Tumor cells lacked clearly defined cell membranes (Figure 1: b2&3, c2–3). Lipids and/or lipid droplets were ample in the cytoplasm of all cases.

The Mitochondria of IDH1-Mutant high-grade Astrocytoma:

Similar to the wildtype GBMs, the 15 *IDH1*-mutant astrocytoma tumors showed abnormal mitochondria (Figure 2). The mitochondrial membranes were uncoupled and in various stages of disintegration. Cristae were absent and the lumina filled with dark amorphous material that seems proteinaceous in nature. Some mitochondria were missing their outer membrane and the inner membrane forming a lattice structure (Figure 2: a3). Some

mitochondria showed lipid inclusion (Figure 2: c3). Lipids and/or lipid droplets were ample in the cytoplasm of all cases. There were myelin inclusions in many cases (Figure 2: a2).

Discussion

Contrary to the widespread conception that only *IDH1*-mt astrocytoma tumors may have aberrant mitochondria, the results of the survey showed that the tumor cells of all surveyed *IDH1*-wt GBM had degenerate mitochondria. The tumor cells of the two *IDH1* genotypes had similarly dysfunctional and uncoupled mitochondria that were at various stages of degeneration and no intact mitochondria were seen in the 30 tumors that were surveyed.

Tumor cells with uncoupled mitochondria have substantial amounts of free lipids and/or lipid droplets accumulation. This is a consistent phenomenon in eukaryotic cells with damaged mitochondria that has been reported on before.[12,14] Although lipid accumulation may be attributed to the *IDH1* mutation; however, in the *IDH1*-wt GBM the exact cause is unknown. We think that the accumulation of lipids and their droplets in all astrocytoma and GBM tumor cells, and sometimes within the mitochondria, is caused by uncoupled mitochondria and incomplete TCA cycle. This usually causes the buildup of acetyl-CoA in the cytoplasm, and its conversion into fatty acid; thus, leading to fatty acid synthesis and its buildup in the cell as lipid droplets and free lipids.[12] Since lipid breakdown takes place in the mitochondria, the presence of a substantial quantity of lipids in the mitochondria (Figure 2: b3 & c3) is an indication that the beta-oxidation pathway of the mitochondria is dysfunctional or the TCA cycle is backed up.[15]

It has been reported that patients with *IDH1*-mt astrocytoma have longer survival than those with the wildtype.[2] This observed longevity could be due to the deleterious effect of the unusable product of the mutated *IDH1*, the 2-hydroxyglutarate (2HG). The *IDH1* mutation compromises the TCA cycle, upregulates the hypoxia inducible factor-1 alpha (HIF1-alpha),[16] and decouples oxidative phosphorylation in mitochondria, which reduces the tumor cell's ability to produce ATP within the mitochondria among several other disadvantages that it bestows. Thus, the *IDH1* mutation forces the tumor cell into a hypoxic phenotype that is primarily dependent on glycolysis and lactic acid fermentation; this limits the amount of NADPH available for protecting the cell from oxidative stress as well. The implications of these biochemical aberrations are clearly visible ultrastructurally in the *IDH1*-mt genotype as lipid inclusions and droplets, glycogen buildup, and degenerate mitochondria.

Although our ultrastructural survey of 30 glioma tumors (15 *IDH1*-wt GBMs and 15 *IDH1*-mt astrocytoma) demonstrated aberrant ultrastructure in the *IDH1*-mt mitochondria, which is an expected finding since the mutation compromises the TCA cycle enzyme; however, the findings have not been as expected for the 15 *IDH1*-wt GBMs since they showed abnormal and degenerate mitochondria as well. This raises several questions: if the two genotypes have aberrant mitochondria, is there an unknown mutation(s) that affect the mitochondria that have not yet been identified? Because the mitochondria are damaged in both genotypes, could the *IDH1* mutation be insignificant, or minimally significant, in causing this damage?

It is important to recognize the limitations of this study; it only included a modest number of specimens (30) and was confined to ultrastructural observations. Although these observations gave us new insights into the nature of glial tumors, answers to the questions raised by the authors can only be addressed by other means of investigation such as molecular, proteomic, and lipidomic techniques.

In conclusion, although many researchers assume that the *IDH1*-mt astrocytoma tumors would have aberrant mitochondria while the *IDH1*-wt GBMs would not; the survey has shown that both genotypes have aberrant uncoupled mitochondria. This may point out that despite the longevity advantage of the IDH1 mutation in astrocytoma patients, its full effect(s) on the tumor cells is not yet entirely elucidated, and at the same time, the main cause of mitochondrial uncoupling in GBM tumors is also unknown.

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Abbreviations

GBM	Glioblastoma
IDH	isocitrate dehydrogenase
IDH-mt	IDH-mutant
IDH-wt	IDH-wildtype
TCA	tricarboxylic acid cycle
AKG	alpha-ketoglutarate

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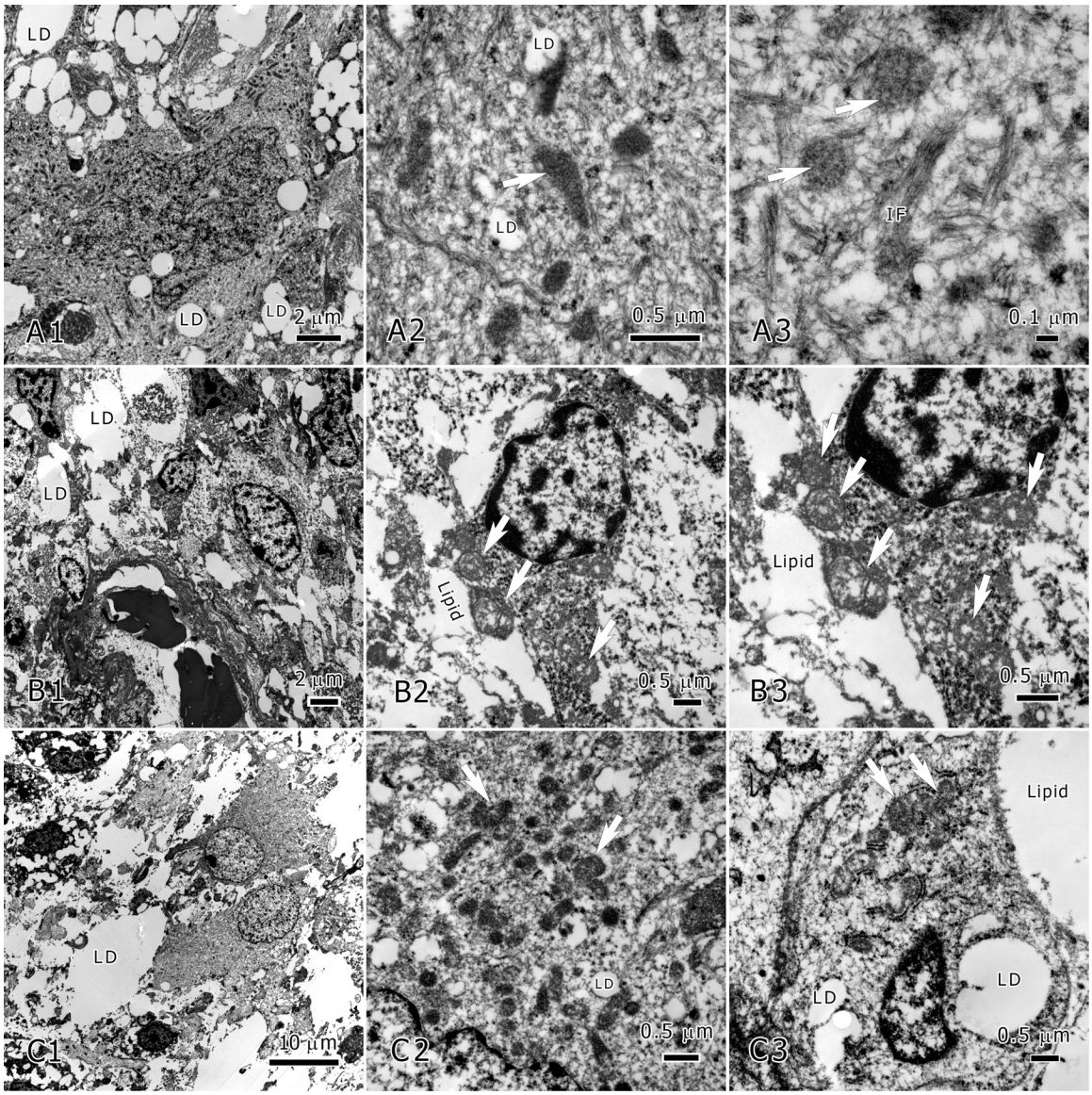

Figure 1.

TEM micrographs of three representative cases of IDH1-wt glioblastoma tumors: first case (a1-a3), second case (b1-b3), and third case (c1-c3). The three cases are similar in their ultrastructural details. The figure shows viable tumor cells with dense cytoplasm (a1, b1, c1), lipid droplets (LD) and lipid inclusions (all 9 images show lipids or droplets), intermediate filaments (IF, a3), and sometimes lobulated nuclei (a1). Mitochondria (white arrows) were degenerate with intact outer membrane but disintegrated inner membrane (b3 & c3); others without outer membrane and with inner membranes forming lattice structure without cristae (a3); some mitochondria contained lipid inclusions (a2 & a3). Tumor cells lacked clearly defined cell membranes (b2&3, c2–3).

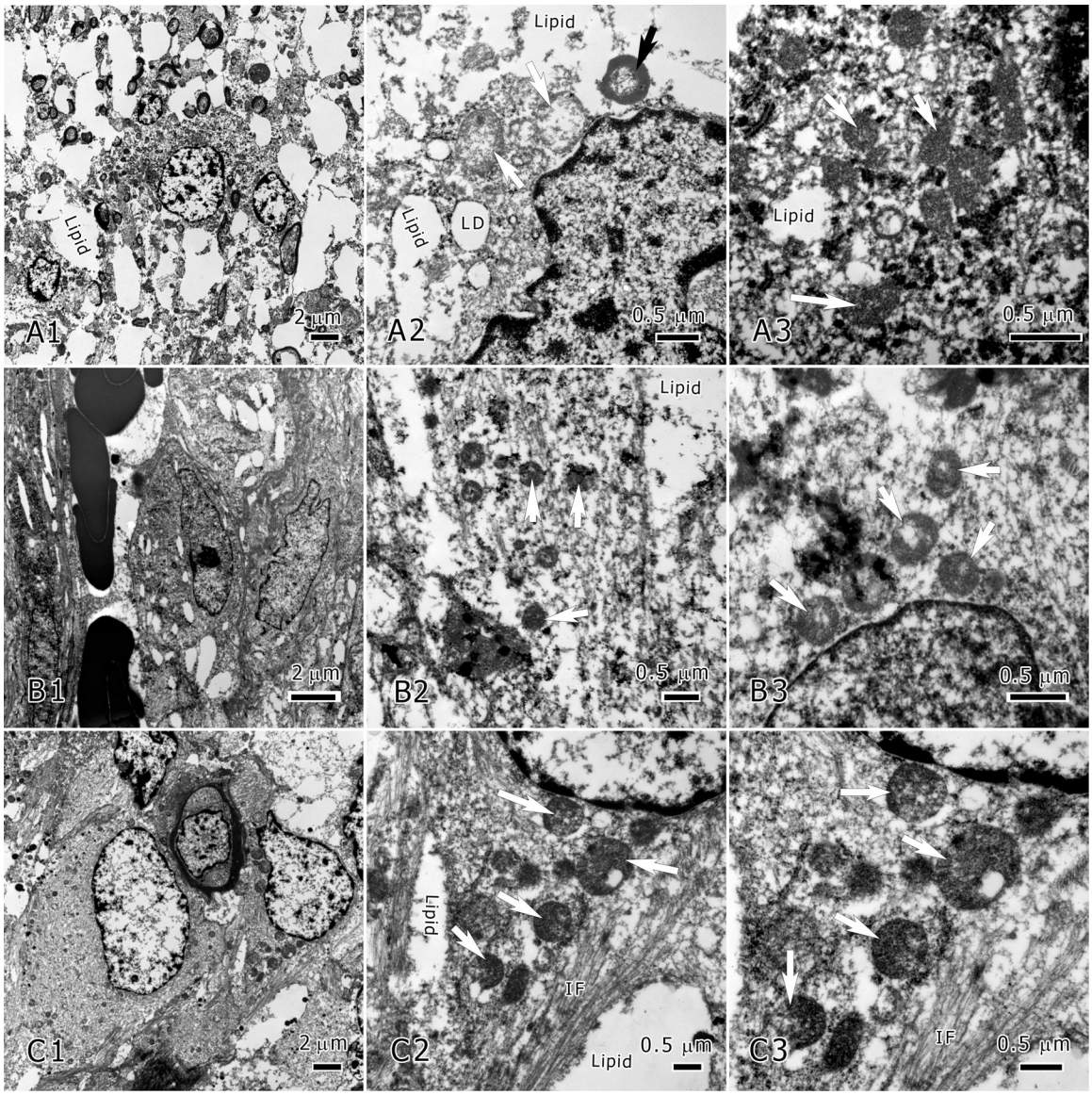

Figure 2.

TEM micrographs of three representative cases of IDH1-mt astrocytoma tumors: first case (a1-A3), second case (b1-b3), and third case (c1-c3). Mitochondria (white arrows) are degenerate in all three cases; cristae are absent and the lumina are filled with dark amorphous material that seems proteinaceous in nature. a3 shows mitochondria missing their outer membrane and the inner membrane forming a lattice structure. c3 shows a mitochondrion with lipid inclusion. Lipids are ample in the cytoplasm of the three cases. a2 shows a myelin inclusion (black arrow).