Sequencing of mtDNA of multiple single mitochondrion isolated from live cells identifies common heteroplasmic sites

Mitochondria are organelles present in all eukaryotic cells, including brain cells, and supply ATP required for cellular physiology through a series of redox reactions known as oxidative phosphorylation. They are unique in their requirement for expression of genes encoded both in the nucleus (1000's of genes) and from their own polyploidy genome (37 genes in human) to function. Mutations in the mitochondrial genomes contained within the organelle are associated with disease, including various neurodegenerative diseases. It is hypothesized that symptom presentation requires a mutation associated with decreased capacity for mitochondrial function to reach a threshold load, since wild type mitochondrial genomes can complement the loss of function. When both wild type and mutated mtDNA are present in the same mitochondrion, cell, or tissue, this is referred to as heteroplasmy. Heteroplasmy status of mtDNA within a single mitochondrion is fluid due to the highly dynamic structural changes of individual mitochondrion, such as fusion with another mitochondrion, or fission into multiple mitochondria. mtDNA is redistributed during these processes and can alter heteroplasmy at the single mitochondrion level.

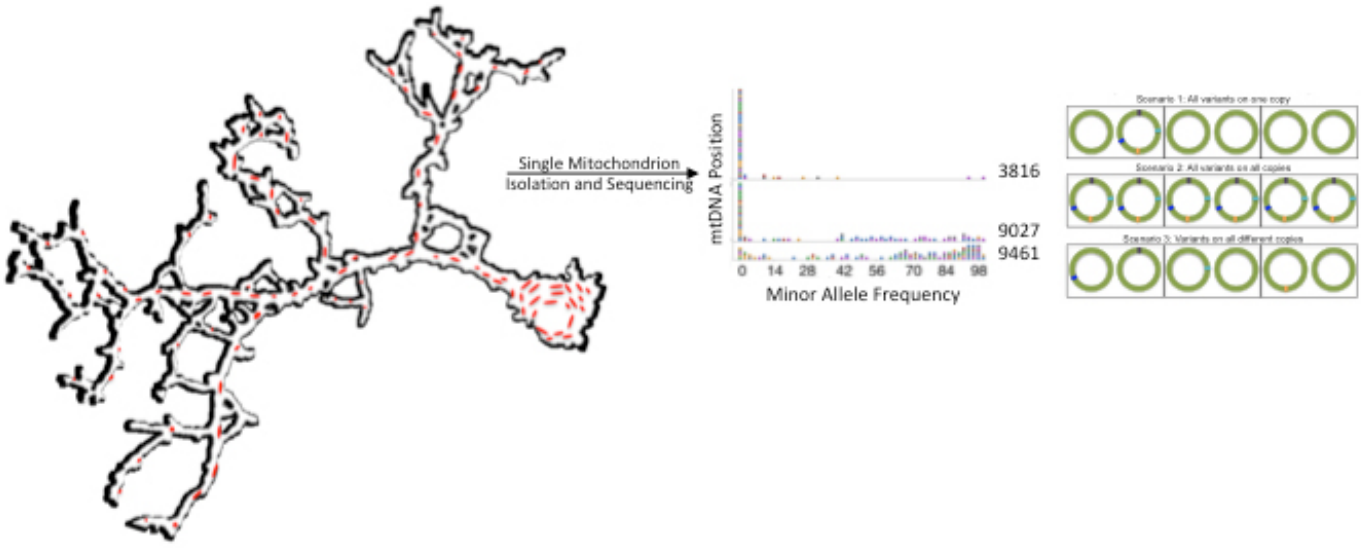

Fig. 1. Single mitochondrion (red ovals) are isolated using a glass pipette and the mtDNA within PCR amplified then sequenced to identify mutations or deviations from the reference sequence. The center dot plot indicates the minor allele frequencies across single mitochondrial samples for

each of the 3 high confidence positions identified in mouse mitochondrion from both neuronal dendrites and astrocytes. The final schematic describes 3 scenarios of how single mutations can be distributed amongst 6 hypothetical mitochondrial genomes contained in a single mitochondrion that would ultimately impact how mutations are distributed following mitochondrial structural remodeling through fission and fusion.

Heteroplasmy at the whole cell level is affected by new mitochondrial DNA synthesis, old mitochondrial degradation, and by stochastic daughter cell mitochondrial inheritance if the parent cell is not post-mitotic and can still undergo cell division. It is unknown how low-frequency heteroplasmic deleterious mutations from a single mitochondrion in a single cell become enriched to the point where they are associated with disease. To identify low-frequency mutations prior to disease presentation, we developed a method for isolation of single mitochondrion from live brain cells in vitro (either mouse or human derived) with subsequent amplification and measurement of mutations contained in their genomes. We found that single mitochondrion isolated from mouse astrocytes, mouse neuronal dendrites or from human cell processes were heteroplasmic at various sites in the mitochondrial genome. Overall, we found high levels of variant alleles of the polyploid genome (~4 polymorphic sites per mitochondrion). The high impact sites found across multiple single mitochondrion isolated from mouse cells included a mutation in the second position of the anticodon stem of the mitochondrial tRNA for glutamine; a missense mutation in the gene encoding cytochrome c oxidase subunit III, a component of the oxidative phosphorylation system; and a mutation in the third position of the start codon of the gene encoding the third subunit of NADH dehydrogenase, also a component of the oxidative phosphorylation system. Mitochondria isolated from in vitro cultured human brain cells prepared from a neurosurgical patient revealed a consistent heteroplasmy at position 309 in a regulatory region of the mtDNA that controls replication and transcription. Our manual approach allowed analysis of up to 10 mitochondrion from a single cell so total mutational burden per cell and how these mutations were distributed across the mitochondrial network could not be assessed. With the future development of high throughput isolation of all of the mitochondrion from a single cell, the distribution of mutational load across the entire mitochondrial system and how this associates with cellular function can be assessed. Understanding of how these mutations propagate in the context of active mitochondrial structural dynamics may allow development of strategies to preferentially expand non-mutated sub-populations in an attempt to prevent or rescue a deleterious mutation-associated phenotype without the need for directed or targeted repair of the mutation.

Jacqueline Morris[1]***, Junhyong Kim***[2]***, James Eberwine***[1]
[1]*Departmentof Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, PA, USA*
[2]*Department of Biology, School of Arts and Sciences, University of Pennsylvania, PA, USA*

Publication

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