MITOCHONDRIA

TIME: 14.30 – 15.15

LOCATION: DOBSON ROOM

MORPHOLOGY AND FUNCTION: TWO FOR THE PRICE OF ONE!

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In the early 20th century biologists were intrigued by the various cellular structures that they encountered. With the development of electron microscopy (EM), they succeeded in imaging the ultrastructural detail of the cell so turned their attention to studying the functions of these structures.

More recently, the majority of literature reported mechanisms and functions of cells and their organelles, with less emphasis on descriptive studies. These two research traditions have remained largely separate but are essential for each other. Mitochondria, the "powerhouse" of the cell, were observed for the first time in the 18th century but it was not until the middle of the 20th century that mitochondria were recognised as playing a central role in cell life. It is now well-established that changes in mitochondrial morphology affect the mitochondrial function of skeletal muscle and vice versa. Transmission EM can be combined with labelling methods to pinpoint the positions or activity of specific molecules, thus linking morphology and function. However, TEM provides 2D information and it is not clear what is happening in 3D.

Therefore, we have developed a labelling method that can be combined with a 3D EM technique, serial block-face scanning electron microscopy (SBFSEM), to correlate 3D mitochondrial morphology with mitochondrial enzyme activity (Cytochrome c, COX) in muscle. This novel technique gives new insights into how mitochondrial function is linked to mitochondrial morphology and mechanisms underpinning human disease.

SHEDDING LIGHT ON MITOCHONDRIAL REDOX: A FLUORESCENT SCREENING METHOD FOR INTERROGATING THE MITOCHONDRIAL NAD+/NADH RATIO

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NAD+ and its reduced counterpart NADH, are involved in redox reactions throughout the cell therefore the ratio between them can act as an indicator of aberrant cellular function. Particularly in cases of mitochondrial dysfunction. Research suggests this redox pair exists in distinct pools throughout the cell, but despite this many studies report changes on a cellular level rather than attributing the fluctuation to specific subcompartments. Considering this, a novel method has been developed using a mitochondrially localised fluorescent NAD+ biosensor and NADH autofluorescence. Consequently, the absolute values for each redox counterpart can be monitored simultaneously in vitro. Following optimisation, this novel technique has been proven to respond to subtle perturbations within the mitochondria, with a focus on the oxidative phosphorylation pathway. Initial results were used to evaluate the screening suitability of the assay, reporting a Z prime value of 0.82, which surpasses the 0.5 threshold required for an accurate assay. To further examine the dynamics of the assay, it was used to interrogate compounds currently thought to increase cellular NAD+. This revealed that inhibition of NAD+ consumers such as PARP1 does not have a significant effect on mitochondrial NAD(H). In the future, this assay will be used to conduct high-throughput screening to identify compounds capable of increasing mitochondrial NAD+ in a complex I deficient redox environment. As no current method exists for interrogating mitochondrial NAD+/NADH in vitro, the assay could serve as a novel approach for drug

discovery and mitochondrial redox interrogation in the future.

topographical scanning of the cellular architecture at the nanoscale.

NANOBIOPSY: EXPANDING UPON CLONAL EXPANSION

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Multiple copies of mitochondria DNA (mtDNA) exist in all mitochondria, which encode proteins that make up or maintain mitochondrial respiratory chain complexes. At birth, in all healthy individuals, the mitochondrial genome exists in homoplamsy where all copies are the same. Over time, however, stochastic damage can introduce mtDNA mutations. Low levels of mutant mtDNA is well tolerated but above a threshold level this leads to mitochondrial dysfunction and ultimately disease.

Clonal expansion refers to how mtDNA mutations can spread and whilst a number of theories explaining this phenomenon have been put forward, the exact mechanism is yet to be elucidated.

An exciting, new theory is the 'perinuclear theory' of clonal expansion: Mitochondria closer to the nucleus are better placed to receive nuclear encoded signals increasing mitochondrial biogenesis – causing an increase in not just the number of mitochondria but also the mutant mtDNA that they contain. To confirm this theory sampling within subcellular foci of deficiency is required and a technique called nanobiopsy offers the potential to allow us to do this.

Nanobiopsy utilises a nanopipette filled with an organic solvent which is immiscible in the cell cytoplasm. 'Electrowetting' involves introducing a sub-physiological voltage across the liquid-liquid interface between the organic solvent and cytoplasm, allowing the aspiration of mitochondria within the cell cytoplasm. Precise placement of the nanopipette is achieved using Scanning Ion Conductance Microscopy, where occlusion of an electrical current, flowing between the nanopipette and references electrodes, allows

PLATELET MITOCHONDRIAL DNA METHYLATION PREDICTS FUTURE CARDIOVASCULAR OUTCOME IN ADULTS WITH OVERWEIGHT AND OBESITY

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The association between obesity and cardiovascular disease (CVD) is proven, but why some adults with obesity develop CVD while others remain disease-free is poorly understood. Here, we investigated whether mitochondrial DNA (mtDNA) methylation in platelets in adults with overweight and obesity predicts those who will subsequently develop CVD. We devised a nested case-control study of 200 adults with overweight or obesity who were CVD-free at Baseline, of whom 84 developed CVD within five years while 116 remained CVD-free. Platelet mtDNA methylation was analysed by bisulfite-pyrosequencing at Baseline in 13 different CpGsites within seven genes. MtDNA methylation at *MT-CO1* nt6807 (OR=1.08, 95% CI 1.02-1.16; P=0.014), *MT-CO3* nt9444 (OR=1.22, 95% CI 1.02-1.46, P=0.042) and *MT-TL1* nt3254 (OR=1.30, 95% CI 1.05-1.61, P=0.008) were higher at Baseline in those who developed CVD during Follow-up, compared with those who remained CVD-free. Combined use of the three loci significantly enhanced risk prediction, with hazard ratios of 1.38 (95% CI 0.68-2.78) and 2.68 (95% CI 1.41-5.08) for individuals with hypermethylation of 1 or 2-3 genes, respectively (P=0.003). Methylation at these sites was independent of conventional CVD risk factors, including inflammation markers, fasting blood glucose concentration and blood pressure. We further tested whether diet can influence mtDNA methylation. Collectively, these markers may represent a novel predictor of CVD in adults with overweight and obesity.