MITOCHONDRIAL REPROGRAMMING IN CANCER

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Tumours must overcome numerous metabolic challenges in order to thrive in nutrient-deprived microenvironments and evade therapeutics. Mitochondria are dynamic organelles that provide the metabolic flexibility and plasticity demanded by cancer cells. Our overall objectives are to understand how mitochondria are reprogrammed at different stages of tumorigenesis and to reveal metabolic vulnerabilities in cancer by targeting mitochondrial metabolite transporters.

During an exciting first year at the Institute, we embarked on our study of mitochondrial reprogramming in cancer cells (Figure 1). Metabolite transporters sit in the impermeable inner mitochondrial membrane and couple the metabolic reactions of the cytosol with the mitochondrial matrix. These transporters and associated regulatory components therefore represent crucial sites of cellular metabolic control. We are performing genetic screens to identify mitochondrial metabolite transporters that influence pancreatic cancer cell growth and survival under different environmental conditions. Extensive metabolic reprogramming occurs during the development of pancreatic cancer, and mitochondria are known to support pancreatic tumour proliferation upon hypoxia and nutrient deprivation. We hope that our screening results will offer new transporters and metabolic pathways as therapeutic targets to test in pre-clinical models of the disease.

The transport and metabolism of mitochondrial nucleotides

One group of metabolites that we have been particularly interested in this year are nucleotides. Mitochondria contain their own genome, packaged into mitochondrial DNA (mtDNA) but lack the ability to synthesise their nucleotides *de novo*. Nucleotides must therefore be imported into mitochondria for the replication and subsequent expression of mtDNA. In addition to providing the building blocks of DNA and RNA, regulated nucleotide transport is required for the exchange of mitochondrial ADP/ATP and GTP for metabolic enzymes. Disturbed mitochondrial nucleotide homeostasis can result in cellular nucleotide imbalance and lead to DNA damage and aberrant innate immune responses. Previous work has shown that enhanced uptake of mitochondrial pyrimidines can trigger the synthesis of mtDNA but also the activation of inflammation pathways. We are keen to

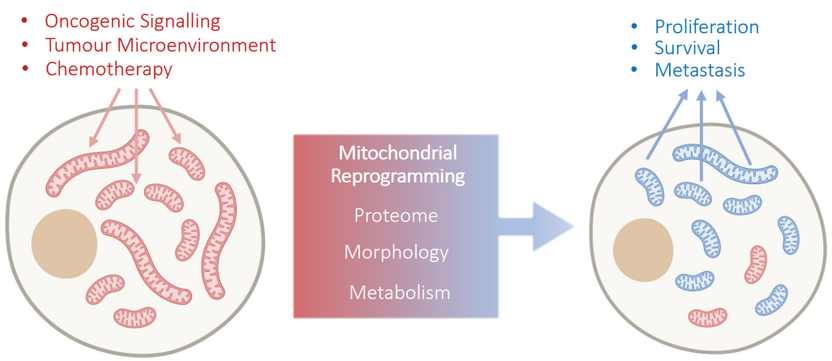

Figure 1
Mitochondria are reprogrammed to facilitate tumour progression and survival

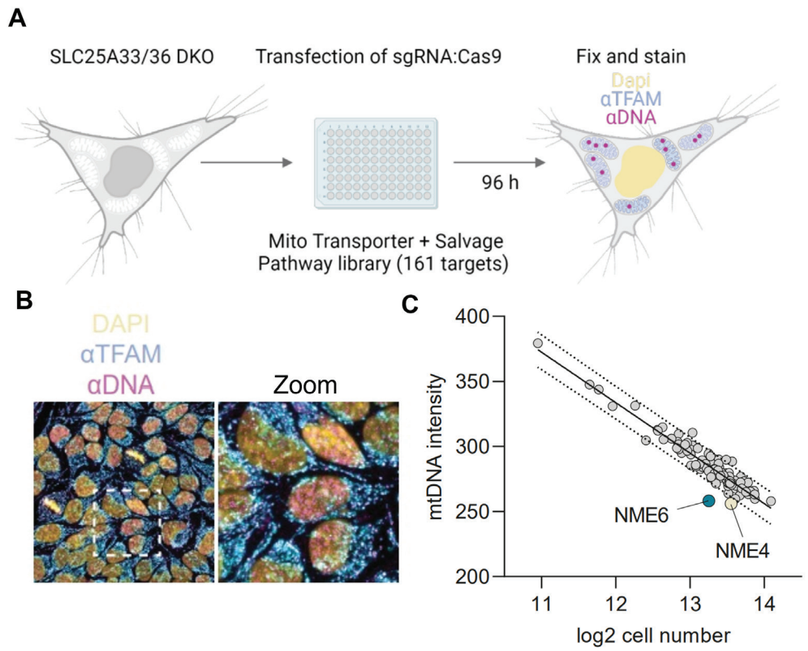

Figure 2
CRISPR-SpCas9 screen reveals regulators of mitochondrial pyrimidine metabolism
A Schematic of the screen conducted in cells lacking both mitochondrial pyrimidine nucleotide transporters, SLC25A33 and SLC25A36 (DKO – double knockout). **B** An anti-DNA antibody was used to detect and quantify mtDNA in cells transfected with a panel of sgRNAs and SpCas9. Mitochondrial nucleoids (packaged mtDNA) were also detected with an antibody against the mtDNA packaging protein, TFAM. **C** Cells transfected with sgRNA targeting the nucleoside diphosphate kinases NME6 and NME4 had reduced levels of mtDNA (data from Grotehans et al., 2022 bioRxiv doi. org/10.1101/2022.11.29.518352).

understand whether mitochondrial pyrimidine transport and metabolism influences tumour immunogenicity and responses to pyrimidine analogue chemotherapies such as 5-fluorouracil and gemcitabine.

Blocking nucleotide supply to suppress mitochondrial activity

We tested what happens to proliferating cells when their mitochondrial pyrimidine import routes are blocked. We were surprised to find that depletion of the two described pyrimidine transporters, SLC25A33 and SLC25A36, had little effect on cell division or mtDNA. One challenge of studying mitochondrial metabolism in mammalian cells is an apparent redundancy in metabolite transporters. Together with the Beatson Advanced Imaging Resource and High-Content Analysis team, we performed a CRISPR-SpCas9 screen of mitochondrial transporters and nucleotide metabolism enzymes to identify genes that regulate mtDNA content when pyrimidine nucleotide import is impaired. Our screen and subsequent experiments revealed that a poorly characterised nucleoside diphosphate kinase, NME6, could preserve mtDNA in pyrimidine depleted conditions (Figure 2). Further work, in collaboration with Prof Thomas Langer (MPI Biology of Ageing, Cologne), revealed that NME6 is constitutively required for the supply of pyrimidines for mitochondrial RNA synthesis. Cells lacking NME6 were deficient in oxidative phosphorylation and could not proliferate in respiration-dependent conditions.
Recent research indicates that perturbing the transcription and translation of mitochondrial genes is a promising strategy to impair tumour proliferation and metastasis. We are therefore excited by our results as NME6 represents a novel node by which we can manipulate mitochondrial gene expression. Our next aim is to target tumours with a particularly high demand on mitochondrial activity and to test if NME6 levels are limiting for tumour progression.