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MITOCHONDRIAL ONCOGENETICS

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Mutations of mitochondrial DNA (mtDNA) are among the most common genetic events in all cancer, however their impact on disease initiation and progression is not understood. Mitochondria perform numerous metabolic functions, relying on faithful expression and maintenance of mtDNA, a small, multi-copy genome separate from the nuclear DNA that is contained exclusively within mitochondria. Mutations of mtDNA and gross changes to mtDNA copy number can lead to profound metabolic alterations - one of the earliest identified hallmarks of cancer – and these changes are observed in >60% of tumours. In order to understand the possible links between mitochondrial genetics and metabolic dysfunction in cancer, our lab studies a range of cancer models using and developing cutting edge mitochondrial genome engineering tools combined with genetic and metabolic analyses. By understanding the relationship between mtDNA and human cancer, we hope to identify new therapeutic targets for clinical application and to inform reallocation of existing treatments based on mtDNA genotype.

Defining the impacts of mtDNA mutations in cancer

Although current model systems for mtDNA mutations in cancer are limited, using model systems in hand we are addressing the effects of mtDNA mutations on cancer initiation, progression and behaviour across a range of established cellular, organoid and *in vivo* models of cancer.

Beyond experimental systems in the lab, using repurposed sequencing data from >40,000 tumours, we have shown that: i) mutations in mtDNA encoded genes were among the most common pan-cancer mutational events, comprising 25 of the 30 most mutated genes in all cancer (Figure 1a), that mtDNA mutational status was unaffected by nuclear DNA mutation burden or MSS/MSI state (Figure 1b,c), that recurrent hotspots defined the patterning of severe mtDNA mutations (Figure 1d) and that mtDNA mutation state offered major prognostic benefit in colorectal cancer (Figure 1e) (Gorelick *et al.*, 2021, *Nature Metabolism*). These findings illustrated some of the major impacts of mitochondrial genetics in cancer for the first time, shining a light on a whole additional genetic system of potential therapeutic targets that have been overlooked in cancer research to date.

Taking this knowledge forward, and using advanced mtDNA engineering techniques, we have now created the first known *in vivo* models of cancer bearing relevant mtDNA mutations across several tissue lineages.

Control of mtDNA copy number

In the nucleus, well-described mechanisms that provide tight control of genome replication are required for cellular and organismal viability. Similarly, mtDNA copy numbers are controlled in a robust, cell-type specific fashion, however, the analogous systems of control underlying regulation of mtDNA genome replication are poorly understood. Cancer cells commonly demonstrate changes in mtDNA copy number, probably due to the metabolic requirements of their tissue lineage and primary site. By developing our understanding of mtDNA copy number regulation and identifying the molecular mechanisms underlying this process, we hope to design future therapeutic strategies underpinned by manipulation of mtDNA copy number.

Genetic transformation of mammalian mitochondria

A major challenge for the field of mitochondrial genetics is the limited set of genetic tools to directly manipulate mtDNA *in situ*. Practically, this means that the experiments we can perform to determine the role of mtDNA mutations in cancer are limited in their scope. In order to develop our understanding of this area of cancer science, we aim to expand the relevant mtDNA genome engineering toolkit.

Publications listed on page 104

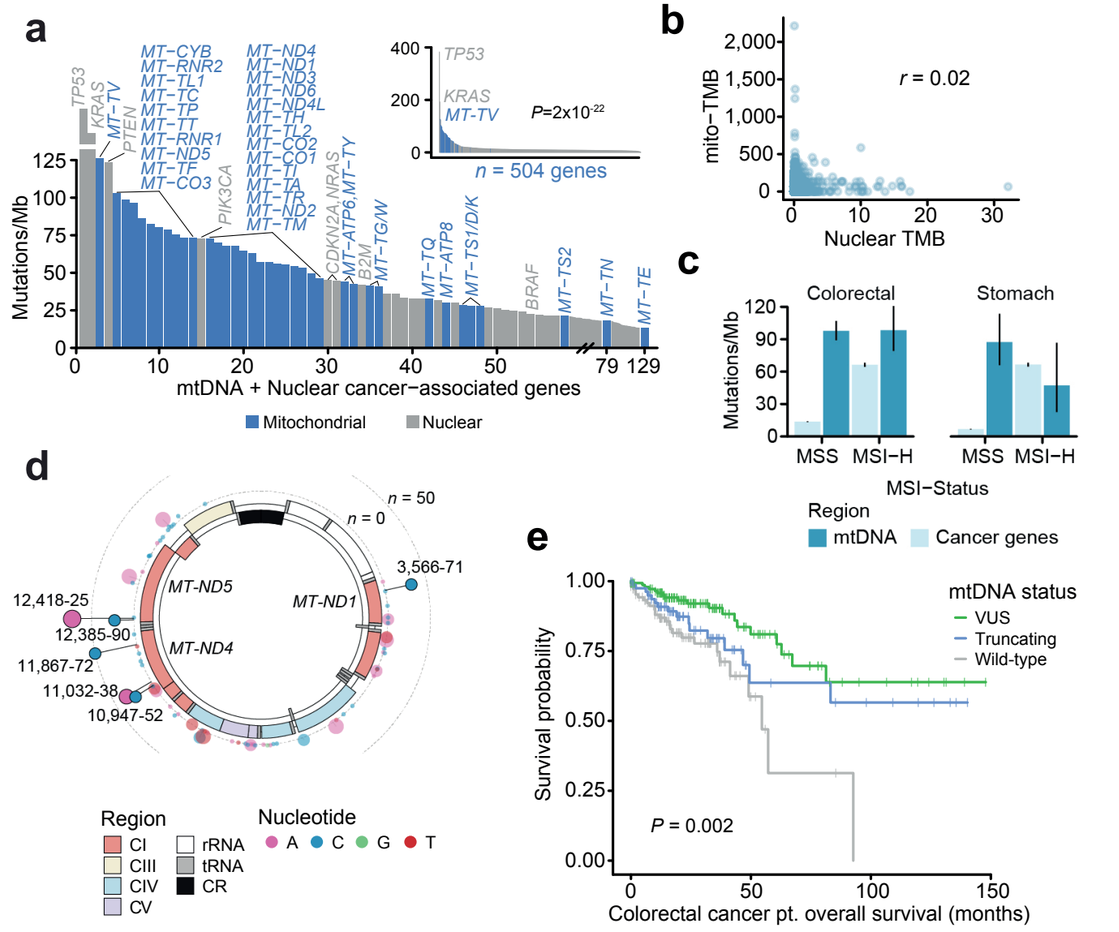

Figure 1

a Mutation rates (Mutations/Mb) of individual mtDNA-encoded genes (blue) and nuclear-encoded cancer-associated genes (grey). Inset plot: mutation rates among 504 genes with mtDNA genes highlighted. Outer plot: closeup of the inset plot in the region containing all 37 mtDNA genes; commonly mutated nuclear cancer genes in this region are labelled for reference. **b** The correlation between TMB (mutations per Mb) among mtDNA (*y* axis) and nuclear-encoded, cancer-associated genes (referred to simply as cancer genes; *x* axis), (n = 3,624 well-covered pan-cancer tumours). **c** TMBs for somatic mtDNA mutations and mutations to cancer-associated genes are compared between microsatellite stable (MSS) and microsatellite unstable (MSI-High) tumours, for both (n colorectal cancer: MSI=65, MSS=318; n stomach adenocarcinomas: MSI=75, MSS=256). Although MSI-high tumours have elevated TMB for nuclear cancer genes, there is no effect on mtDNA TMB. Moreover, mtDNA TMB is similar to (or exceeds) that of nuclear cancer associated genes in both cancer types. Error bars are 95% exact Poisson confidence intervals. **d** Circular mtDNA genome annotated with locations of homopolymer repeat loci ≥5bp in length. Dot height from the circular mtDNA genome indicates the number of affected samples, dot colour indicates the identity of the repeated nucleotide (A, C, G, T), dot width indicates the length of the repeat region (5-8bp). The 6 solid-colour homopolymer loci highlighted are statistically enriched hotspots for frameshift indels, and when combined are the site of ~40% of all mtDNA truncating mutations in cancer. **e** Survival analysis of 344 Stage 1-3 colorectal cancer patients from The Cancer Genome Atlas (TCGA), stratified by mtDNA status (Wild-type n = 108; Truncating n = 84; VUS n = 152). Data from [Gorelick *et al.*, 2021]. VUS, variant of unknown significance (any other potentially pathogenic mtDNA mutation that is not a truncating variant).