Novel Mechanisms of Nucleoside Analog Associated Mitochondrial DNA Mutation

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Introduction

Mitochondrial DNA (mtDNA) mutation is a key feature of human aging [1]. NRTI therapy may lead to accelerated accumulation of mtDNA mutations [2], but the mechanisms are unknown.

Methods

We used a transmitochondrial cybrid cell line containing a single 7.5kb mtDNA deletion mutation [3] (**Fig. 1**), and fibroblasts from elderly individuals containing the m.414T>G point mutation. Cells were exposed to NRTIs for 32 days. MtDNA content was determined by real-time PCR. Deletion mutations were analysed by real-time PCR and long-range PCR. Point mutations were analysed by pyrosequencing (Qiagen) and massively parallel resequencing (Illumina MiSeq).

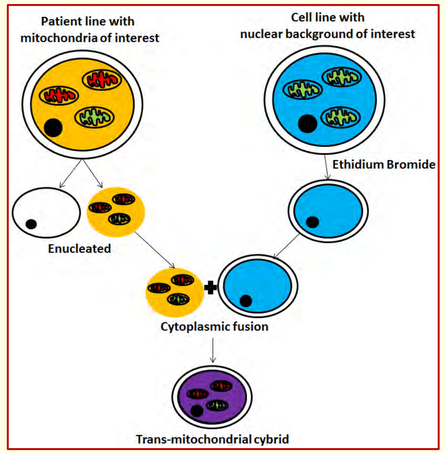


Figure 1. Generation of trans-mitochondrial cell line containing a single large-scale mtDNA deletion mutation.

Results

- In ddI-treated cybrids, heteroplasmy level of the deletion mutation increased from 75% to 96% (SD 1.6%, p<0.01).
- This was due to selective depletion of wild-type mtDNA content (to 14% of baseline, SD 3%, p<0.001) but preservation of mutant mtDNA (**Fig. 2**).
- Cybrids treated with physiological doses of d4T, AZT or TDF showed no changes in deletion heteroplasmy, however 10x physiological dose d4T did increase heteroplasmy to 86% (SD 4%).
- There was no evidence of new deletion mutation formation with any NRTI at normal or 10x dosing.
- In fibroblasts there was no significant change in the heteroplasmy level (~50%) of the m.414T>G point mutation with any of the NRTI exposures (**Fig. 3**).
- Deep resequencing revealed multiple additional low-level (<5% heteroplasmy) point mutations.
- When subjected to mtDNA depletion (due to ddI), followed by repopulation of mtDNA content (i.e. a molecular bottleneck), significant shifts in low-level mutations were seen (ddI, mean shift 6.3%, SD 2.8%; untreated, mean 2.7%, SD 1.2%; p=0.01) (**Fig. 4**).
- There was no new point mutation formation.

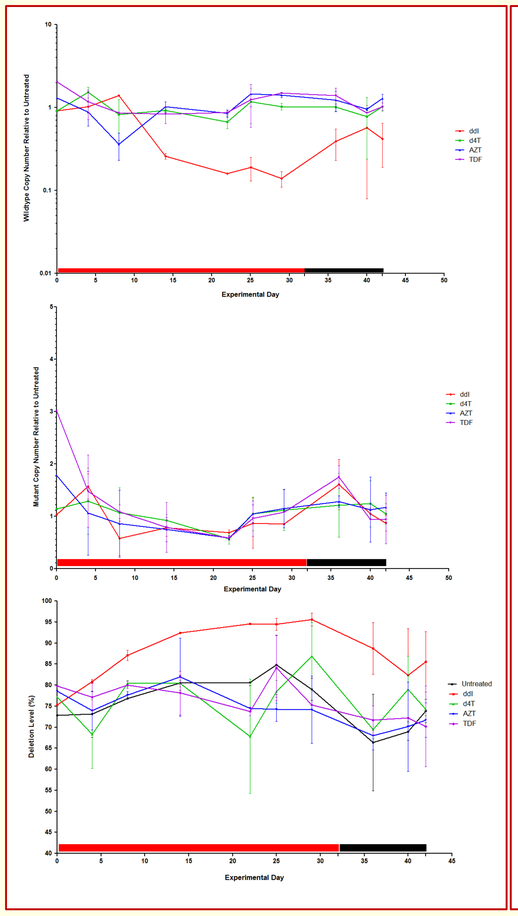


Figure 2. Effect of NRTI exposure on deletion mutation. Wild-type mtDNA is depleted by ddI (top), but not mutant mtDNA (middle). This results in an increase in heteroplasmy level of the deletion mutation (bottom). Red bar = drug exposure period.

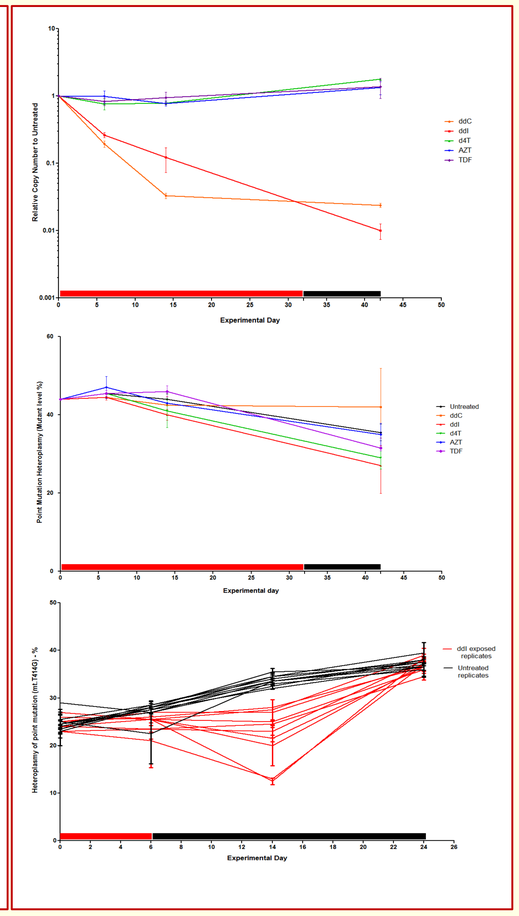


Figure 3. Effect of NRTI exposure on point mutation. Total mtDNA is depleted by ddI or ddC (top), but there is no overall change in mutation heteroplasmy level (middle). However, spread of heteroplasmy levels is increased suggestive of a molecular bottleneck effect (bottom).

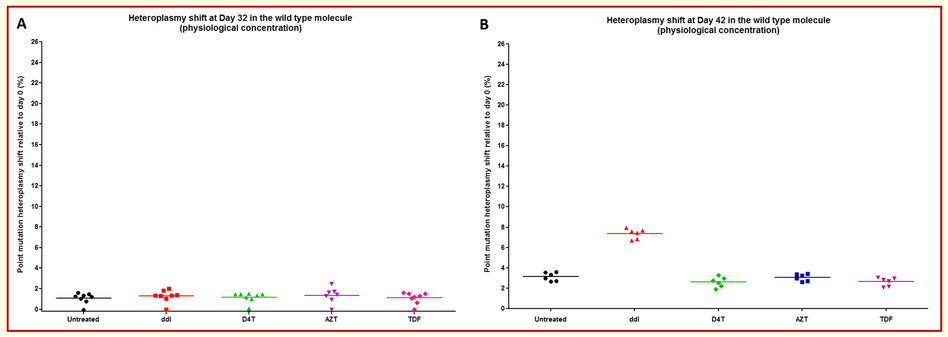


Figure 4. Effect of NRTI exposure on low-level point mutations detected by deep resequencing of mtDNA. Mutation heteroplasmy levels are shown at the end of NRTI treatment (left panel) and following removal of the NRTI (right panel). ddI-treated cells have been through an mtDNA depletion phase followed by repopulation of mtDNA content (i.e. a molecular bottleneck). This results in a significant shift in mutation heteroplasmy levels after the bottleneck.

Conclusions

- Despite prolonged and high-dose exposure to NRTIs, there is no mutagenesis in mtDNA.

However, we observed:

- A selective advantage to the replication of deleted mtDNA
- The enhanced segregation of mtDNA point mutations through a molecular bottleneck mechanism.
- Both phenomena may *in vivo* lead to accelerated clonal expansion of pre-existing (age-associated) mtDNA mutations within individual cells.
- These data provide a plausible mechanism for increased mtDNA mutations observed in NRTI-treated patients.
- Such mutations may lead to accelerated physiological decline during aging.

References

[1] Brierly *et al* (1998) *Ann Neurol* 43(2):217-23. [2] Payne *et al* (2011) *Nat Genet* 43(8):806-10. [3] Diaz *et al* (2002) *Nucleic Acids Res* 30(21):4626-33.

Funding

BP: MRC and NIHR, UK. PFC: Wellcome Trust, MRC, and NIHR, UK. KG: partial funding from AstraZeneca.