Targeted inhibition of human mitochondrial nuclease EXOG, as a means of mitochondrial DNA repair process modulation

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Mitochondrial DNA (mtDNA) integrity is shaped by several processes including replication, turnover and mitochondrial quality control. Among the repair pathways operating in mitochondria, base excision repair (BER) is the best characterized. In some cancers, including lung adenocarcinoma, elevated mtDNA integrity is associated with cancer-cell survival. mtDNA BER relies in part on enzymes shared with the nuclear pathway, however the 5'-exonuclease EXOG is mitochondria-specific. EXOG contributes to mtDNA BER and has been implicated in the processing of specific 5' repair intermediates. The loss of its activity was reported to result in the accumulation of unrepaired single-strand breaks and, ultimately, apoptosis. These features make EXOG a candidate anti-cancer target: selective inhibition of a mitochondria-specific repair activity could sensitize cancer cells to DNA-damaging chemotherapeutics. To date, however, no selective EXOG inhibitor has been identified.

The aim of this work was to identify a small-molecule modulator of EXOG activity that could serve as a starting point for such an agent. Two complementary strategies were used: high-throughput screening (HTS) of the LOPAC®1280 library and structure-based virtual screening of the BioAscent Compound Collection.

HTS identified aurintricarboxylic acid (ATA) as an inhibitor of both the endonuclease and the 5′-exonuclease activities of EXOG. Its effect on a range of DNA and RNA substrates was characterized, the apparent K_d of the interaction was determined, and a mechanism of inhibition was proposed. ATA bound the free enzyme with high apparent affinity, markedly increased its thermal stability, and, on preincubation, lowered the affinity of EXOG for DNA - observations consistent with a model in which ATA interferes with EXOG-DNA complex formation, possibly at or near the active site.

Co-crystallization and soaking did not yield interpretable electron density for the ligand, so a direct structural view of the binding site was not obtained. Residues indicated by *in silico* docking were tested by site-directed mutagenesis. However, none of the variants showed reduced sensitivity to ATA, and their contribution to binding could not be confirmed.

Critically, ATA proved highly non-specific, also inhibiting several other DNA- and RNA-processing enzymes and showing no measurable selectivity for EXOG. Nevertheless, combining ATA with camptothecin (CPT) in a lung adenocarcinoma cell line (A549) decreased

mtDNA integrity, metabolic activity and cell viability - data consistent with, but not directly demonstrating, impairment of mtDNA repair.

Virtual screening yielded three candidate inhibitors, but on further testing these also inhibited unrelated enzymes and were identified as aggregators, consequently, none was validated as a selective inhibitor.

Overall, this work did not identify a selective EXOG inhibitor. It established ATA as a potent but non-specific inhibitor of EXOG and characterized its likely mode of action. ATA-camptothecin combination reduced metabolic activity and mtDNA integrity in A549 cells, which supports the broader strategy of sensitizing cancer cells by reducing mtDNA integrity.