Peptide-Mediated Mitochondrial Delivery of Temozolomide for Evading Cellular Resistance Mechanism

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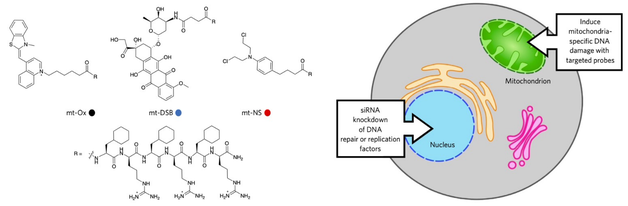
Mitochondria-Penetrating Peptide

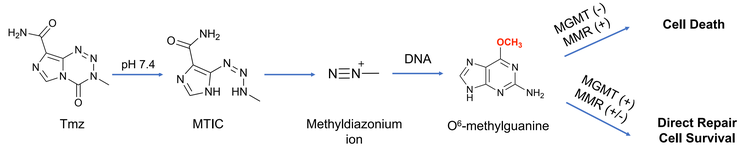
- ***Mitochondria*** are responsible for generating cellular energy (ATP), propagating reactive oxygen species (ROS), activating programmed cell death (apoptosis), as well as ***house their own small, circular genome (mtDNA)***
- This makes the mitochondria an interesting drug target for ***DNA damaging agents***
- Our lab has developed a ***mitochondria-penetrating peptide vector ($(Fxr)_3$)*** that can deliver DNA damaging agents to the otherwise ***impenetrable*** organelle; possesses balance between ***cationic and lipophilic character***
- Several mitochondria-targeted probes that induce different types of DNA damage have been developed in our lab that demonstrate either ***enhanced toxicity or an altered mechanism of action*** compared to their nuclear-targeting parent (e.g. doxorubicin, cisplatin, chlorambucil)[1-4]



- Through RNA interference experiments, ***novel nuclear DNA repair and replication factors*** have been discovered to be ***involved in mtDNA maintenance*** using mitochondria-targeted chemical probes[5]
- This has sparked interested in developing **novel probes** to further study the ***proteomic response to mtDNA damage***

Temozolomide & MGMT

- ***Temozolomide (Tmz)*** is a ***nuclear DNA alkylating agent*** used as treatment for glioblastoma multiforme (GBM)
- Tmz methylates O^6-guanine on DNA, resulting in the ***cytotoxic O^6-methylguanine lesion (O^6-MeG)***
- O^6-MeG forms a ***mismatch with thymine*** in DNA replication that causes futile cycling of the ***mismatch repair pathway (MMR)***, ultimately leading to ***replication stalling, DNA double-strand breaks and cell death***[6]
- ***Resistance to Tmz*** treatment is observed in ~50% of GBM due to expression of ***direct repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT)***, that removes the O^6-methylguanine lesion caused by Tmz[7]

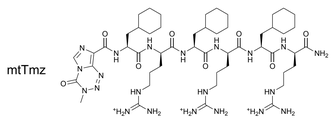


- MGMT has only been found in the nucleus, although ***repair of O^6-MeG has been observed in mitochondria***[8-10]

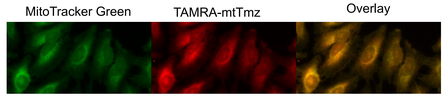
Research Question: *can mitochondrial targeting of Tmz allow for identification of MGMT-associated DNA repair in mitochondria, or identify mitochondria as a potential therapeutic target in MGMT-expressing GBM?*

Methods & Results

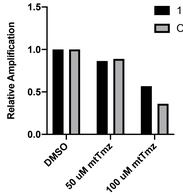
Probe Structure & Subcellular Localization

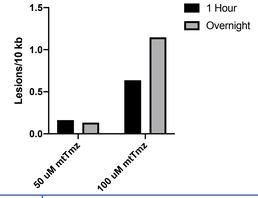


➢ **Mitochondria-targeted temozolomide (mtTmz) localizes to mitochondria in HeLa cells**



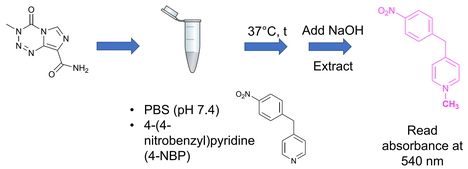
DNA Damage



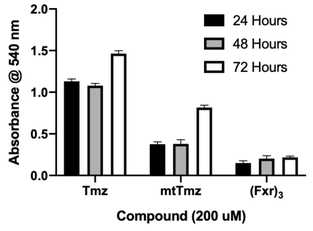


➢ **PCR-based assay shows mtDNA damage caused by mtTmz. The introduction of lesions on DNA hinders the progression of the polymerase enzyme in the PCR reaction. DNA damage can be detected by relative amplification of gene fragments compared to non-treated control. The lesion frequency on mtDNA can also be determined**

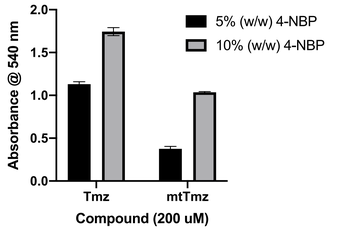
Alkylation Activity



➢ **Chemical alkylation assay demonstrates alkylation ability and behaviour of mtTmz compared to Tmz. In the presence of an alkylating agent, 4-NBP becomes methylated and produces a colour change that can be measured at 540 nm. The intensity of the colour change is proportional to alkylation rate**

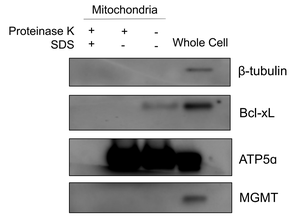


✓ Time-dependent



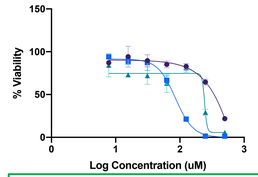
✓ Substrate-dependent

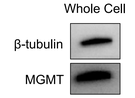
Mitochondrial MGMT Expression



➢ **Proteinase K digestion assay in HeLa cells demonstrates that under mtTmz treated conditions MGMT does not translocate from nucleus to mitochondria**

Dose-Response





➢ **Mitochondrial targeting in MGMT expressing HeLa cells results in increased cytotoxicity compared to nuclear targeting**

Conclusions & Future Work

- This work highlights the development and characterization of a mitochondria-targeted alkylating agent featuring a peptide delivery vector conjugated to Tmz
- The biological assays performed in a MGMT expressing cell line suggest that MGMT does not localize to mitochondria, and is not involved in mtDNA repair of Tmz-induced damage
- Future work includes:
  - Using patient-derived GBM stem cells as a clinically relevant model
  - RNA knockdown of MGMT, as well as targeting MGMT to mitochondria via expression vector to further confirm these results
  - Using mtTmz as a tool to probe for other DNA repair factors that could be active in mitochondria, i.e. mismatch repair

Acknowledgements

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