Unveiling Novel Mitochondrial CpG-DNA Sequences for Trauma Immunotherapy

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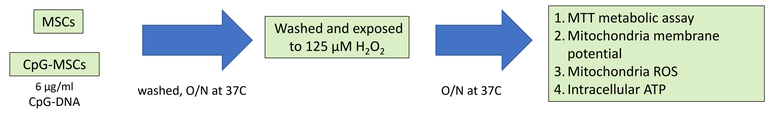
Background and Introduction

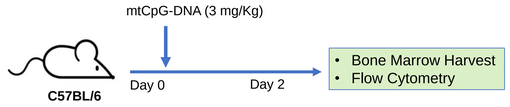
- Trauma induces an immune response resulting in an increased susceptibility to infections.
- CpG-DNA is a TLR-9 agonist shown to amplify emergency granulopoiesis in immune-compromised individuals
- Our previous work demonstrated survival benefits with a class A CpG-DNA sequence 2336 (CpG2336) in irradiated and burn injured mice.
- We identified 34 unique class A type CpG-DNA sequences in mitochondria (mtCpG).
- *We hypothesize that mtCpG has the potential to improve immune function following trauma.*

Methods

- Human MSCs were pre-treated with 6 µg/mL GpC-DNA (control), CpG2336, or 34 different mtCpG sequences.
- After 1 hour, MSCs were washed and exposed to H_2O_2 to induce oxidative injury.
- Mitochondrial function assays were done at 24 hours to measure cellular metabolism (MTT), membrane potential (JC-1), mitochondrial reactive oxygen species (ROS) production (MitoSOX), and intracellular ATP production.



- For *in vivo* testing, 3 male C57BL/6 mice per group were subcutaneously injected with a subset of mtCpG sequences (3, 11, 15, 16, 27, 33) at 3 mg/Kg; 2 mice were included as untreated controls.
- Bone marrow (BM) was collected at two days post injection for detailed BM stem and progenitor cell phenotyping by flow cytometry.



Results

- Findings from *in vitro* studies revealed a subset of mtCpG protected metabolic function in damaged human MSCs similar to CpG2336 (Figure 1).
- Results from *in vivo* studies revealed high and significant increases in mesenchymal stromal cells (MSCs) for mice treated with mtCpGseq11 and mtCpGseq33 (Figure 2).
- Hematopoietic stem cells (LSKs) and some progenitor cells were significantly increased in mice treated with mtCpGseq3, mtCpGseq11, mtCpGseq16, and mtCpGseq33.

Mitochondrial CpG-DNA demonstrates regenerative activity on bone marrow mesenchymal stromal cells (MSCs) and hematopoietic stem cells (HSCs) for trained immunity

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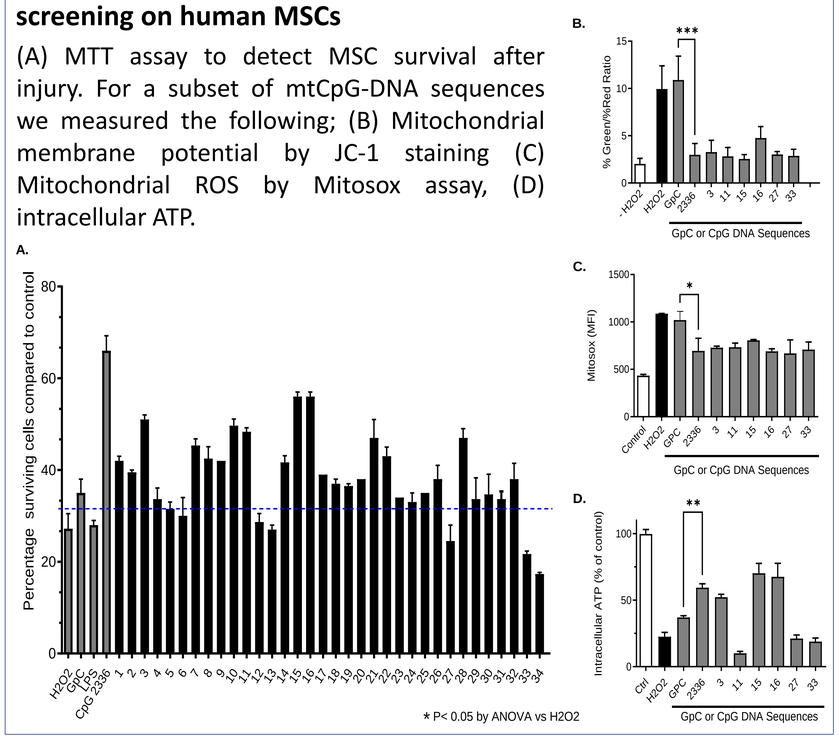
Figures and Tables

Figure 1. Mitochondrial CpG-DNA (mtCpG-DNA) bioactivity screening on human MSCs

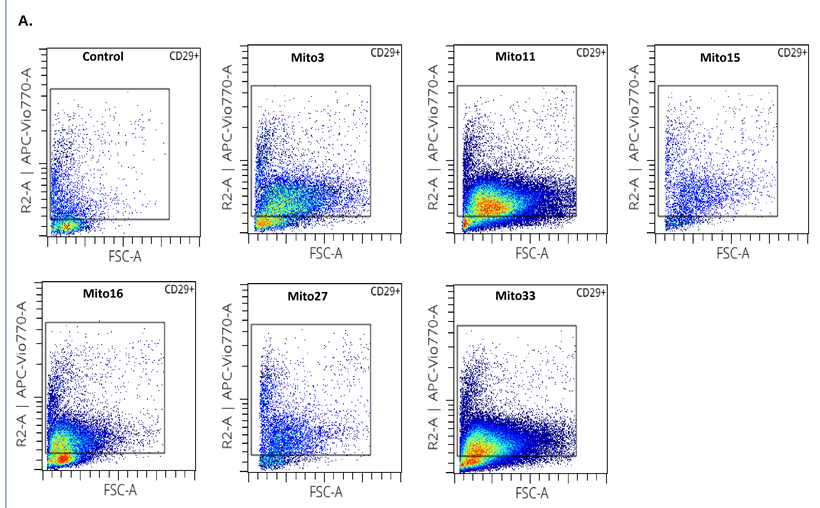
(A) MTT assay to detect MSC survival after injury. For a subset of mtCpG-DNA sequences we measured the following; (B) Mitochondrial membrane potential by JC-1 staining (C) Mitochondrial ROS by Mitosox assay, (D) intracellular ATP.

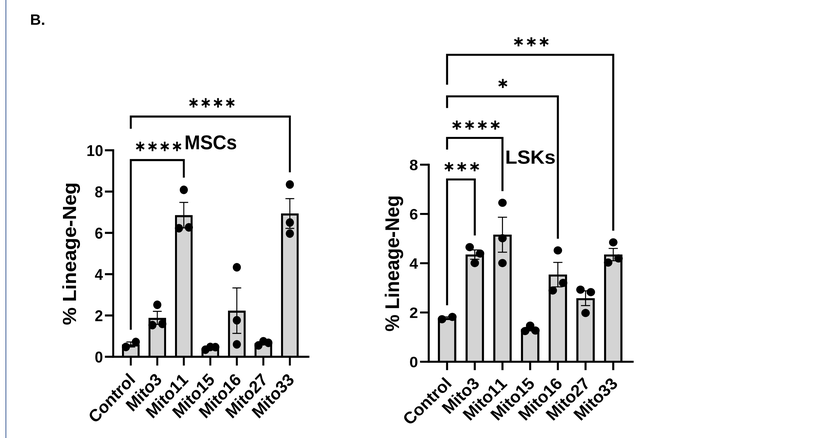


Figures and Tables Continued

Figure 2. In vivo screen for a subset of mitochondrial CpG-DNA on bone marrow MSC and HSC activity in mice.

(A) FACS plots showing MSC number differences in equal subsampled stains from mice injected with the indicated mtCpG-DNA sequences. (B) Plot showing MSC and LSK, a subset of HSCs, staining differences for mice injected with mtCpG-DNA sequences.





Conclusions and Future Directions

1. mtCpG-DNA sequences have biological activity on human MSCs similar to a canonical class A CpG-DNA sequence (CpG2336).
2. Certain mtCpG-DNA sequences showed potent bone marrow regenerative activity in mice.
3. This discovery opens the potential to advance mtCpG-DNA as a way to improve immune function in trauma patients.
4. Ongoing studies are addressing the activity of mtCpG-DNA as an immunotherapy for trauma-induced immune dysfunction in a mouse model.