Expressing *Dm*-dNK in a mouse model a strategy to reverse the depletion of mtDNA caused by nucleoside kinase deficiency.

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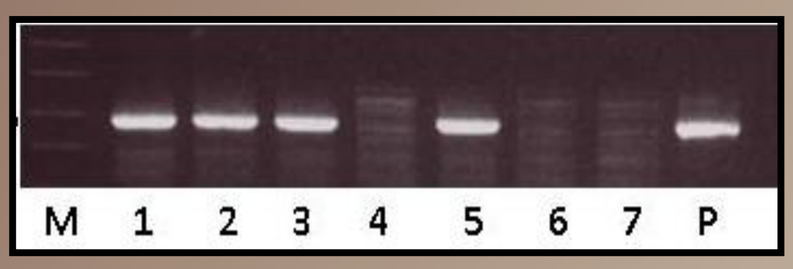
AIM

To investigate the expression of *Dm*-dNK in mice.

BACKGROUND

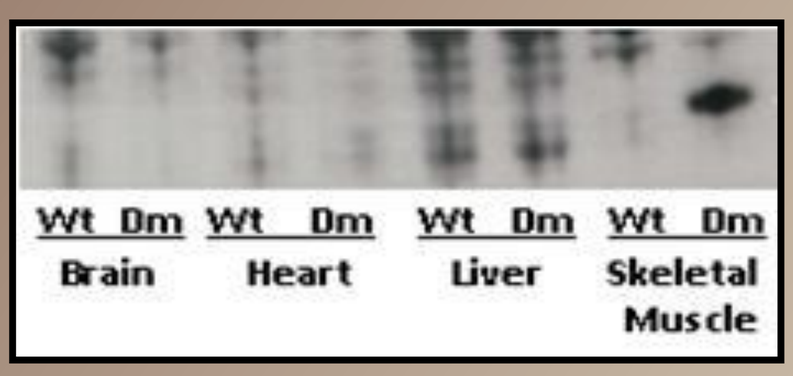
- Mammals contain four deoxynucleoside kinase (dNKs) for the phosphorylation of different deoxyribonucleosides into deoxyribonucleoside monophosphates (dNMPs), which are precursors of the deoxyribonecleoside triphosphates (dNTPs).
- Mutations in the nuclear encoded dNKs; mitochondrial deoxyguanosine kinase (DGUOK) and thymidine kinase 2 (TK2), have been associated with heterogenous group of mitochondrial disorders called Mitochondrial DNA depletion syndrome (MDS).
- *Drosophila melanogaster* has a single multisubstrate nucleoside kinase (*Dm*-dNK), that can phosphorylate all the natural nucleosides. This enzyme has broad substrate specificities and high catalytic rates.

a



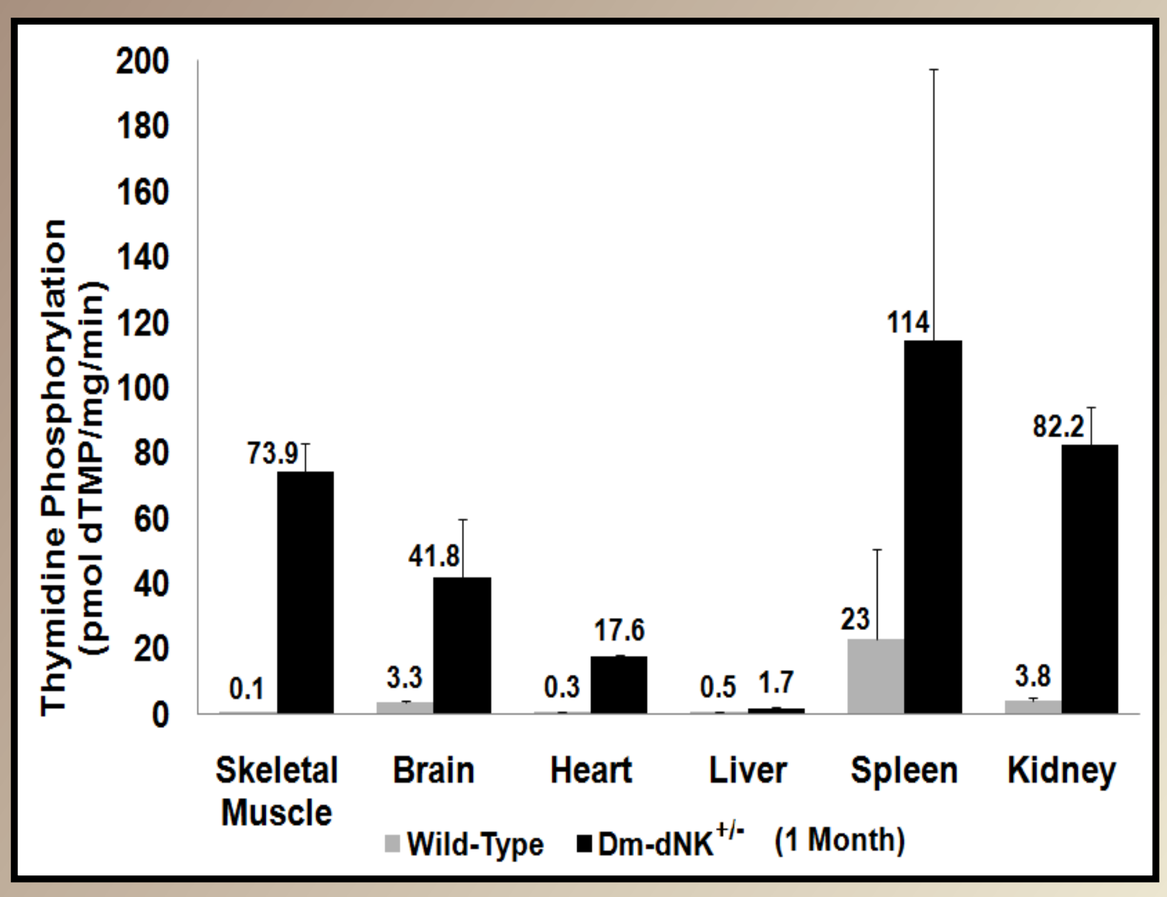
≈ 850 base pairs

b



≈ 28 kDa

c



RESULTS

a. The *Dm*-dNK gene is expressed in gene level (60% positives).

b. *Dm*-dNK protein (≈ 28 kDa) expression could be detected only in the skeletal muscle.

c. Thymidine phosphorylation higher in *Dm*-dNK$^{+/-}$ mice than in wild-type mice.

- ✓ *Dm*-dNK is highly expressed in kidney, skeletal muscle and brain of *Dm*-dNK$^{+/-}$ mice.
- ✓ *Dm*-dNK enzyme loses activity after 3 months in brain, and after 5 months in other tissues.
- ✓ There was no change in the mitochondrial DNA levels, mortality, growth rate and organ weights of the *Dm*-dNK transgenic mice.

METHODS

1. PCR – genotyping of *Dm*-dNK transgenic mice using specific primers.
2. Western Blotting - anti-histidine 1° Ab targeted against His-tag of the protein.
3. Thymidine phosphorylating activity
   - Tissues : brain, heart, liver, skeletal muscle, spleen and kidney
   - Time points : 1 month, 3.5 months and 5 months old mice.
   - Substrate : [methyl-3H]thymidine.

CONCLUSION

Dm-dNK can be expressed in brain, heart, skeletal muscle, kidney, liver and spleen.

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