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

The aim of this study was to investigate the expression of *Dm*-dNK in mice.

CONCLUSION

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

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BACKGROUND

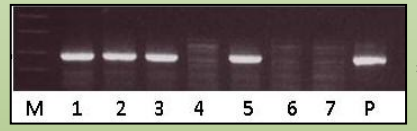
Mammals contain 4 deoxynucleoside kinase (dNKs) for the phosphorylation of different deoxyribonucleosides into deoxyribonucleoside monophosphates (dNMPs), which are precursors of the deoxyribonecleoside triphosphates (dNTPs).

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.

RESULTS

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



≈ 850 base pairs

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

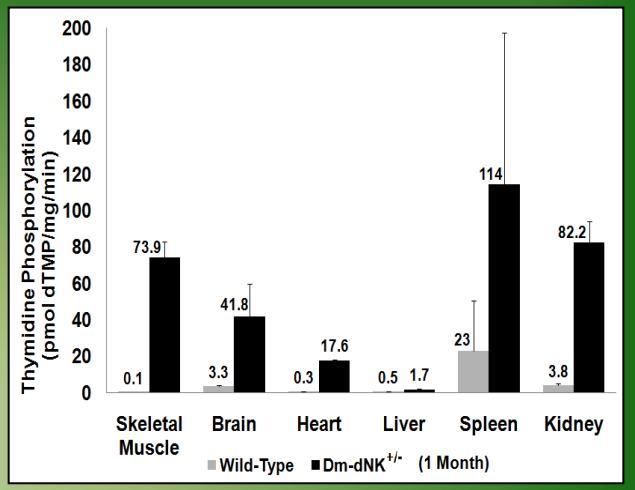
≈ 28 kDa

✓*Dm*-dNK enzyme activity higher than wild-type enzyme activity (TK1, TK2).

✓*Dm*-dNK is highly expressed in kidney, skeletal muscle and brain of mice.

✓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 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. *Dm*-dNK enzyme 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.