The heterologous expression of a *Toxoplasma gondii* oxopurine transporter reveals it binds nucleobases and nucleosides in different manners

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1.Introduction

- *Toxoplasma gondii* causes Toxoplasmosis, it is Intracellular parasite.
- The cat is the definite host, and warm-blooded animals including humans as intermediate host, asymptomatic, unbale to synthesis purines.
- The life cycle of *T. gondii* is : non-feline (asexual, acute stage) tachyzoites and chronic stage bradyzoites (tissue cysts); feline (sexual) which is the sporozoites (in oocysts
- First line treatment is a combination of Pyrimethamine sulfadiazine and folinic acid, only active against Tachyzoites.
- Four ENTs genes in veg strain named; TGVEG_244440;TGVEG_233130;TGVEG_359630 and TGVEG_288540
- 1/3 of world population infected by the parasite.
- Adenosine is favourite purine source.
- TgAT1 first adenosine transporter with low affinity for nucleosides (Schwab et al., 1995).
- TgAT2 second adenosine transporter with high affinity for purine and pyrimidine nucleosides.
- TgNBT1 first high affinity nucleobase transporter (Hypoxanthine, guanine and xanthine (De Koning et al., 2003)

2.Objectives

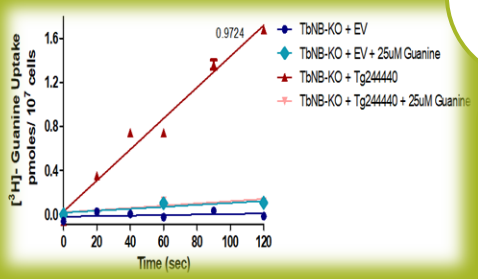
- Heterologous expression of 4 ENTs *T.gondii* and characterise them in depth and here we focused on TGVEG_2444400 in TbNBT-KO procyclic form.
- Identify the substrate for TGVEG_244440 and characterise the gene using different nucleoside and nucleobase analogues.

3.Material and methods

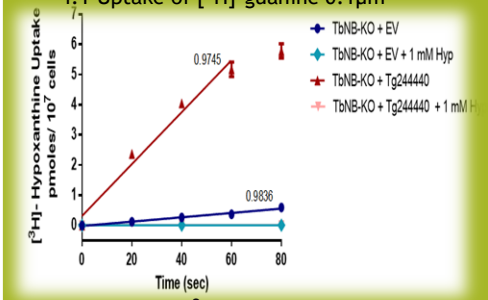
- Tg244440 was amplified from *T. gondii* cDNA RH strain
- Subcloned in plasmid Phd1336, Blasticidin selection.
- Expression in (TbNBT-KO) procyclic form.
- Uptake assay (transport assay) using different radioisotopes [^{3}H], adenosine, 50 nM of [^{3}H]-guanine g, 100 nM of [^{3}H]-hypoxanthine, or [^{3}H]-thymidine.

4.Results

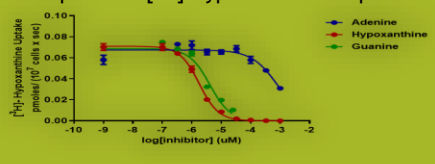
- Tg244440 is a high-affinity oxopurine transporter (*Ki* guanine (1.25 ± 0.16 µM) and (*Ki* Hypoxanthine 2.26 ± 0.36 µM).
- Fully characterisation using more than 40 nucleobase and nucleoside compounds.
- Inhibitory effect on Hypoxanthine> 250 µM guanosine and 1000 µM inosine>> the uptake of hypoxanthine is almost completely abolished.



4.1 Uptake of [^{3}H]-guanine 0.1µm



4.2 Uptake of [^{3}H]-hypoxanthine 0.1µm



4.3 Uptake of 0.1 uM [^{3}H]-hypoxanthine in the absence or presence of varying concentrations of unlabelled hypoxanthine, adenine and guanine

5.Discussion

- Tg244440 showed to have 10-fold higher affinity for adenosine (*Ki* 68.18) than for adenine *Ki* (262.52) , which might indicate the transporter interacts differently with adenine and with adenosine.
- Chiang et al., 1999 is showed that TGVEG-24440 has a low affinity for adenosine, with similar affinity for inosine, and even lower affinity for guanosine, guanine and hypoxanthine transporter however our result showed it is oxopurine transport.

6.Conclusion

- TGVEG_244440 is oxopurine transporter.
- Tg244440 binds purine nucleobases and nucleosides in different manners

7.Refernces:

Chiang, C. W. *et al.* (1999) 'The adenosine transporter of Toxoplasma gondii. Identification by insertional mutagenesis, cloning, and recombinant expression', *Journal of Biological Chemistry*, 274, pp. 35255-35261. doi: 10.1074/jbc.274.49.35255.

De Koning, H. P., Al-Salabi, M. I., Cohen, A. M., Coombs, G. H. and Wastling, J. M. (2003).'Identification and characterisation of high affinity nucleoside and nucleobase transporters in *Toxoplasma gondii*', *International Journal for Parasitology*, 33(8), pp. 821-831.

Schwab, J., Afifi Afifi, M., Pizzorno, G., Handschumacher, R. and Joiner, K. (1995). Toxoplasma gondii tachyzoites possess an unusual plasma membrane adenosine transporter. *Molecular and Biochemical Parasitology*, 70(1-2), pp.59-69.

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