Alkaline phosphatase multi-tasks in the brain

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INTRODUCTION:

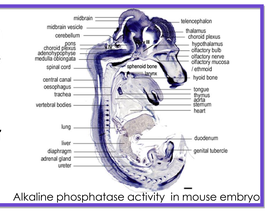
There are several genes for **alkaline phosphatase (AP).** One of them codes for **TNAP (Tissue Non-specific Alkaline Phosphatase)**. It is a ubiquitous ectoenzyme located in different tissues in the body. In the brain, this enzyme is GPI anchored on the neuronal membrane, especially on node of Ranvier and synapses *(Fonta et al., 2005)*.

Mutations of the TNAP gene are responsible for **hypophosphatasia**, a rare disease characterized by defective bone mineralization *(Millan, 2006)* caused by pyrophosphate accumulation. In the severe form, hypophosphatasia is associated with epileptic seizures *(Whyte, 1995)*. This neurological disorder could be explained by a decrease in GABA level due to lack of pyridoxal 5'phosphate (PLP) dephosphorylation.

Inactivation of the TNAP gene in mice **(Akp2)** produces an animal model of severe hypophosphatasia with bone mineralization defects and epileptic seizures *(Waymire et al. 1995, Narisawa et al. 1997)*. These mice usually die before too weeks of age.

We use two approaches to understand the function of TNAP in the brain :

- **Metabolomic** research in order to determine **new TNAP substrates and products that are directly or indirectly affected** by **TNAP**, **using mice invalidated for TNAP gene**.
- **Neuronal cell culture** to study the effect of blocking **TNAP activity on cell proliferation and differentiation**.



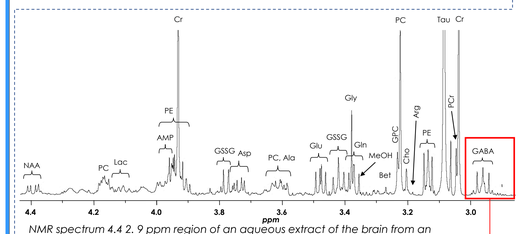
Alkaline phosphatase activity in mouse embryo

METABOLOMIC EXPERIMENTS

Materials and Methods

Akp2 (+/+) n=11
Akp2 (-/-) n=9
Akp2 (+/-) n=12

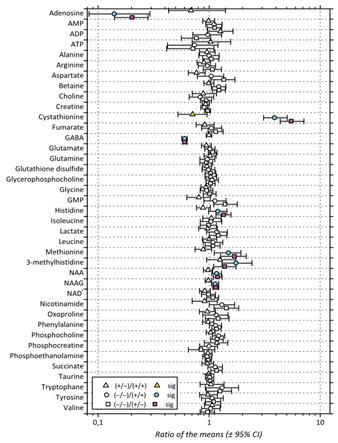
Brain dissection → Sonication and centrifugation → Aqueous extract → ^{1}H NMR analysis



NMR spectrum 4.4 2. 9 ppm region of an aqueous extract of the brain from an Akp2 (+/+) mouse

Results

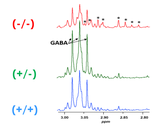
39 metabolites identified in whole brain extract



- **8 metabolites** significantly impacted by TNAP gene invalidation.
- Akp2 (+/-) / (+/+) mice have very **similar metabolomes.**
- Very large changes in Knockout mice for **GABA, cystathionine and adenosine concentration** compared to Akp2 (+/+) and Akp2 (+/-).

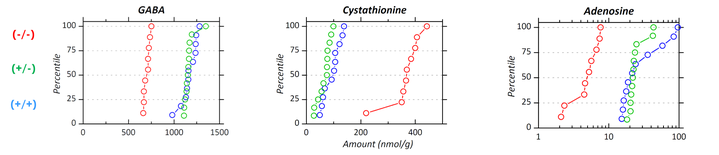
If ratio<1 metabolite concentration decreased.
If ratio>1 metabolite concentration increased.
Bars encompass to the 95% confidence interval of the ratio. Colored symbols indicate significant variations between different mice genotypes.

Qualitative comparison of ^{1}H NMR spectra



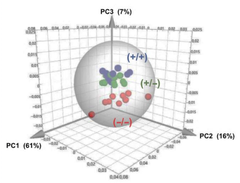
❖GABA levels were decreased in Akp2 -/- mice compared to others.

3 metabolites strongly impacted by TNAP gene invalidation

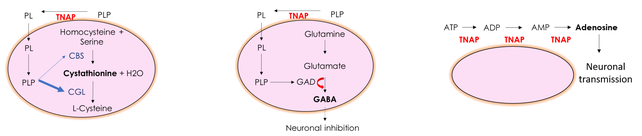


Concentration decreased by 40 % | Concentration increased by 450 % | Concentration decreased by 80%

Cluster analysis revealed segregation of Akp2 -/- vs Akp2 +/- and +/+ mice



❖Unsupervised statistical analysis (PCA) indicated that the metabolome of Akp2-/- mice was distinct from that of Akp2 +/- and +/+ mice.



➢ PLP dephosphorylation by TNAP is required for cystathionine and GABA production

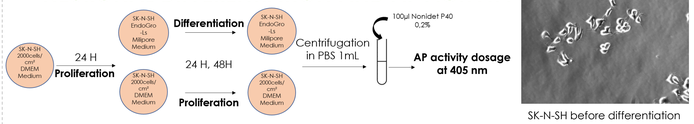
➢ TNAP ectonucleotidase function produces adenosine.

➢*In parallel with its regulatory role in neuronal synaptic transmission, TNAP could be involved in inflammation.*

CELL CULTURE EXPERIMENTS

Materials and methods

NEURONAL DIFFERENTIATION AND AP ACTIVITY

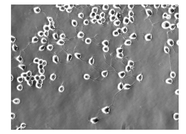


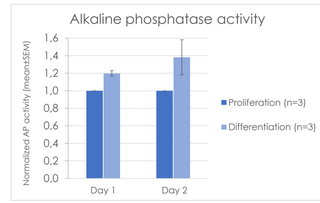
DMEM medium contains penicillin, streptomycin and fetal calf serum (10%)
EndoGro-LS Millipore medium induced the differentiation of cells SK-N-SH to neurons.

AP activity was measured with a buffer (pH 10.3) containing 10 mM of AP substrate (para nitrophenyl phosphate, pNPP) and 1mM of $MgCl_2$.

Results are expressed as hydrolyzed pNpp nmoles per minute and per mg of proteins.

SK-N-SH before differentiation

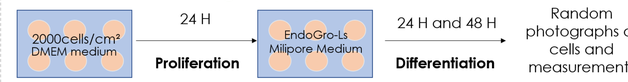

SK-N-SH at day 2 of differentiation



❖ AP activity increased during differentiation.

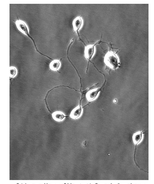
➢ AP could be involved in cell differentiation.

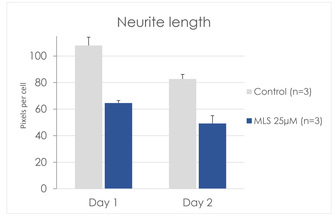
Control of neurite growth by AP



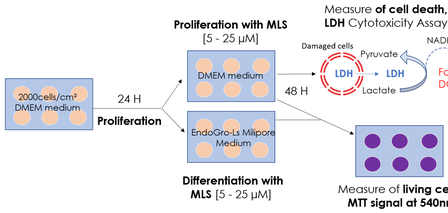
(TNAP specific inhibitor. Sergienko et al 2009)

Neurites longer than twice the diameter of cell bodies were measured in10 pictures for control and MLS conditions using ImageJ program. The results are expressed as pixels per cell. This measure represents the total mean length of neurites per cell.

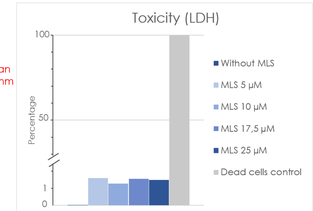

SK cells with MLS at 1 day



MLS effects on cell toxicity and viability :



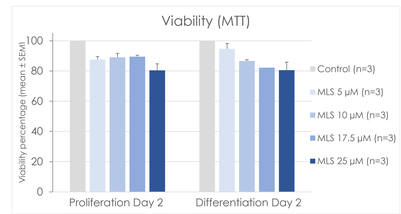
The level of formazan is directly proportional to the amount of LDH (Lactate deshydrogenase) released into the medium.
MTT (3-(4,5- Dimethylthiazol-2yl)-2,5-diphenyltetrazolium Bromide) is reduced by mitochondrial succinate dehydrogenase and forms a purple dye.



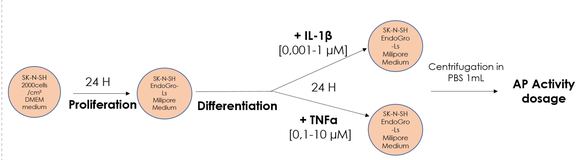
MLS is weakly toxic and has a dose dependent negative effect on cellular viability.

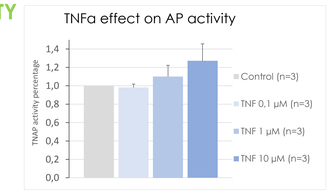
❖ Inhibition of AP with MLS led to a decrease of neurite length.

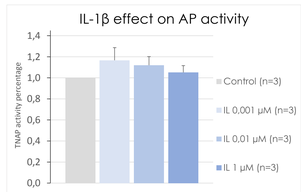
➢ AP could be involved in neuron maturation



PROINFLAMMATORY CYTOKINE EFFECT ON AP ACTIVITY







➢ Inflammatory factors could regulate AP activity.

❖Low doses of IL-1β increased AP activity.

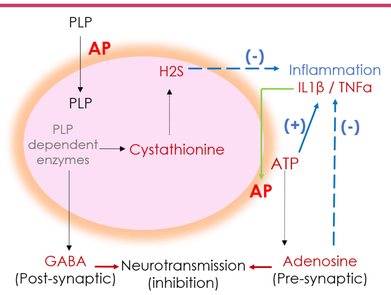
❖TNFa increased AP activity in a dose dependent manner

CONCLUSION

❖ The metabolomic approach showed that TNAP deficiency **reduces the level of GABA** and **adenosine** and **increases cystathionine level** in the brain.

❖ The cell culture approach indicated that AP influences **neurite length during differentiation** and also that AP activity is **regulated by inflammatory factors**.

➢ AP in the brain is involved in **neuronal inhibition** via GABA and adenosine production as well as in **neurite maturation**. Besides AP may play a role in **inflammation regulation** via proinflammatory cytokines, cystathionine metabolism and nucleotide hydrolysis.



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