Restoration of BDNF metabolism to improve MeCP2 knock-out mice symptoms

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Mecp2 and Bdnf

- Bdnf (Brain derived neurotrophic factor) is a neurotrophic factor essential for neuronal survival and synaptic connections (LTP/LTD).

- Bdnf is directly regulated by Mecp2 (Chen et al., 2003, Martinovitch et al, 2003)

- A lack of Mecp2 leads to:
  - A decrease of the Bdnf levels in the brain (Chen et al., 2003; Chang et al., 2006)
  - A defect in the axonal transport of the Bdnf (Roux et al., 2012; Xu et al., 2014)
Mecp2 and Bdnf

- Problem: BDNF does not cross the Blood Brain Barrier (BBB).

Chang et al, 2006
Indirect stimulation of Bdnf (Ampakines)

- Use of pharmacological compounds able to cross the BBB and to stimulate the production of BDNF.

David Katz
Indirect stimulation of Bdnf (Ampakines)

- Ampakines can increase BDNF levels.
- Restoration of a normal respiratory rhythm (after 3-days of treatment).
- No survival data.
- A pharmaceutical company (Cortex Pharmaceuticals) is responsible for clinical development.
Use of IGF-1 to mimic the BDNF action
Use of IGF-1 to mimic the BDNF action

- Improvement of the excitability properties of neurons
Use of IGF-1 to mimic the BDNF action

Safety, pharmacokinetics, and preliminary assessment of efficacy of mecamsermin (recombinant human IGF-1) for the treatment of Rett syndrome

Ongoing clinical trials (increased risk of Leukaemia?)
Stimulation of BDNF metabolism

- Is it possible to stimulate the Bdnf transport to the synapse?
- Is it possible to use gene therapy to increase BDNF level?
Stimulation of axonal BDNF transport

➢ Is it possible to stimulate the Bdnf transport to the synapse?
Knock-in mouse model

Mutation of Huntingtin

- **S421D** (Serine-Aspartic, HTTSD): constitutive phosphorylation
- **S421A** (Serine-Alanine, HTTSA): absence of phosphorylation

Neuronal culture microfluidic device

Videomicroscopy of axonal Bdnf transport
Huntingtin phosphorylation rescues BDNF transport in a Mecp2-deficient corticostriatal circuit

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- **WT**
- **HTT<sub>SD</sub>**
- **HTT<sub>SA</sub>**
- **WT siCt**
- **WT siMecp2**
- **HTT<sub>SD</sub> siMecp2**
- **HTT<sub>SA</sub> siMecp2**
In vivo phosphorylation

Crossing Mecp2+/- with HTT$_{SA}$ or HTT$_{SD}$ mice
Constitutive phosphorylation of HTT rescues the corticostriatal BDNF transport.
Constitutive phosphorylation of HTT improves the phenotype of the Mecp2-deficient mice
Constitutive phosphorylation of HTT improves the phenotype of the Mecp2-deficient mice
Constitutive phosphorylation of HTT improves the phenotype of the Mecp2-deficient mice
Pharmacological stimulation of HTT phosphorylation improves BDNF transport in a Mecp2-deficient corticostriatal circuit

Effet of FK506 on the axonal transport of Bdnf

Pardo et al., 2006
In vivo pharmacological stimulation of HTT phosphorylation improves the phenotype of the MeCP2-deficient mice

 FK506 increases HTT-P level

Lifespan

Body weight

Breathing
In vivo pharmacological stimulation of HTT phosphorylation improves the phenotype of the Mecp2-deficient mice
The inability to phosphorylate huntingtin prevents phenotype improvements due to FK506 treatment in *Mecp2-deficient mice*

- Treatment of *Mecp2* y/− HTT<sub>SA</sub> with FK506

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Breathing (Apneas)
The inability to phosphorylate huntingtin prevents phenotype improvements due to FK506 treatment in *Mecp2-deficient mice*.
Conclusion

- The genetic and pharmacological approaches show that the phosphorylation of HTT at S421 significantly improves the RTT phenotype.

- Even if the use of FK506 appears interesting its use may be limited due to the side effects (immunosuppressant).

- Instead of playing with inhibition of the dephosphorylation we could alternatively increase the phosphorylation (IGF1/SGK1/AKT...).
Use of AAV-BDNF to treat Mecp2-deficient mice

- AAV=Adeno Associated Virus
- Does not cause illness
- No integration into the genome
- Low immune response
Use of AAV-BDNF to treat Mecp2-deficient mice

- Why use a AAV-BDNF vector rather than a vector that would put back a functional Mecp2 gene in place of the mutated one?

- Other and we have used AAV-Mecp2 vectors with some success. Nevertheless, the number of infected cells by AAV vectors is weak (6-8%) and only(mainly) the infected cells will be cured (cell autonomous). On the other hand, BDNF is secreted and AAV-BDNF infected cells can affect positively the non infected ones.

- Unfortunately, AAV vectors infect drastically the liver and overdosage of Mecp2 lead to hepatotoxicity.
Use of AAV-BDNF to treat Mecp2-deficient mice

In fact the dosage of Mecp2 is something complicated to control.
Use of AAV9-BDNF to treat Mecp2-deficient mice

Examen post-mortem (28j post-injection)
AAV9-BDNF improves the phenotype of the Mecp2-deficient mice

Lifespan

Survival AAV BDNF

- WT
- KO AAV BDNF
- KO

Body weight

Breathing (Apneas)
AAV9-BDNF improves the phenotype of the Mecp2-deficient mice

Motor coordination

Motor activity
AAV9-BDNF improves the phenotype of the MeCP2-deficient mice

Circadian motor activity
Conclusion

- The AAV9-BDNF vector appears to be a promising therapeutic agent and an interesting alternative to the use of conventional AAV-Mecp2 vectors.

- We are currently evaluating the molecular and cellular effects of the AAV9-BDNF vector.

- We will have to replicate the treatment with female mice.
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