Apigenin as Candidate Prenatal Treatment for Trisomy 21: Effects in Human Amniocytes and the Ts1Cje Mouse Model

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Background

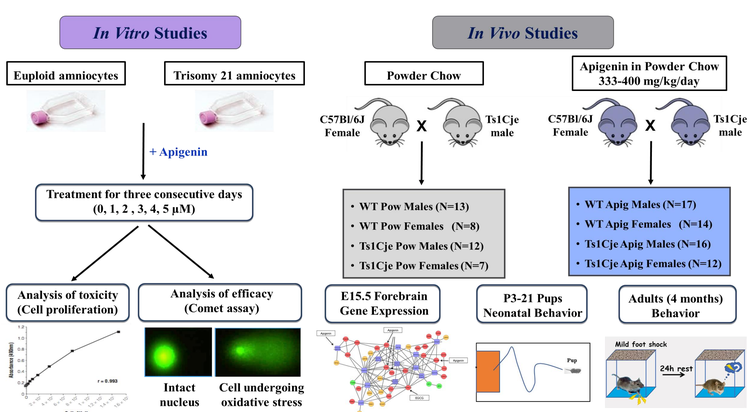
Fetuses with trisomy 21 (T21) have atypical brain development that is apparent as early as the second trimester[1]. We hypothesize that by integrating dysregulated gene expression and pathways common to humans and mouse models of Down syndrome (DS) we can discover novel targets for prenatal therapy. Previously, we demonstrated that pathway abnormalities associated with DS were the result of gene-dosage specific effects and a global stress response with activation of compensatory mechanisms[2]. To counteract these genome-wide abnormalities, we used the Connectivity Map database to discover molecules that could be repurposed to rescue the transcriptome and promote more typical brain development in individuals with DS[2,3]. One of the molecules that had the most consistent negative scores (hence, negating the dysregulated gene expression signatures in DS) across tissues and species was apigenin.

Objectives

Investigate the potential therapeutic effects of apigenin

- ***In Vitro:*** by analyzing the effects of apigenin on the redox homeostasis in human amniocytes derived from living fetuses with T21 and gestational age and sex matched euploid (Eup) fetuses.
- ***In Vivo*** by analyzing the effects of apigenin on embryonic forebrain gene expression and neonatal and adult behavioral outcomes in the Ts1Cje mouse model of DS.

Study Design



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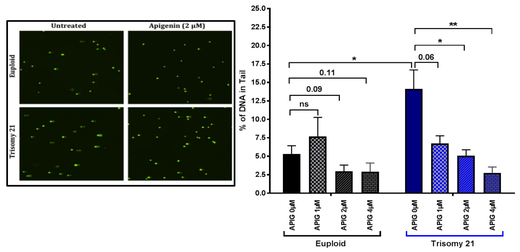
Results

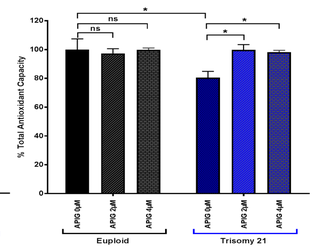
In Vitro Findings

1) Apigenin Rescues Redox Homeostasis in T21 Amniocytes

Apigenin reduces oxidative stress in T21 amniocytes in a dose-dependent manner (T21=5 versus Eup=5)

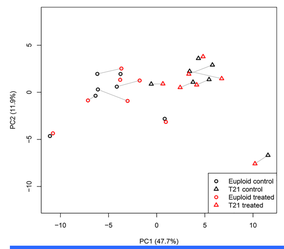


Apigenin promotes physiological anti-oxidant defense in T21 amniocytes



2) Apigenin Induces Mild Global Gene Expression Changes in T21 Amniocytes

Gene expression was analyzed after 2 uM apigenin treatment using HTA 2.0 arrays (T21=7 versus Eup=7)
For each amniocyte line, the untreated (black) and apigenin-treated (red) pairs are connected with a solid line

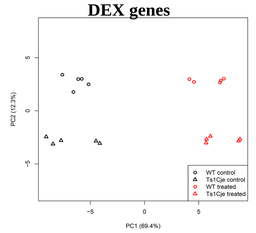


- **Apigenin induces mild global gene expression changes in treated versus untreated T21 and Eup amniocytes.**
- **Apigenin-induced gene expression changes show variability between individual amniocytes lines as shown by the connecting lines in the Principal Component Analysis (PCA).**

In Vivo Findings

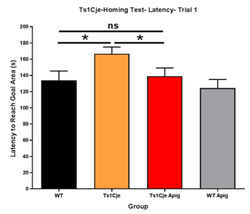
1) E15.5 Gene Expression

Apigenin induced significant gene expression changes in Ts1Cje and WT E15.5 forebrain over 1,300 DEX genes



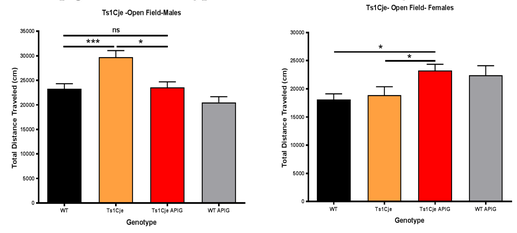
2) Neonatal Behavior

Apigenin rescues spatial olfactory memory (homing test) in Ts1Cje pups



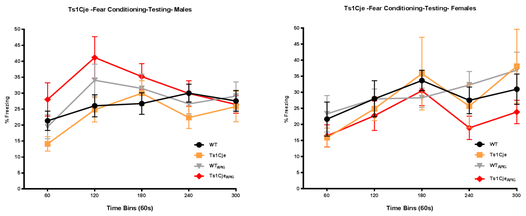
3) Adult Behavior (Open Field)

- **Ts1Cje males, but not females, showed hyperactivity versus WT mice**
- **Apigenin rescued exploratory behavior abnormalities in Ts1Cje males**
- **Apigenin induced hyperactive behavior in Ts1Cje Females**



4) Adult Behavior (Fear Conditioning)

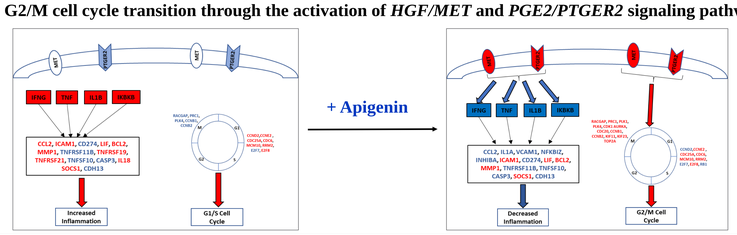
- **Ts1Cje males showed significant long-term memory deficits**
- **Ts1Cje females exhibited milder deficits compared to males**
- **Apigenin significantly improved long-term memory only in Ts1Cje males**



Common Apigenin Mechanisms of Action In Vitro and In Vivo

In Vitro Mechanisms of Action

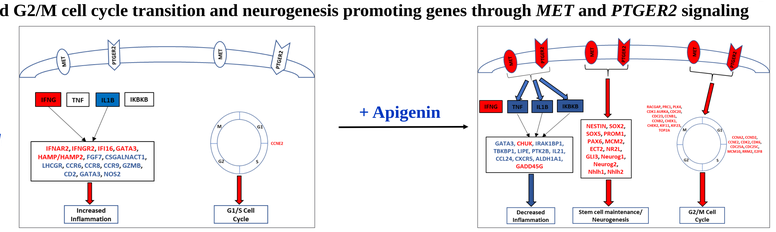
- **T21 amniocytes show increased expression of pro-inflammatory genes, up-regulation of genes associated with G1/S and down-regulation of genes associated with G2/M cell cycle transition versus Eup amniocytes**
- **Apigenin treatment significantly down-regulated the expression of pro-inflammatory genes and promoted G2/M cell cycle transition through the activation of *HGF/MET* and *PGE2/PTGER2* signaling pathways**



Red: Up-regulated
Blue: Down-regulated

In Vivo Mechanisms of Action

- **In Ts1Cje E15.5 embryos, the expression of pro-inflammatory genes is increased along with the up-regulation of the G1/S gene *CCNE2***
- **Similar to its *in vitro* effects, apigenin treatment down-regulated the expression of pro-inflammatory and up-regulated G2/M cell cycle transition and neurogenesis promoting genes through *MET* and *PTGER2* signaling**



Conclusions and Future Directions

- ***In vitro***, apigenin treatment restored redox homeostasis, inhibited pro-inflammatory genes and up-regulated the expression of G2/M promoting genes.
- ***In vivo***, apigenin treatment improved spatial olfactory memory in Ts1Cje pups and had sex-specific effects on behavioral outcomes in Ts1Cje adults.
- Apigenin has common mechanisms of action *in vitro* and *in vivo* through the activation of *PGE2/PTGER2* and *HGF/MET* signaling pathways.
- Ongoing studies are focused on validating these mechanisms of action and understanding of the sex-specific effects of apigenin in the Ts1Cje mouse.

References

1. B. Schmidt-Sidor, et al. Brain growth in Down syndrome subjects 15 to 22 weeks of gestational age and birth to 60 months. Clin Neuropathol 9, 181-190 (1990)
2. F. Guedj et al. An integrated human/murine transcriptome and pathway approach to identify prenatal treatments for Down syndrome. Sci Rep 6, 32353 (2016).
3. J. Lamb et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 313, 1929-1935 (2006).

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158--PO

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