**Epigenetic regulation of estrogen receptor alpha via DNA repair gene Gadd45b following neonatal hypoxic ischemic encephalopathy**

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**BACKGROUND**

Neonatal hypoxia ischemia (HI) related encephalopathy is an important cause of life-long mortality and morbidity. Female newborn brains may be relatively resistant to the detrimental effects of HI while male newborn brains are more susceptible. Our recent findings reveal that HI increases hippocampal estrogen receptor α (ERα) expression leading to neuroprotection only in the female mice hippocampi through crosstalk with the neurotrophin receptor, tyrosine kinase B (TrkB). One potential mechanism leading to increased expressions of ERα post-HI in female hippocampi could be through epigenetic mechanisms. Objective of this study is to determine the hippocampal epigenetic enzyme mRNA expressions and their association with ERα mRNA expression post-HI.

**METHODS**

We investigated the mRNA expressions of the panel of methylating and demethylating enzymes post-HI. The panel included the classic epigenetic enzymes such as tet1, tet2, dnmt1, dnmt3a and dnmt3b along with DNA repair gene Gadd45b. Our hypothesis is that Gadd45b upregulation is required for ERα upregulation leading to TrkB-mediated neuroprotection in female hippocampi following neonatal HI.

Neonatal mouse model of HI was induced in P9 C57BL/6J male and female wild type (WT) and gadd44d KO mice by using Vannucci’s HI model. gRT-PCR was performed with mRNAs isolated using RNAeasy® mini kit (Qiagen, Hilden, Germany) from hippocampal samples obtained on day 1 and 3 post-HI. RNA was converted to cDNA using superscript VI synthesis kit (Life Technologies) and gene expressions of gadd45b, tet1, tet2, dnmt1, dnmt3a and dnmt3b and ERα determined using a predesign Taqman probe kit (Applied Biosystem, Thermofisher) on an ABI V7A 7 real time PCR system (Applied Biosystem). GAPDH and Ywhaz were used as housekeeping genes.

**RESULTS**

- There is no significant sex differences seen in tet1, tet2, dnmt1, dnmt3a and dnmt3b mRNA expressions in male and female hippocampi on day 1 and 3 post-HI.
- Gadd45b mRNA expression was significantly upregulated in the ipsilateral female hippocampi compared to male on day 1 post-HI (p<0.01).
- Sex differences in increased hippocampal ERα mRNA expression was eliminated in Gadd45b KO mice (p<0.05).
- This data suggest that Gadd45b maybe epigenetically regulating ERα upregulation following neonatal HI.

**CONCLUSIONS**

The DNA repair enzyme, Gadd45b, is upregulated in neonatal female mice hippocampi compared to male. Sex differences in ERα mRNA expression is eliminated in Gadd45b KO female mice hippocampi post-HI. These results suggest that Gadd45b maybe epigenetically regulating ERα upregulation following neonatal HI.

Further studies will be performed to determine the methylation status of the ERα promoter regions in the hippocampi following in vivo HI. In addition, we will investigate the role of Gadd45b in demethylating ERα promoter regions leading to ERα upregulation in hippocampal neurons following in vivo ischemia.

**ADDITIONAL KEY INFORMATION**

Figure Legend: Hippocampal epigenetic enzyme mRNA expressions following HI. There were no significant sex differences observed on day 1 (A) or 3 (B) post-HI in tet1, tet2, dnmt1, dnmt3a and dnmt3b mRNA expressions in hippocampi (n=3-4). The difference between male and female IL Dnmt3b expressions was not significant (p<0.07) on day 3 post-HI. mRNA expressions were normalized to male sham CL. D. ERα upregulation is highest in WT female HI (p<0.05). Female ERα mRNA expression decreases to male levels in Gadd44d KO female hippocampi 3 days post-HI (n=3-5). mRNA expressions were normalized to male sham WT CL.

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**References**

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