Induction of Nrf2 Activity May Decrease the Risk of Pollutant-Induced Neurodevelopmental Disorders

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ABSTRACT

- Pregnant women are commonly exposed to chemicals and pollutants that can increase the risk of the fetus developing a neurodevelopmental disorder[1,2]. For example, fetal exposure to the anti-epileptic drug valproic acid (VPA) increases the risk of developing an Autism Spectrum Disorder (ASD)[1,2].
- VPA is a well-established model for toxicant-induced neurodevelopmental abnormalities in a developing fetus.
- We hypothesize that upregulation of cellular detoxification and antioxidant pathways via increased expression of the Nrf2 transcription factor may prevent VPA-induced neurodevelopmental abnormalities.
- To test this hypothesis, we grew human cortical spheroids (HCSs), and induced Nrf2 expression using sulforaphane (SFN), a nutrient obtained from cruciferous vegetables[3,4]. We exposed HCSs to VPA alone, VPA and SFN, SFN alone, or solvent control
- We expect to see that SFN-treated brains develop normally even when also exposed to VPA, while those exposed to VPA alone will develop a hyperexcitable phenotype characteristic of VPA-induced ASD.
- Should our data support our hypothesis, it would indicate that pregnant women who are exposed to toxicants could benefit from sulforaphane supplementation to reduce the risk that their offspring will develop neurodevelopmental abnormalities.
- Our results are of particular importance because ASD prevalence increased more than two-fold over the last two decades[5], and environmental pollutants could be a significant factor in this dramatic rise.
- Future experiments will test whether sulforaphane is protective against other pollutants and ASD-associated risk factors.

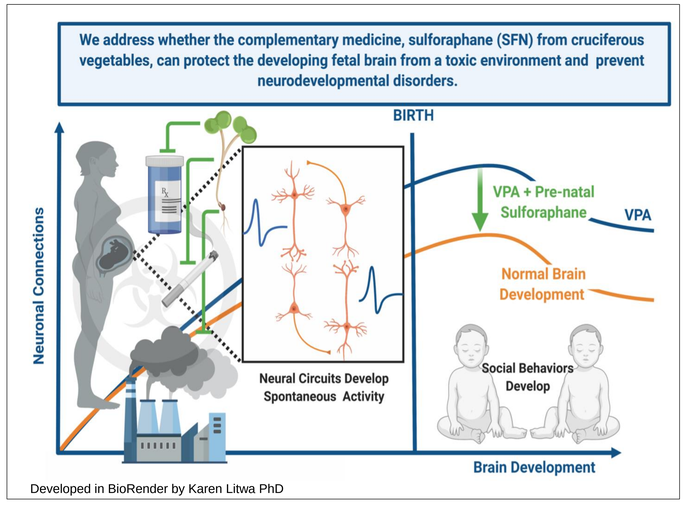


Fig. 1 Exposure to chemicals such as environmental pollutants, pesticides, and drugs is impossible to avoid completely during pregnancy. SFN may reduce the harmful effects to the developing fetal brain resulting from such exposures.

RESULTS

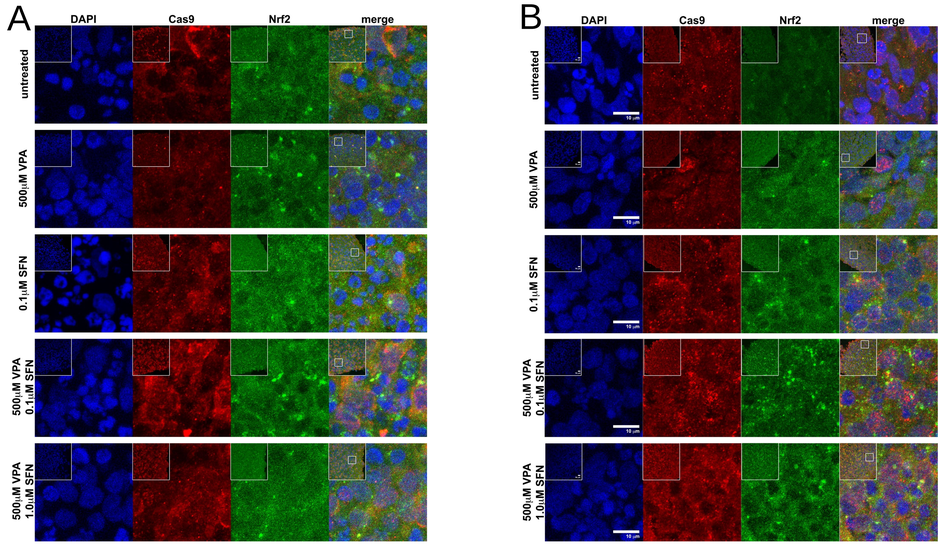


Fig. 2 dCas9 coupled to scrambled sgRNA (**A)** and Nrf2 sgRNA (**B**) were introduced into HCSs. At day 90, HCSs were sectioned and later immunostained for DAPI, Cas9, and Nrf2. Colocalization of Nrf2 and DAPI were measured to determine the relative amounts of Nrf2 activity in the nuclei. As expected, Nrf2 was seen in greater quantity in the nucleus of scrambled sgRNA HCSs (**A**).

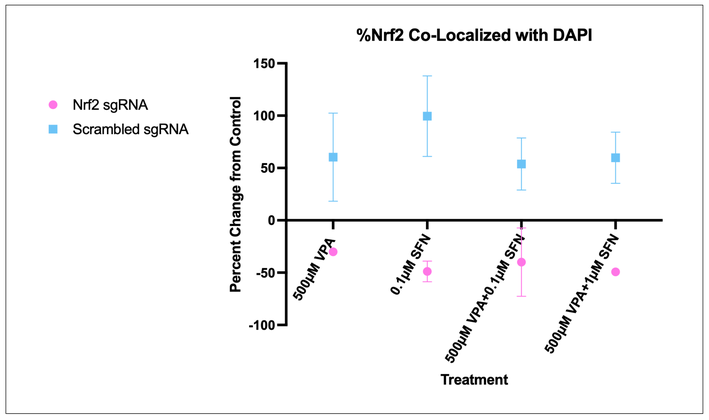
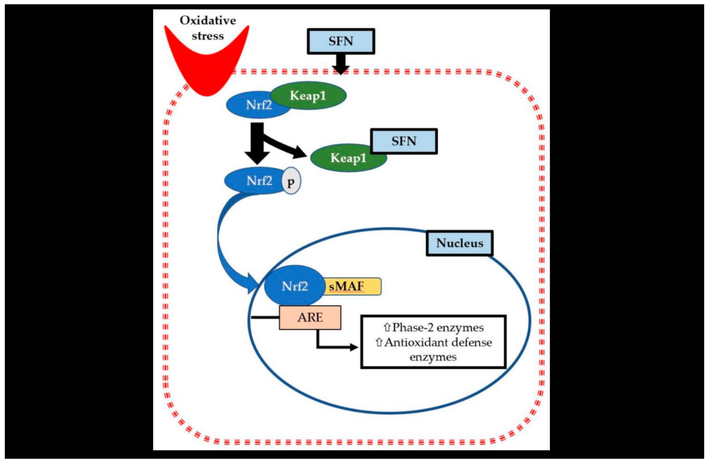


Fig. 3 The percent of Nrf2 that colocalized with DAPI was greater in all treatment groups as in HCSs transduced with dCas9 and scrambled sgRNA as compared to those with Nrf2 sgRNA. This confirms that the Nrf2 sgRNA serves to suppress Nrf2 expression.

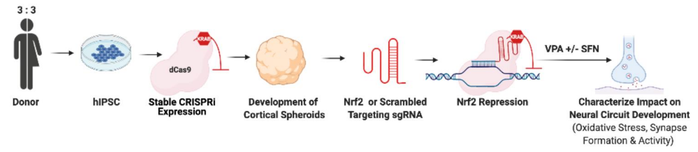


Ruhee RT, Suzuki K, 2020.

Fig. 4 Sulforaphane causes Nrf2 phosphorylation and translocation to the nucleus of the cell. This active form of Nrf2 is able to act as a transcription factor to promote the production of enzymes involved in cellular detoxification and free radical elimination[10]

MATERIALS & METHODS

- We used human cortical brain spheroids as a model with valproic acid as the inducer of ASD related phenotypic changes. VPA models have been shown to induce both genetic and cellular changes associated with ASD by increasing oxidative stress and altering synapse-related gene expression[7-9].
- We used a lentivirus to introduce dCas9 coupled to KRAB, a transcriptional repressor, and guide RNA (sgRNA). The guide RNA targeted either Nrf2 or had no specific target with a scrambled sgRNA.
- Within each of the two Cas9 treatment groups, the brain spheroids were treated with either VPA alone, SFN alone, VPA+SFN, or solvent control.
- Nrf2 expression was evaluated with immunostaining as well as synapse formation. Expression and synapse formation were analyzed using LSM 700 confocal microscopy followed by analysis with ImageJ.



DISCUSSION

- Should our final results support our hypothesis, they would indicate SFN offers a safe and low-cost way to decrease the risk of developing Autism Spectrum Disorders and other neurodevelopmental abnormalities
- Sulforaphane has already been indicated for the treatment of Autism Spectrum Disorder, showing reduced cognitive and behavioral symptoms in patients with ASD[6].
- SFN's effectiveness as a treatment of ASD are seen due to sulforaphane's ability to reduce neuroinflammation, mitochondrial dysfunction, and oxidative stress[6].
- In the future, we will evaluate Nrf2 levels using qRT-PCR to validate the data collected via image analysis

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