Prenatal Supplementation in the Presence of an EDC Changes Protein Expression in the Placenta

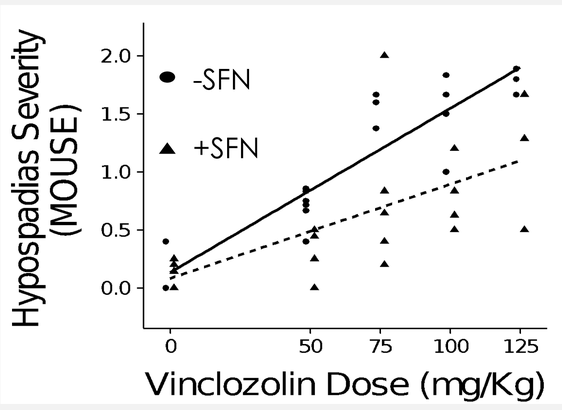


Figure 1. Prenatal supplementation with sulforaphane decreases vinclozolin induced hypospadias severity. Vinclozolin is a model endocrine disrupting chemical used to induce hypospadias 100% of the time.

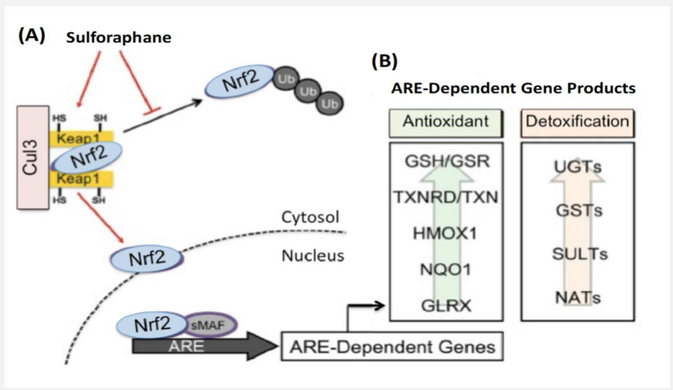


Figure 2. Mechanism of sulforaphane protection. In the presence of SFN, Nrf-2 enters the nucleus, pairs with small Maf (sMaf), and binds to antioxidant response elements (ARE) to initiate transcription. (B) ARE-mediated gene products.

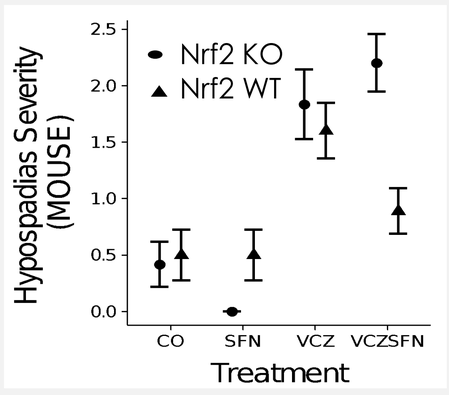


Figure 3. Nrf2 is required for the sulforaphane induced rescue. Only WT individuals exposed to vinclozolin were rescued by sulforaphane.

Figure 4. Where is this rescue occurring?. Pups with different genotypes have a different reaction to vinclozolin plus sulforaphane, so the pup is not being protected solely by the dam. The next place to search is the interface between dam and pup, the placenta.

Introduction

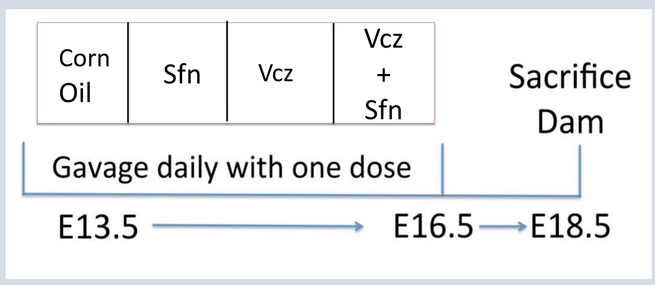
There is no accepted preventative therapy for pollutant-induced malformations, identifying a prenatal regimen to protect the fetus from general environmental toxicant exposure is vital.

- The placenta is a transient endocrine organ that is vital to fetal and adult health.
- Exchange and detox capabilities of the placenta make it a tissue of high importance when looking for the mechanism of prenatal protection from pollutants.
- Protein changes can show how the placenta functions under stress and in a rescue scenario.

We test the hypothesis that sulforaphane changes expression of Nrf2 responsive proteins in the presence of vinclozolin exposure.

Methods

Female and male mice that were heterozygous for Nrf2 were mated to produce wild type and Nrf2 knock out fetuses. Pregnant dams were gavaged.



Placentas from each treatment were sent for proteomics (N≥ 4). Data from KO and WT individuals within each treatment were compared to identify Nrf2 responsive proteins (66 total identified). Nrf2 responsive proteins were then compared in the vinclozolin exposed only treatment and the vinclozolin plus sulforaphane treatment group.

Results

Myg1	UPF0160 protein MYG1, mitochondrial
Ppfibp2	Isoform 4 of Liprin-beta-2
Dusp3	Dual-specificity protein phosphatase 3
Gng5	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-5
Taf15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor

Figure 1: Five proteins showed significantly different expression in the vinclozolin exposed (V) treatment from the vinclozolin + sulforaphane (V+S) treated group. Nrf2 responsive proteins were first identified from the comparison of wildtype Nrf2 pups to knockout Nrf2 pups. These 66 Nrf2 proteins were compared in the V treatment and the V+S.

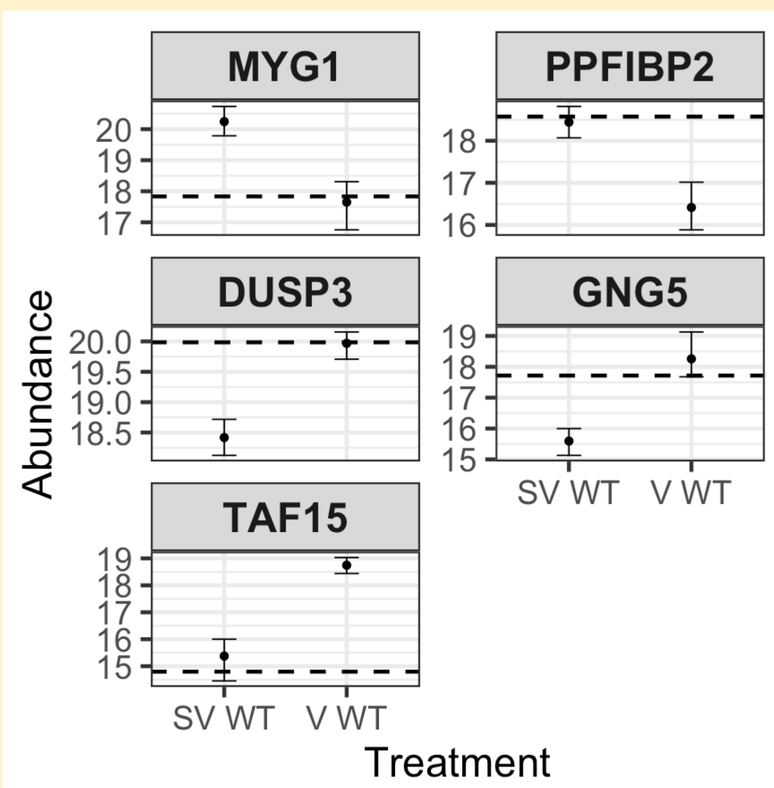


Figure 2: Protein abundance changes between the two treatments occurs in 3 different patterns relative to CO abundance. For Dusp3 and Gng5 proteins sulforaphane supplementation decreases protein abundance relative to V treatment and the corn oil (CO) control (dashed line). For Taf15 and Ppf1bp2, supplementation brings abundance to CO levels. For Myg1 supplementation is reduced relative to V and CO.

Discussion

This work takes an important step towards determining how we can protect the placenta from EDCs. The first pattern we see is the change we expected to see, an increase in protein abundance with supplementation of sulforaphane. This was the expected change as Nrf2 is a transcription factor and should be activated by sulforaphane to increase protective gene products. The second pattern is also expected as the supplementation of sulforaphane rescues the genitalia to a normal phenotype aligning with the protein abundance being brought to corn oil levels. The third pattern was unexpected. In this pattern we see sulforaphane supplementation decreasing protein abundance. To investigate the rescue activity of sulforaphane supplementation further the functions and pathways of these significantly different proteins will be investigated.

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