Sulforaphane Rewires Central Metabolism to Support Liver Antioxidant Response

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Introduction

Diets rich in cruciferous vegetables are linked with a reduction of a wide range of diseases; such as prostate and bladder cancer along with cardiovascular diseases [1]. These benefits are mainly attributed by Sulforaphane (SF), derived from sulphur-rich phytochemicals present in broccoli [2].

Evidence from human intervention studies, suggests that metabolic regulation by SF may play a big role in the observed protective effects [3], but the molecular pathways involved are not fully understood. One potential mediator may be the antioxidant transcription factor NRF2.

The aim of this study was to determine the molecular mechanisms by which SF regulates liver energy metabolism upon exposure to varying glucose concentrations that represent different cellular metabolic states.

Methods

Illumina pair-end RNA-Seq in HepG2 human liver cells treated with physiological concentrations of SF (10µM) under Basal (5.5 mM) and High (25 mM) Glucose. Differentially Expressed Genes and pathways were identified through *edgeR, limma* and Gene Set Enrichment Analysis (GSEA).

Untargeted metabolomics was performed using Gas Chromatography coupled to Mass Spectrometry (GC-MS). Reduced Glutathione was assessed using Triple Quad Liquid Chromatography Mass Spectrometry LC-MS/MS.

Fig.1

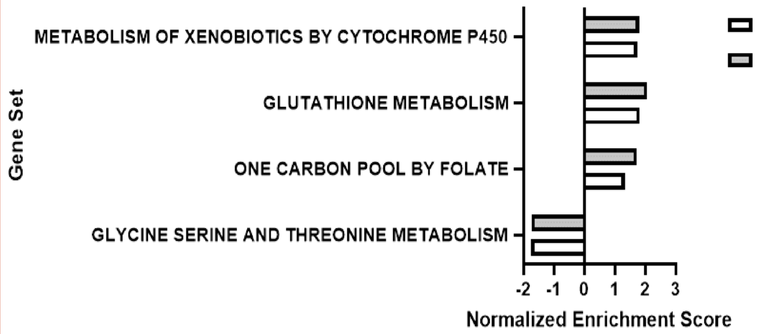

Fig 1. Transcriptome analysis of HepG2 cells treated with 10uM SF under basal (3.3mM) and High (25mM) Glucose levels. Pathways shown are enriched in SF-treated cells (q<0.05)

Fig.2

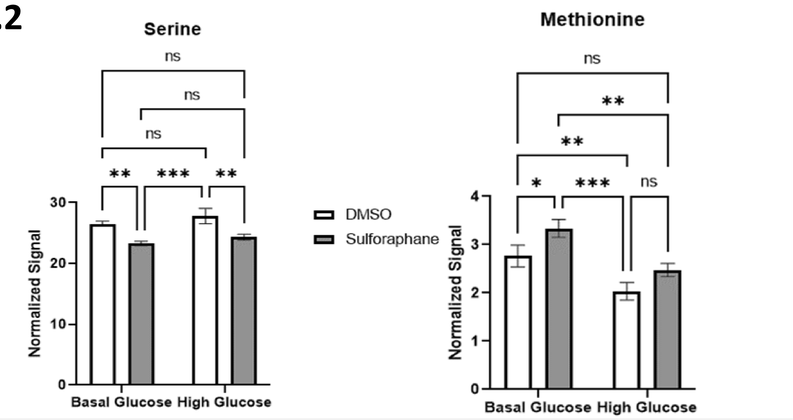

Fig.2 Untargeted Metabolomics of HepG2 cells treated with 10uM SF under basal (3.3mM) and High (25mM) Glucose levels. Serine: Basal Glucose DMSO vs Basal Glucose SF p=0.0047 and High Glucose DMSO vs High Glucose SF p=0.0059. Methionine: Basal Glucose DMSO vs Basal Glucose SF p=0.031

Fig.3

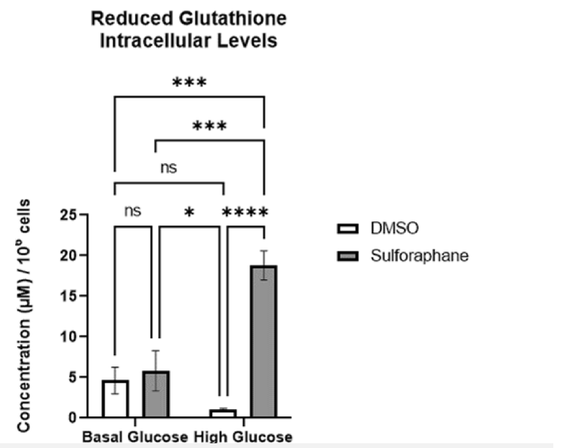

Fig.3 LC-MS/MS analysis of HepG2 cells treated with 10uM SF under basal (3.3mM) and High (25mM) Glucose levels. Reduced Glutathione High Glucose DMSO vs High Glucose SF p <0.001

Results

- SF resulted in a transcriptional downregulation of the Glycine, Serine and Threonine pathway in both glucose environments, along with upregulation in One Carbon pool by Folate pathway in High Glucose only (Fig. 1)
- Untargeted Metabolomics, revealed that in both High and Basal glucose conditions SF interferes with One Carbon Metabolism (increases intracellular methionine and decreases serine) (Fig2)
- SF treatment resulted in a large increase in Reduced Glutathione in High but not Basal Glucose (Fig3)

Conclusion and Future Work

- This is the first study showing that SF rewires Central Metabolism to support the antioxidant response, by upregulating certain genes in One Carbon Metabolism involved in NADH/NADPH production, and by altering key metabolites, thereby supporting antioxidant Glutathione Metabolism.
- Future work is underway, using CRISPR/Cas9 genome edited HepG2 NRF2 knockout cells, to elucidate the role of NRF2 in mediating regulation of One Carbon Metabolism by SF.

References

[1] Traka MH and Mithen RF, 2009. Phytochem. Rev 8, 269–282. [2] Yagishita, et al., 2019. Molecules, 24(19), 3593. [3] Traka MH, et al., 2019.. Am J Clin Nutr. 1;109(4):1133-1144.