Brusatol downregulates HER1 signalling pathway via NRF2-mediated inhibition leading to reduced ovarian cancer proliferation

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Introduction

- Ovarian cancer is the fifth leading cause of cancer death in women worldwide
- Nuclear factor (NRF2) and HER family regulate the normal cellular proliferation and determine the cancer initiation and progression
- Overexpression of both HER1 and NRF2 are reported in ovarian cancer
- Studies have implicated both NRF2 and HER1 in resistance of numerous cancers to chemotherapeutic agents
- Brusatol has recently been shown to inhibit NRF2 signalling, reduce tumour burden, and ameliorate chemoresistance

Objectives

- To develop novel tools to study the regulation of HER1 family receptor by NRF2
- To test the effect of brusatol on HER1 and NRF2 as targets for ovarian cancer therapy;
- To explore how HER1 alongside NFR2 can be regulated; and
- To come out with novel interventions that may overcome chemoresistance in ovarian cancer

Methodology

- Bioinformatics
- Luciferase gene reporter assay
- Immunoblotting (Western blotting)
- ATP dependent cell viability assay
- Reactive oxygen species (ROS) assay
- Total Glutathione assay

Experimental **Setting**

- Human ovarian cancer cell lines, PEO1 and SKOV3 were maintained in RPMI 1640 media supplemented with 10% foetal bovine serum (FBS) and 1% pen/strep in 5% CO2 and incubated at 37°C. Before experimental treatments, cells were grown for 24 h in RPMI 1640 media and then treated with tert-Butylhydroquinone (tBHQ; Sigma) or Brusatol (Carbosynth) to a final concentration as required with media. Following this, Dual luciferase assay, immunoblotting cytotoxicity assay, ROS detection and Glutathione assay were performed in this study.

Results and Discussion

1. HER1

Figure 1.0: Bioinformatics analyses of HER1 promoter identified putative NRF2 binding sites

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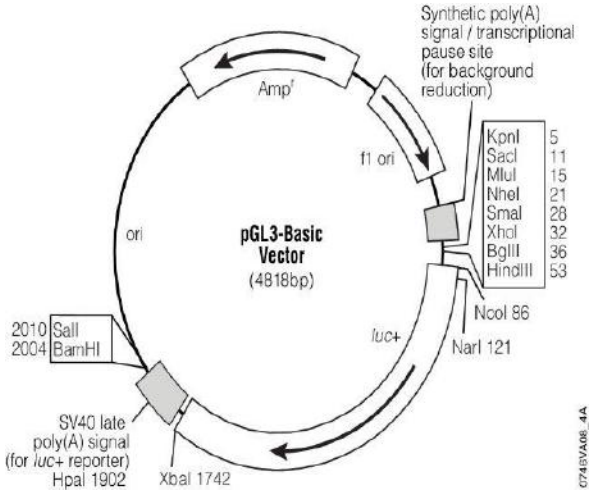

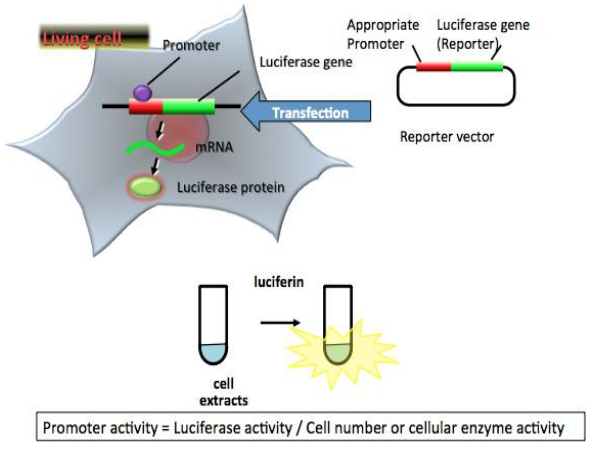

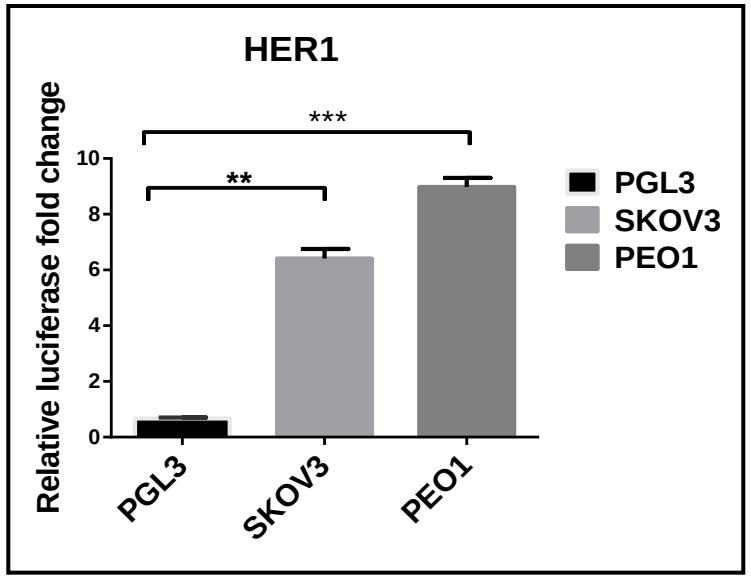

Figure 2.0: HER1 Basal promoter activity (Luciferase activity) in ovarian cancer cell lines

Results and Discussion

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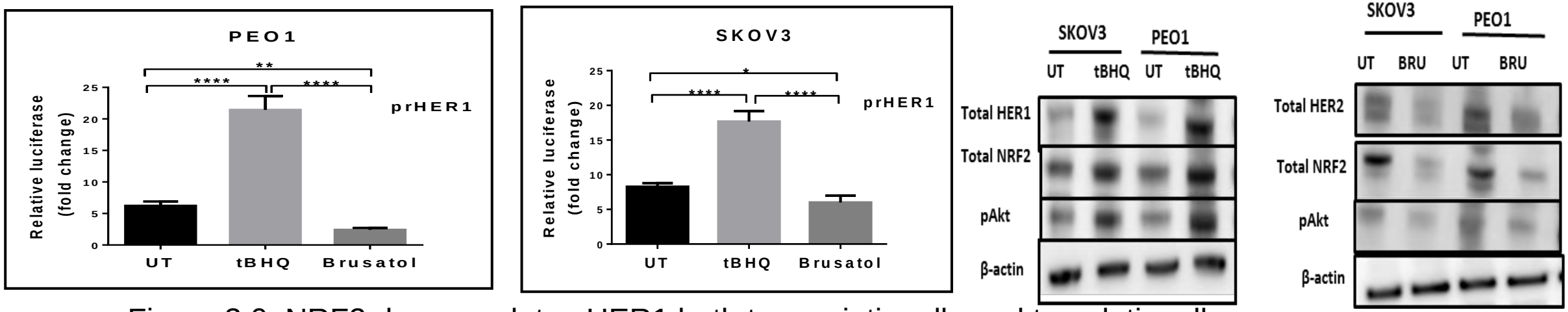

Figure 3.0: NRF2 downregulates HER1 both transcriptionally and translationally

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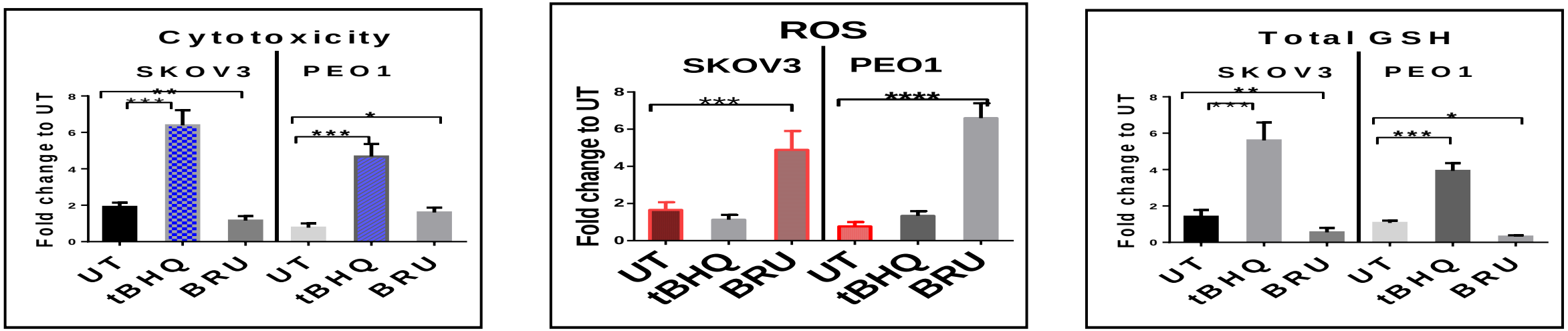

Figure 4.0: Treatments with brusatol reduced cancer cell growth, induced ROS and depleted total glutathione

Conclusions & Recommendations

In conclusion, the findings demonstrates:

- NRF2 regulates HER1 receptor
- A cross-talk between ROS, NRF2 and HER1 receptor
- Importance of NRF2 and HER1 as a key anticancer targets
- Inhibition of NRF2 function could improve cancer treatments

The main recommendations are:

- Further experiments such as gel shift assay and ChIP assay should be employed to investigate the interaction between other relevant transcription factors such AP-1, sp1 and ETF and their possible roles in the transcription of HER1
- To better recommend the use of brusatol in clinical settings, there is a need for more collective research to explore whether these inhibitor can reliably be used in future either alone or as efficient sensitizing agents in combination with chemotherapy and radiotherapy to improve not only ovarian cancer treatment, but also other cancer treatments.

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