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Activation of the integrated stress response by the developmental HSF1 pathway inhibitor NXP800

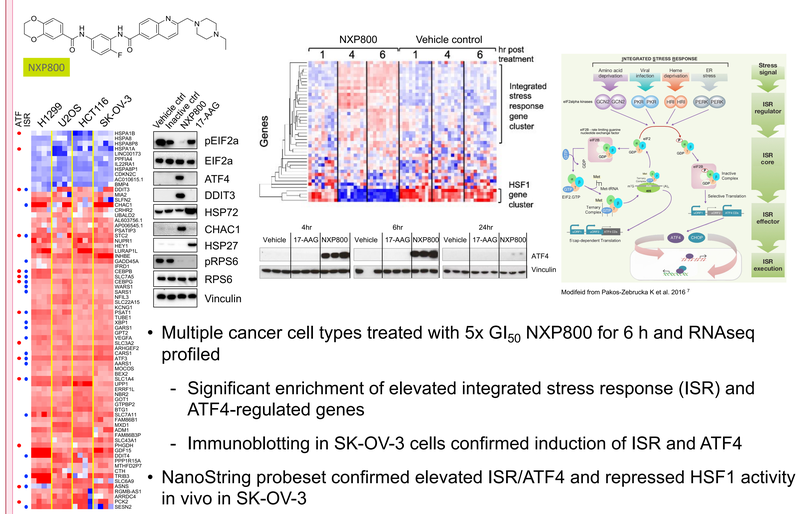
Marissa V Powers, Swee Y Sharp, Eirini- Maria Lampraki, Toby Roe, Robert te Poele, Loredana Pellegrino, Maria Taskinen, Suzanne Eccles, Florence Reynaud, Matthew Cheeseman, Keith Jones, Paul A Clarke and Paul Workman

INTRODUCTION

- HSF1 is an ancient transcription factor regulating the classical eukaryotic Heat Shock Response (HSR) and is a master regulator of proteotoxic stress[1]
- In response to oncogenic stress, HSF1 is hijacked in cancer cells to regulate a gene set overlapping with but distinct from the canonical HSR[2]
- Depleting HSF1 inhibits the oncogenic effects of p53 loss and RAS activation in mouse models[3]
- HSF1 is amplified, over-expressed and activated in human cancers and these and the oncogenic stress signature predict clinical outcome in numerous human cancers, including ovarian[2,4]
- We identified NXP800, the first-in-class orally active inhibitor of HSF1 pathway activation now in phase I clinical trial using a cell based phenotypic pathway screen[5,6]

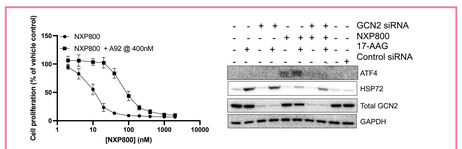
RESULTS

Identifying protein and gene signature PD biomarkers for NXP800

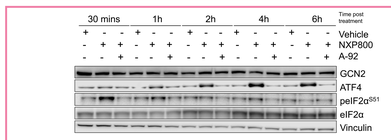


- Multiple cancer cell types treated with 5x GI_{50} NXP800 for 6 h and RNAseq profiled
  - Significant enrichment of elevated integrated stress response (ISR) and ATF4-regulated genes
  - Immunoblotting in SK-OV-3 cells confirmed induction of ISR and ATF4
- NanoString probeset confirmed elevated ISR/ATF4 and repressed HSF1 activity in vivo in SK-OV-3
  - In vivo immunoblotting confirmed induction of ATF4 protein
- Overall NXP800 induces prolonged activation of ISR and blocks HSF1 activation

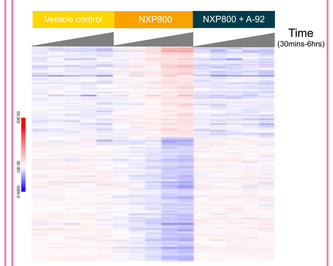
NXP800 stimulates GCN2 to activate the ISR



- NXP800 activates GCN2 to induce expression of ATF4
- Combination of NXP800 and GCN2 inhibitor A92 is antagonistic in SK-OV-3 cells
- PERK, PKR and HRI not affected by NXP800 (data not shown)

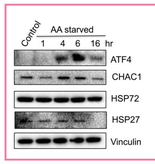


- NXP800 induces phosphorylation of eIF2α and ATF4 induction which is blocked by GCN2 inhibition by A92

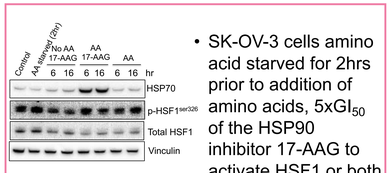


- NXP800 induces global phospho-proteome changes in SK-OV-3 human ovarian carcinoma cells
- Changes are reversed when combined with GCN2 inhibition

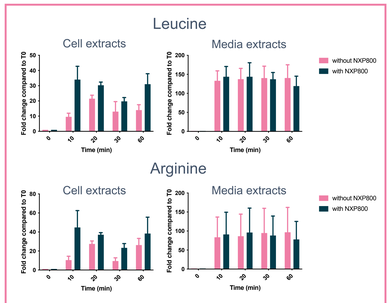
NXP800 does not activate GCN2 by altering amino acid uptake



- SK-OV-3 cells amino acid starved for up to 16hrs
- Activation of the ISR/ATF4 evident from 4hrs

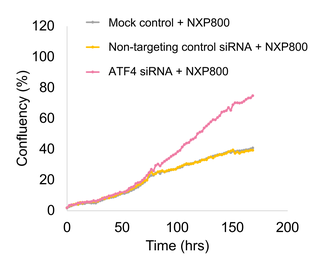


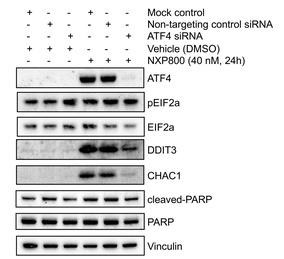
- SK-OV-3 cells amino acid starved for 2hrs prior to addition of amino acids, 5xGI_{50} of the HSP90 inhibitor 17-AAG to activate HSF1 or both for 6-16hrs
- Amino acid starvation blocks activation of HSF1



- Uptake of Leucine or Arginine in SK-OV-3 cells following amino acid starvation measured by mass spectrometry
- NXP800 does not inhibit amino acid uptake

ATF4 induction contributes to the anti-proliferative activity of NXP800





- SK-OV-3 cells treated with NXP800 following siRNA mediated silencing of ATF4
- Significant reduction in the anti-proliferative activity of NXP800 when ATF4 induction was prevented

PHASE 1 CLINICAL TRIAL OF NXP800

Phase 1 trial comprising dose-escalation (Phase 1a) and expansion (Phase 1b) phases commenced and first patient treated in January 2022 (www.clinicaltrials.gov/ct2/show/NCT05226507)

SUMMARY AND FUTURE PLANS

- NXP800 is the first in-class, orally active inhibitor of HSF1 activation which is important for cancer cells
- Biomarkers discovered and validated provide insight to the mechanism of action of NXP800
- Orthogonal approaches indicate potent inhibition of HSF1 activation via stimulation of the ISR
- NXP800 induces phosphorylation of eIF2a via activation of the stress activated kinase GCN2
- Inhibition of GCN2 by A92 blocks activation of the ISR and reverses changes in the global phosphoproteome induced by NXP800
- Induction of ATF4 contributes to the anti-proliferative mechanism of NXP800
- NXP800 does not activate GCN2 by interfering with amino acid uptake; current studies are investigating the precise mechanism of action

Contact

The Institute of Cancer Research
Division of Cancer Therapeutics
15 Cotswold Road, London, SM2 5NG
paul.workman@icr.ac.uk or paul.clarke@icr.ac.uk

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