Glucocorticoids promote DNA repair to reduce efficacy of radiotherapy in Glioblastoma

Kathryn McGinnis[1], Syed Murtuza Baker[2], Andrew Berry[2], Thomas Ward[1], Magnus Rattray[2], David Ray[2], Graham Cook[1], Jacquelyn Bond[1] and Laura Matthews[1]

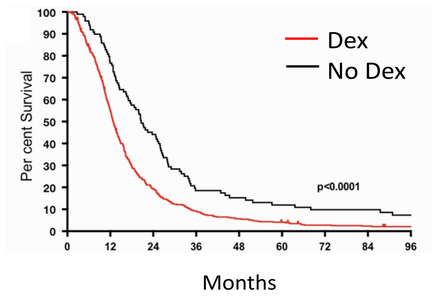
[1]School of Medicine, Faculty of Medicine and Health, University of Leeds, LS9 7TF, UK
[2]School of Medicine, Faculty of Biology, Medicine and Health, University of Manchester, M13 9PT, UK

UNIVERSITY OF LEEDS

Steroid use in GBM correlates with poor outcome

Glucocorticoids (Gc) are steroid hormones and potent anti-inflammatory drugs.

Gc, including Dexamethasone, are given to reduce cerebral oedema caused by brain tumours, such as glioblastoma, which is a highly aggressive form of brain cancer.



Patients receiving Dex have reduced rates of survival[1].

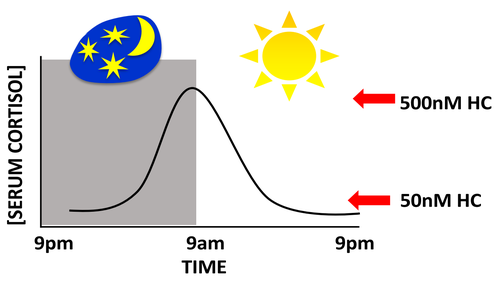
Several studies have questioned safe use of Gc as part of standard therapy, i.e. alongside radiotherapy and chemotherapy

Aims and Experimental Design

To characterize the cellular response of GBM cells to Gc treatment at the transcriptional level using RNA-seq.

1) To measure genome wide transcriptional responses of GBM cells to steroids
2) To identify pathways regulated by steroids in GBM cells relevant to cancer
3) To investigate the effect of Gc on therapeutic efficacy

The study aims to investigate the GBM cell response to both the endogenous Gc, cortisol, and the synthetic Gc, Dex, which forms part of therapy.



50nM and 500nM Hydrocortisone (HC) mimics the physiological evening and morning levels of circulating cortisol respectively.

100nM Dexamethasone (Dex) mimics the dosage of synthetic Gc given as therapy in GBM.

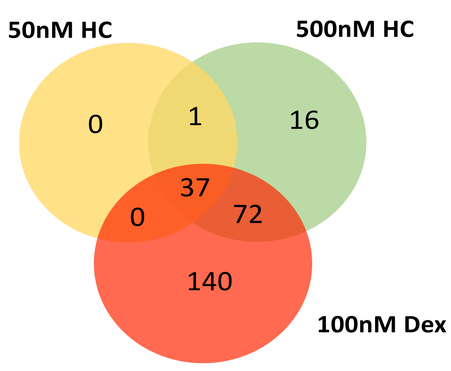
Genome wide transcriptional response to steroids in GBM

GBM (M059K) cells were treated for 4 hours with vehicle, low HC, high HC or Dex and analysed by RNA-seq.

In total, we identify 266 genes that are regulated by Gc in GBM cells.

- 37 genes were regulated by all 3 treatments
- 72 genes were only regulated in response to peak HC and Dex.
- 140 genes were regulated in response to Dex alone.

Gc regulated genes (>1.5 fold, <0.05 FDR)



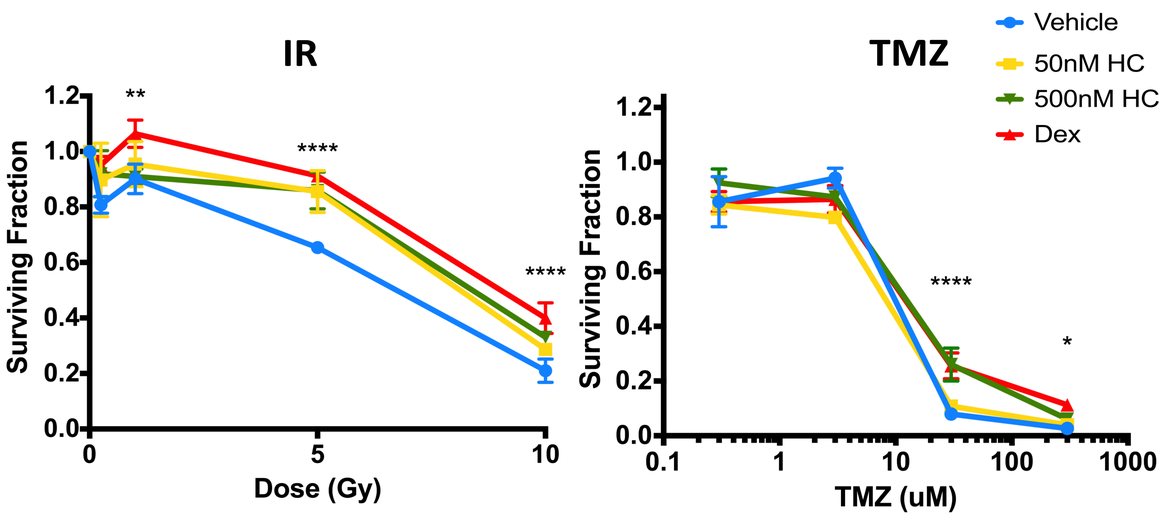
Gene ontology analysis of the Gc regulated genes using Enrichr identifies key pathways under Gc control in GBM, relevant to inflammation (green) and cancer (red).

KEGG Database	NCI-Nature Database
NFκB signalling pathway	IL6-mediated signalling events
Transcriptional misregulation in cancer	AP-1 transcription factor network
Chagas disease	Direct p53 effectors

Gc regulate genes which may contribute to therapeutic resistance

Effect of steroids on cancer cell survival

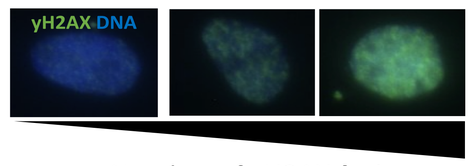
M059K cells were irradiated (IR) or treated with chemotherapy, temozolomide (TMZ) following Gc or vehicle treatment. Cell survival was analysed by MTT assay (n=3).

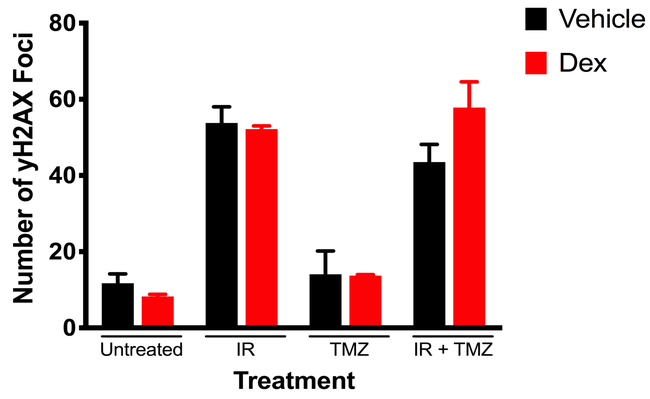


Gc increase survival of GBM cells following IR and TMZ

Effects of steroids on DNA damage

M059K cells were irradiated (IR) or treated with chemotherapy, temozolomide (TMZ) following Gc or vehicle treatment. DNA damage was analysed by quantifying the average number of yH2AX foci per nucleus 2 hours post-IR/TMZ treatment (n=2). Representative images are shown.

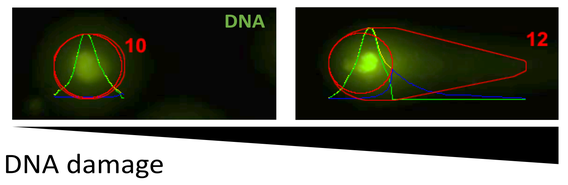




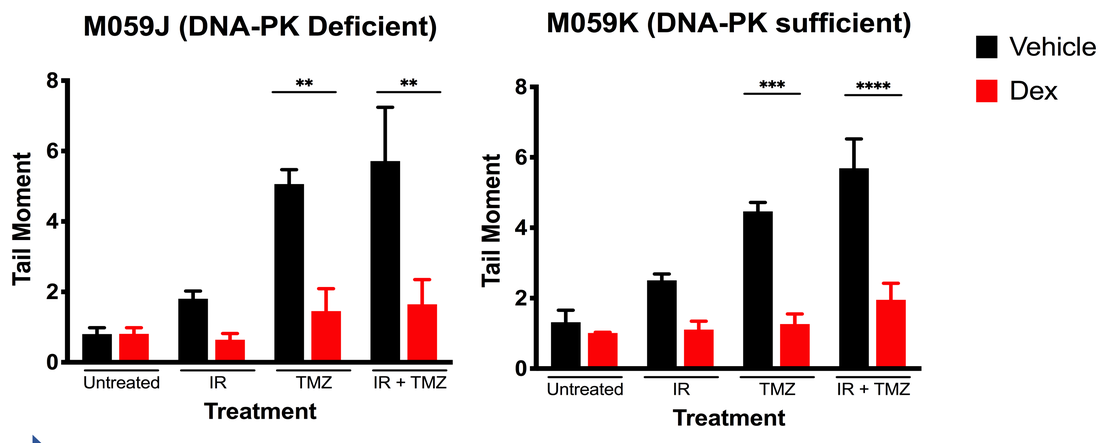
Gc do not prevent DNA damage in response to IR and TMZ

Effects of steroids on DNA repair

M059J and M059K cells were irradiated (IR) or treated with chemotherapy, temozolomide (TMZ) following Gc or vehicle treatment. DNA repair was analysed by comet assay 24 hours post-IR/TMZ treatment (n=3). Tail moments were used as a measure of DNA damage per nucleus.



Tail moment = comet length x Tail DNA %



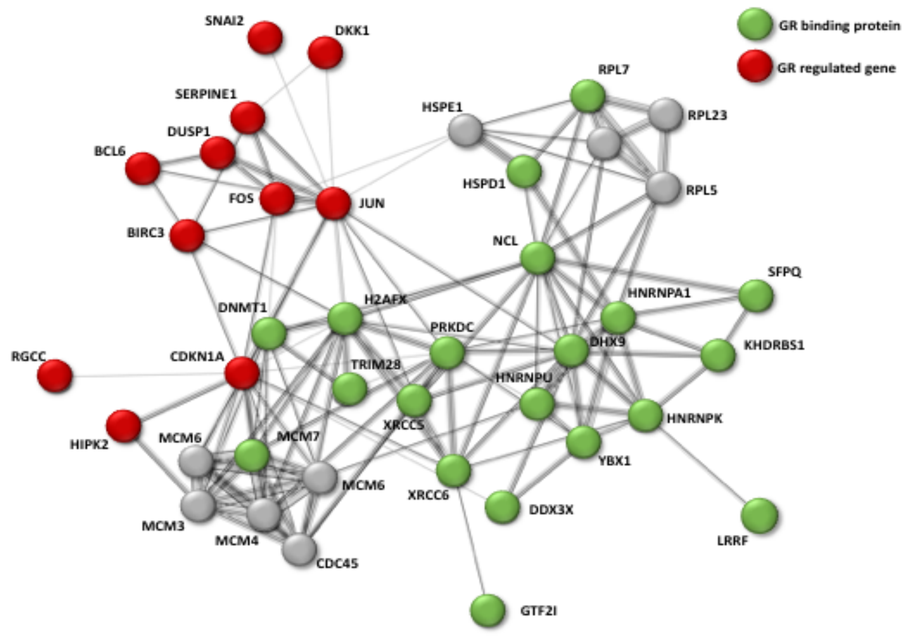
Gc promote DNA repair following IR and TMZ treatment

Conclusions

- Gc regulate the expression of cell cycle and DNA repair genes in GBM cells.
- Pre-treatment with Gc reduces the efficacy of radio- and chemotherapy through the upregulation of DNA repair.

Future Work

Gc-regulated genes (red) identified by the RNA-seq were combined with our previously published list of GR binding proteins (green) to identify potential candidates linking Gc action to DNA repair pathways.



Gc may affect DNA repair through multiple mechanisms

Future studies will systematically test the role of each candidate effector outlined above in facilitating DNA repair following Gc treatment.

Through this, we will confirm the safe use of Gc in cancer treatment

References

1. Pitter KL, Tamagno I, Alikhanyan K, et al. Corticosteroids compromise survival in glioblastoma. *Brain*. 2016;139(5):1458-1471.