Glucocorticoid Receptor(GCR) activation increases resistance to chemotherapy in HNSCC, and detection of NR3C1 mRNA expression using deep learning can inform optimization of chemotherapy

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BACKGROUND

- HNSCC account for nearly 350,000 per annum deaths from cancer worldwide.
- GCR agonists, such as dexamethasone, are widely used to treat inflammation and inhibit chemotherapy-related side effects among cancer patients.
- Glucocorticoids are nonetheless thought to increase antiapoptotic behavior via NR3C1(GCR) activation.
- Deep learning, an emerging filed of AI, is being deployed for pathological image analysis to examine cellular features.

PURPOSE

- To investigate whether NR3C1 activation by dexamethasone increases resistance to chemotherapy.
- To train a Convolutional Neural Network (CNN) to segregate between high NR3C1 gene expression from low using the Xception architecture to inform chemotherapy optimization.

METHODS

Cell Line: SCC74A(highly metastatic)

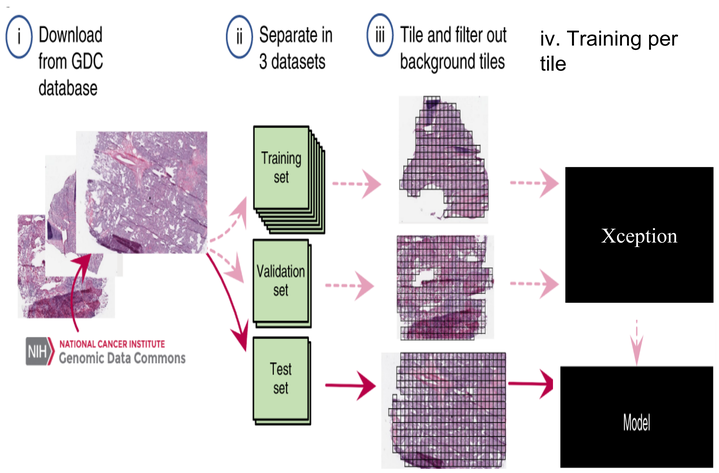
Drug Treatment:

- Cells are treated with dex (50nM) 1h prior to treatment with chemotherapy (cisplatin 1uM).
- Incucyte S3 system is used to scan images for cell confluence at different time points post treatment to determine % confluence.

Deep Learning Pipeline:

- HNSCC slide images were downloaded from GDC and separated into training and validation cohorts.
- Use the Xception CNN to train the model to identify correctly high vs low NR3C1 from annotated slides from The Cancer Genome Atlas(TCGA).

Fig. 1. Deep learning pipeline for identifying NR3C1 mRNA expression levels.



RESULTS

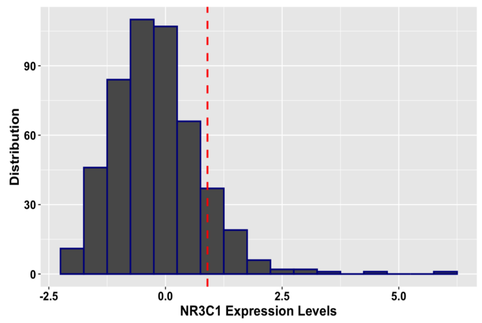


Fig.2. Distribution of NR3C1 mRNA expression levels. The values are z-score normalized. Data obtained from CDG (TCGA data). The red dashed line indicates the cutoff point used to create a categorical variable, high vs low, for model training.

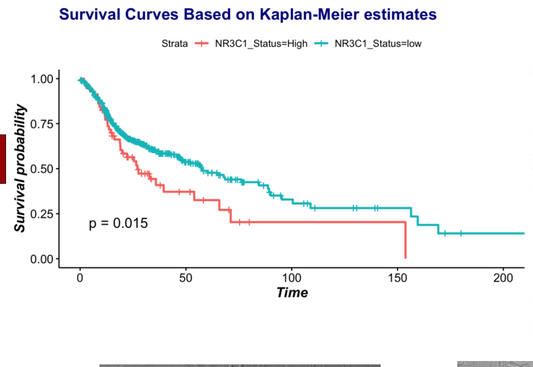


Fig. 3. Survival Curve for patients with distinct NR3C1 statuses. The survival probability demonstrates that cancer patients with low NR3C1 expression have higher prognosis. p = 0.015

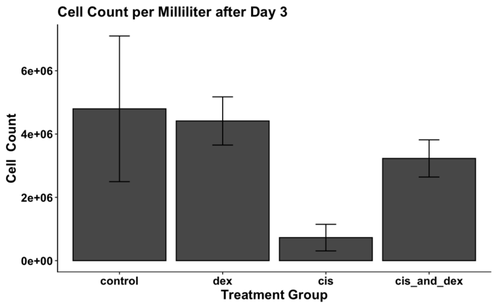


Fig.4. Distribution of cell counting on day 3. Cells were treated with dex (50nM) 1 hour before treatment with chemotherapy drug, cisplatin (1uM). Results analysis shows that cis effectively kills cancer cells (p-value <0.05 in comparison with control or dex). Additionally, the results demonstrate that dex inhibits cell apoptosis for chemotherapy-treated cells through NR3C1 activation. P-value = 0.061. Error bars are SEM.

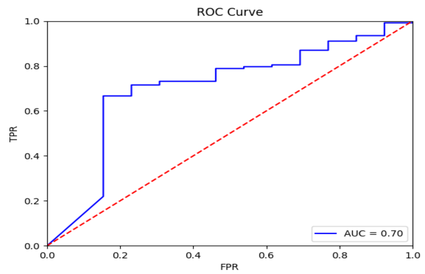


Fig.9. Receiver Operating Characteristic (ROC) curve for the classification of NR3C1 mRNA as either high or low. The y-axis shows true positive rate and the x-axis shows the false positive rate. 417 whole-slide images from TCGA were used. The slides were tessellated using 598px and 604uM. To minimize error and increase the model's performance, the data was split into 3 folds using k-fold cross- validation. The validation accuracy is 0.84. Results are retrieved from k-fold 2 after 10 epochs. Although not optimal performance, the deep learning model considerably identifies high versus low NR3C1 mRNA expression (0.70 AUC).

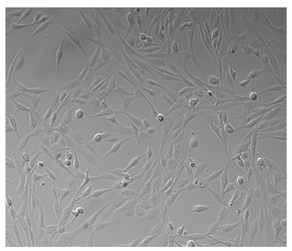


Fig.5. Cell confluence for control cells after day 3. The image shows that control cells proliferated after 3 days. Fig.4. shows cell count for for control cells. The small round-shaped objects are dead cells.

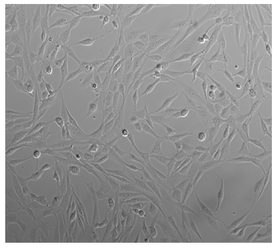


Fig.6.Cell confluence for dex-treated cells(50nM) after 3 days. The image above confirms no significant difference between control cells and dex-treated cells.

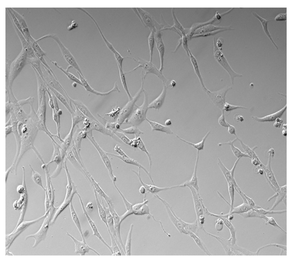


Fig.7. Cell confluence for cisplatin(1uM)-treated cells. There are significantly less cells for cisplatin-treated cells in comparison with control cells.

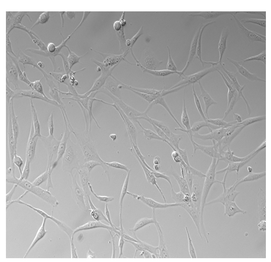


Fig.8. Cell confluence for cells treated with dex(50nM) cisplatin(1uM). In comparison with cis-treated cells, this group shows less dead cells and high confluence.

CONCLUSIONS & FURTHER STUDIES

1. We have successfully investigated and proven that dexamethasone induces an anti-apoptotic behavior among SCC74A after treatment with chemotherapy.
   Relevance: Although effective as an anti-inflammatory substance and its capacity to prevent chemotherapy side effects, dexamethasone should be administered with carefulness because it can cause poor prognosis among cancer patients, especially those with high NR3C1 expression.

2. We have also deployed a convolutional neural network to classify high versus low glucocorticoid receptor expression.
   Relevance: The first part of the research studied and confirmed that dex activates NR3C1 and reverses the effect of chemotherapy. Being able to classify very efficiently NR3C1 gene expression levels using deep learning will inform clinical strategies for chemotherapy optimization for patients with distinct NR3C1 mRNA expression levels.
3. Further studies will focus on fine-tuning hyperparameters to improve the model's performance.

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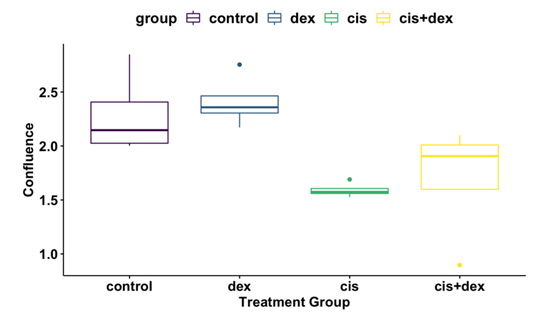

Fig.6. One-way ANOVA of cell confluence after day 1 following treatment with chemotherapy. $P < 0.05$ (control – cis). The difference between cis+dex and cis is not statistically significant (p=0.88).