A Bioinformatics Approach Highlights the Potential of Retinoids in Glioblastoma Growth Regulation

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Coláiste na hOllscoile Corcaigh

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BACKGROUND

Glioma, the most commonly diagnosed primary brain cancer in Ireland and worldwide, carries a poor prognosis. Glioma is classified by WHO grade and histological origin of the cancer. Treatment options are limited and face many physical, physiological, and pharmacological obstacles. Glioblastoma, the most aggressive form of glioma (grade IV), arises through progression from a lower grade glioma or directly from cancer stem cells. Only 5% of patients survive beyond five years from their diagnosis [1].

While the tumours may present heterogeneous molecular signatures, cancer stem cells isolated from these tumours can be classified into three subgroups, mesenchymal, classical and proneural, on the basis of the genes expressed in these cells [2].

Retinoic acid (RA) and its synthetic analogues, the retinoids, are potent lipophilic differentiation agents that have been considered as potential therapeutic agents for glioma. Previous attempts to use RA to treat glioma have reported mixed outcomes [3]. RA signals through complex pathways involving multiple proteins (figure 1), leading to changes in gene expression and cellular function. Three genes exist for the nuclear retinoic acid receptors (RARs), *RARA, RARB* and *RARG,* that have been shown to affect cell growth differentially.

It is possible that the use of pan-RAR ligands promoted cell growth in some tumours while supressing growth in others. We propose that the selective stimulation of parts of the RA pathway may potentially trigger terminal differentiation of the glioma cells. There is potential to identify not only the RAR isoforms that are capable of inducing terminal differention but also the retinoids that are most effective.

Using gene expression analysis, we identified components of the RA pathway, whose expression is altered in tumours of various grades. These alterations could potentially explain the mixed response of tumours to RA and motivates further research into stratification of glioma with respect to their response to retinoids.

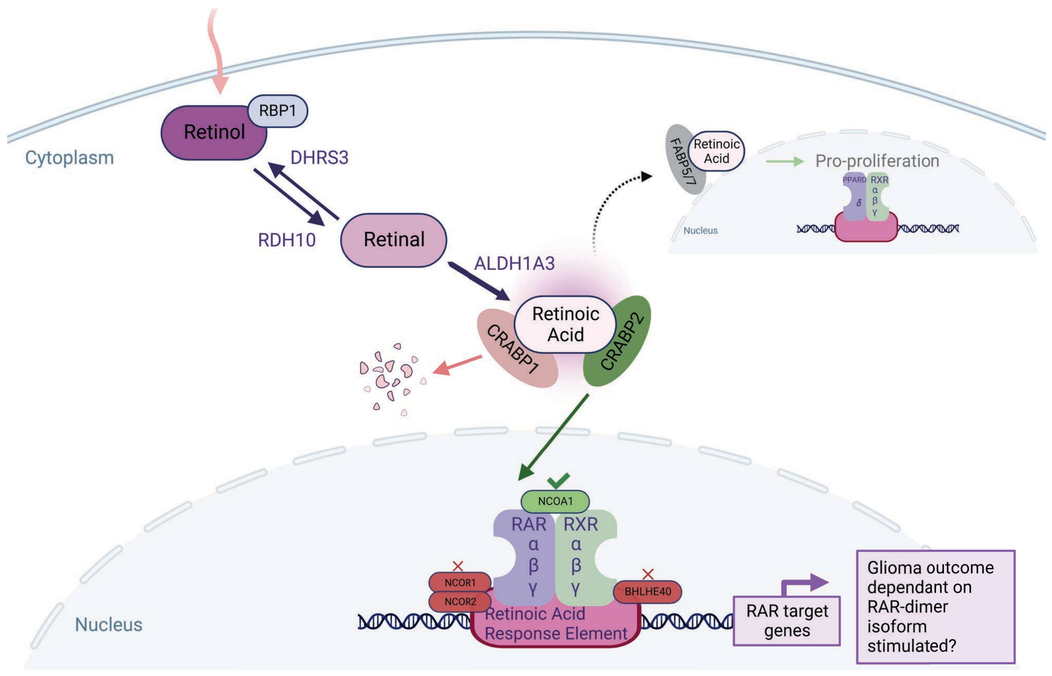


Figure 1 - The retinoic acid signalling pathways

METHODS

TCGA RNA-Seq data [4] (figure 2) was obtained in the form of FPKM and converted using the following formula (equivalent to transcripts per kilobase per million):

$$\frac{gene\ counts\ in\ FPKM}{sum\ of\ total\ sample\ counts} \times 10^6$$

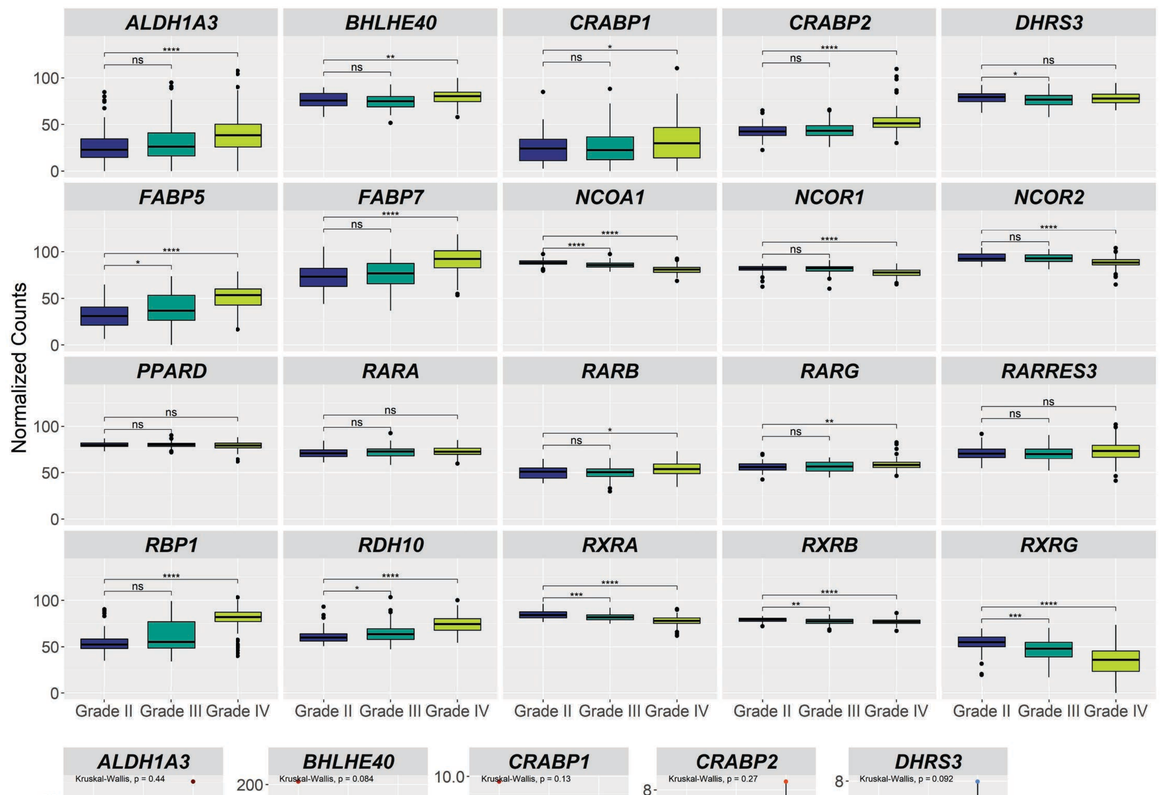
RNA-Seq data was obtained from QIMR Berghofer for the Q-Cell panel [5] (figure 3) in the form of raw counts and was normalised with the following formula (equivalent to transcripts per kilobase per million):

$$A \times \frac{1}{\sum(A)} \times 10^6$$

$$\text{Where } A = \frac{total\ reads\ mapped\ per\ gene}{gene\ length\ in\ kb}$$

All data analysed in R (version 4.2.1). Expression data for key genes of the RA signalling pathways was extracted for WHO grades II, III and IV (TCGA) and cell type (QCell) and analysed by non-parametric statistics (TCGA: Wilcoxon signed-rank test, Bonferroni-adjusted; QCell: Kruskal-Wallis)

RESULTS



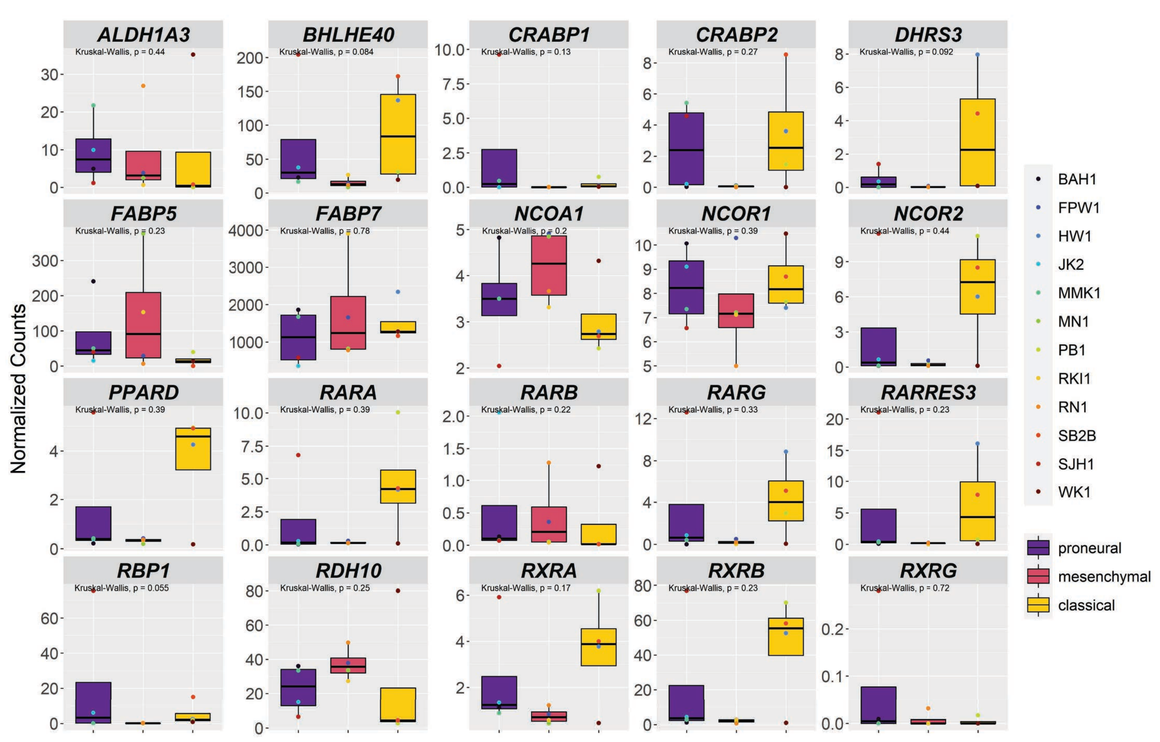


Figure 2 (Top): Expression data of key genes in the RA pathway in the TCGA RNA-Seq data set.
Figure 3 (Bottom): Expression data of key genes in the RA pathway in the QIMR Berghofer Q-Cell data set (all $p > 0.05$).
QIMR: N = 4; TCGA: Grade II N = 55, Grade III N = 114, Grade IV N = 152.
**** $p <= 0.0001$; *** $p <= 0.001$; ** $p <= 0.01$; * $p <= 0.05$; ns: $p > 0.05$ (Wilcoxon signed-rank test, Bonferroni-adjusted).

CONCLUSIONS

The RA pathway is altered in glioma cells in a grade-dependent manner. This research indicates that there is opportunity for RA to reach the nucleus of the cell and lead to downstream transcription of the RA-inducible genes and suggests that RAR expression is increased in higher grade glioma (HGG). As seen in figure 2, the expression of the RXRs is decreased in HGG compared to low grade glioma (LGG), with the exception of RXRB. In HGG, the transport of RA by CRABP2 has potential to be interfered with by the increased expression of FABP5 and FABP7, which direct RA towards the pro-proliferative PPAR pathway.

As reflected in figure 3, there is insufficient data to draw conclusions as to any subgroup-dependent effects on the pathway. This data highlights the need for elucidating the growth effects mediated by selective stimulation of the RAR isoforms in glioma cell subgroups and defining downstream isoform-specific effects.

ACKNOWLEDGEMENTS

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