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OMICS RESEARCH SYMPOSIUM

Bioinformatics Analysis to understand the transcriptional variations and molecular pathways underlying IDH-mutant Glioblastoma

Akanksha Singh[1], Harpreet Kaur[2], Elia Brodsky[2], Mohit Mazumder[2]

Introduction

Glioblastoma (GBM) is an aggressive form of primary central nervous system malignant brain tumor. Survival rate of patients with glioblastoma is unsatisfactory and has limited treatment and therapy options. **(Birkó *et. al,* 2020) (Grossman et. *al,* 2010) (Schneider *et. al,* 2010) (Chinot *et. al,* 2014)**

According to the **CBTRUS (Central Brain Tumour Registry of the United States) 2013** report, the average annual age adjusted incidence rate (IR) of **GBM is 3.19/100,000** population, which is regards as the highest incidence rate among malignant brain and CNS tumors. **(Kanderi *et al,* 2021)**

Glioblastoma generally consists of two types **primary and secondary subtypes** that are originated due to modification of genetic pathways which affects the patients at various ages. Primary GBMs account for almost eighty percent of GBMs and occur in patients with a age of sixty-two yrs while secondary glioblastoma occurs from lower-grade astrocytoma or oligodendroglioma in the patients with a average age of forty-five years. The World Health Organization demonstrated GBM as a grade IV cancer characterized as malignant, mitotically active, and predisposed to necrosis (natural cell death).

FUNCTION OF IDH (Isocitrate dehydrogenase)

Isocitrate de-hydrogenases predominantly occur in cytoplasm and peroxisome is an essential enzyme of Krebs cycle that reduces nicotinamide adenine dinucleotide phosphate (NADP+) -dependent into nicotine amide adenine di-nucleotide phosphate (NADPH) and helps in catalysing the oxidative de-carboxylation of isocitrate to α-ketoglutarate (α-KG). **(Kaminska *et al,* 2019)**

Manufacture of NADPH in the human-brain accounts for almost 65%. **(Bleeker *et al,* 2010)** IDH1 enzymes help balancing redox reaction and protecting an overall cell by oxidation (oxidative stress) and its damages and also protects damages against DNA. **(Koh *et al,* 2004) (Minard *et al,*1999).** This is done by providing or generating adequate amounts of strong reductive agents such as NADPH and α-KG**. (Mettellus *et al,* 2011)** Furthermore, their products (NADPH and α-KG) interact with glutathione- and thioredoxin-producing systems produce a reduced glutathione (GSH) and peroxiredoxin. **(Mettellus *et al,* 2011)** Generation of NADPH by IDH1 is also involved in metabolism of lipids and also provides cellular defense against a reactive oxygen species (ROS) induced during lipid oxidation. **(Koh *et al,* 2004) (Mettellus *et al,* 2011)** IDH1 also accounts for glutamine metabolism under hypoxia and electron transport chain alterations (ETS). **(Kaminska *et al,* 2019)**

According to classification of WHO, it is noticed that in almost 12% of glioma samples, it has been depicted that in the IDH1 gene an unexpected spectrum of mutations, among which somatic, recurrent mutations in grades II and grade III. **(Parsons *et al,* 2008)**

Appearance of D-2HG considered as major intracellular effector in IDH1 mutated glioma and regarded as one of the oncometabolite (accumulation of small molecule or enantiomer that participates in normal metabolism and involved in process of cancerogenesis), which is responsible for alteration of epigenetics as well as made cellular state a permissible for transforming towards malignant**. (Garrett M. *et al,* 2021) (Losman *et al,* 2013) (Flavahan *et al,* 2016)**

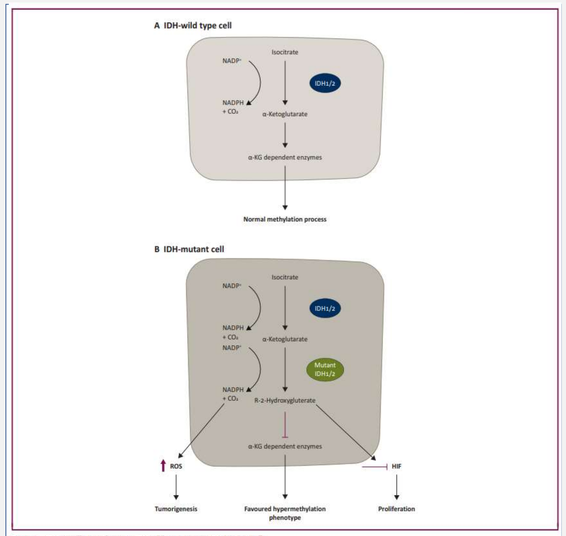


Figure 1. ***IDH* signalling pathway in *IDH*-wild-type versus *IDH*-mutant cells.**
Unlike aberrant *IDH*-mutant intracellular signalling, wild-type *IDH* expression elicits no major effects on cellular metabolism, production of ROS, tumorigenesis or proliferation. Cells expressing wild-type *IDH* favour a normal methylation pattern, compared with the favoured hypermethylation phenotype of *IDH*-mutant cells. HIF, hypoxia-inducible factor; KG, ketoglutarate; ROS, reactive oxygen species.

Methods

Dataset

Dataset for study was taken from the **Gene Expression Omnibus (GEO)** Database. The **GSE147352** series with the supplementary file DESeq normalized counts has been extracted. Transcriptome profiling of human glioma and normal brain tissues by rRNA-deleted total RNAseq with a total of 118 brain tissue samples (85 adult glioblastomas, 18 lower grade gliomas, and 15 normal brain tissues) by high throughput sequencing from Illumina HiSeq 4000.

EXPLORATORY ANALYSIS

- Done by running utilities pipeline on t-bioInfo server.
- Data was normalized using Quantile normalization and then PCA was performed to get the get the visuals among the samples.
- Exploratory analysis done on all the groups then followed by two groups (IDH1 Wt vs IDH1 Mut).

COMPARATIVE ANALYSIS

- Comparative analysis done on (IDH1 Wt vs IDH1 Mut) to analyse the sample by running the Dseq2 pipeline on t-Bioinfo server.
- Comparative analysis- consists of pipelines, DEseq2, Enrichment analysis, GSEA Analysis.
- Usually done to obtain differentially expressed genes, KEGG pathways, GO enriched genes, Network plot among the groups.

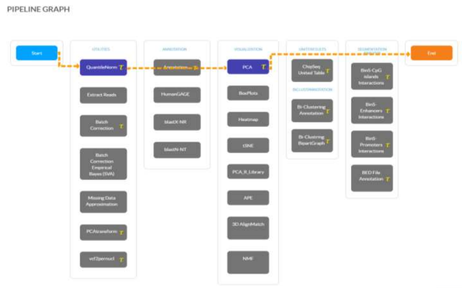


Figure 2.: Exploratory analysis pipeline2

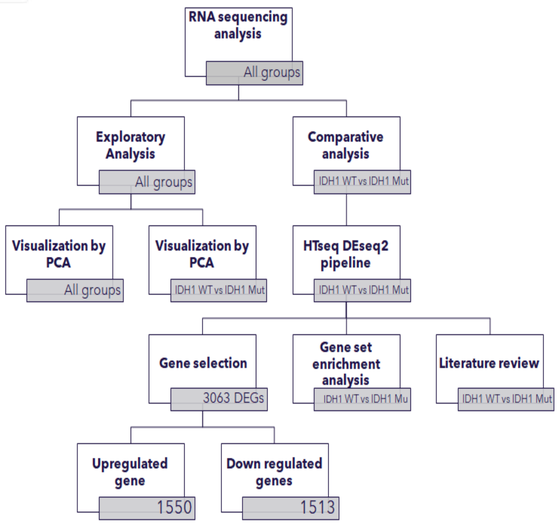


Figure 4.: methodology illustrated workflow to get the differentially expressed genes

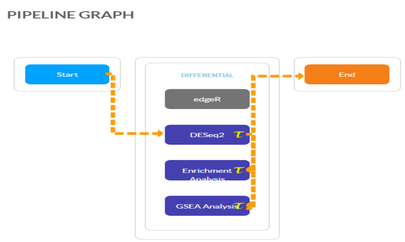


Figure 3.: Comparative analysis

Results

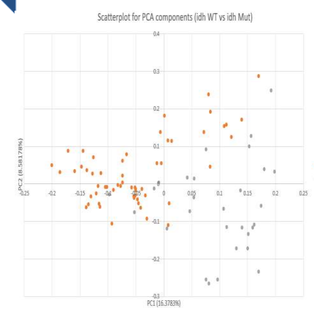


Fig. 5 PCA scatter plot

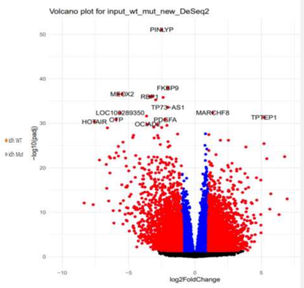


Fig. 6 Volcano plot

PCA Scatter plot

- Second Scatter plot was generated on **IDH1 WT** and **IDH1 Mut** with **PC1 (16.3783%)** and **PC 2 (8.58178%).**
- No clear clustering was generated between the groups.
- No signs of outliers.

VOLCANO PLOT

- Performed under the two conditions: the **adjusted P-value** at y – axis versus **log2-foldchange** at X – axis.
- It determine significant **differentially expressed genes (DGEs).**
- Genes which are having n**egative log2fold change value** will be considered as **downregulated genes** whereas **positive log2fold change value** to be considered as **upregulated genes**.

KEGG - Kyoto Encyclopaedia of Genes and Genomes

- **Count** shows the **number of genes** involved in the pathway
- **Colour** represents the **p-adjusted value**. (determine significant genes)

GO PATHWAY

- Perform enrichment analysis on gene sets.
- The **circles (dot)** in the network indicate the **count or number of genes** present and the **colour** of the circular dots (genes) provides information about the **p-adj value** of the genes.

NETWORK PLOT

- Its is basically a graph that Analyses the pathways in the network based on fold change value.
- **Size of dot** in the **core** depicts the **number of genes** involved in particular pathway.
- **Colour** indicates the **fold change value.**
- Higher fold change higher the gene expressed in pathway.

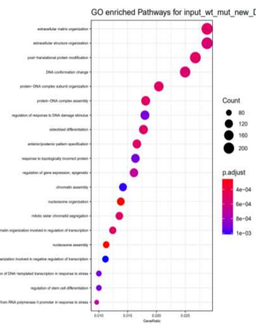


Fig. 9 GO enriched pathway

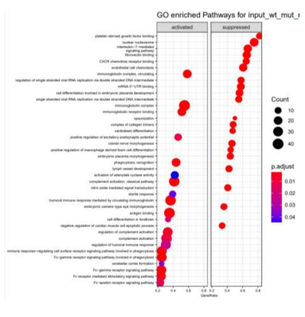


Fig. 10 GO enriched pathway - upregulated vs down regulated genes.

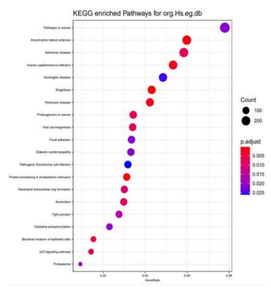


Fig. 7 KEGG enriched pathway

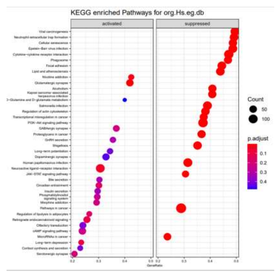


Fig. 8 KEGG enriched pathways – upregulated vs down regulated

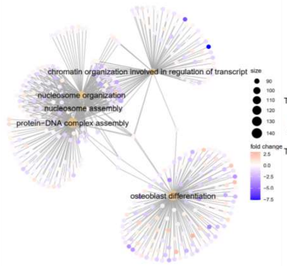


Fig. 11 Network plot

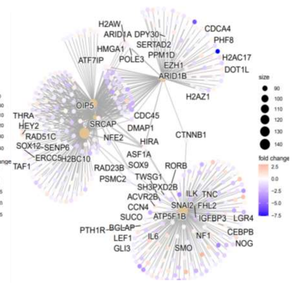
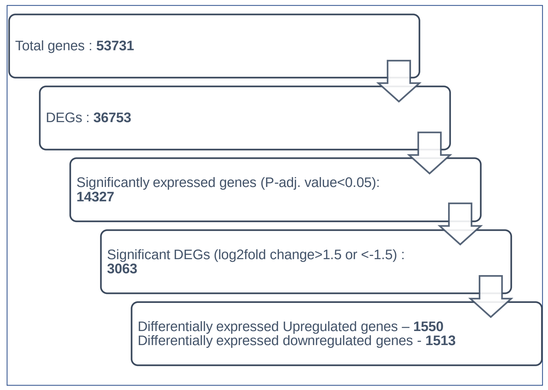


Fig. 12 Network plot – representation of genes involved in pathway

Conclusions

- **KEY FINDINGS**
  - From KEGG analysis possible association can be made between suppressed pathway of **viral carcinogen** and **neutrophil extracellular** trap formation in glioblastoma.
  - From GO analysis possible association can be made between
    - ✓activated pathway of immunoglobin complex.
    - ✓suppressed pathway of platelet-derived growth factor and nuclear nucleosomes.
- **FUTURE DIRECTIONS**
  - Much work needed in future to access the precise affect of IDH1 mut. Pathway on progression of glioblastoma.
  - Researchers can perform experiments focusing on these particular gene found in the study and corelate their up regulated and down regulated with disease progression.



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