Uncovering an Association Between Oligodendroglioma and the Human Papillomavirus (HPV) Through Molecular Subpopulation Analysis Using NetraAI

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BACKGROUND

OLIGODENDROGLIOMA HETEROGENEITY

Oligodendrogliomas (OGs), a rare subset of primary brain tumors constituting ~5% of all brain tumors, exhibit complex genomic heterogeneity, contributing to varied clinical outcomes. These tumors are stratified into three molecular subtypes based on *IDH* mutation and *1p/19q* co-deletion, with additional heterogeneity related to *EGFR, PTEN, 10q* deletion, and several other markers that impact survival, invasiveness, and progression.

NETRA AI: UNVEILING PATIENT INSIGHTS

NetraAI, driven by its Attractor AI algorithms, is a machine learning (ML) system, offering actionable insights into patient subpopulations. Its unique capability lies in extracting valuable knowledge from small datasets that are truly generizable. NetraAI avoids overfitting by identifying which patients it can learn from and derives “Causal Clusters” of variables. It generates hypotheses, statistically significant collections of these causal clusters of variables along with the identified subpopulations of patients. This can be used by physicians to explore oncology patient subpopulations, discover novel drug targes, identify study confounds, and understand heterogeneous outcomes.

OBJECTIVES

We seek to identify variables that explain glioma patient outcomes. To achieve this objective, we aim to:

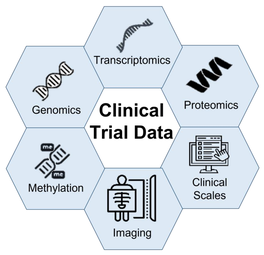
- Leverage the exceptional capabilities of Attractor AI to *decompose patient populations into explainable and unexplainable segments.* The process is used to generate hypotheses to explain what might impact patient outcomes and to help personalize clinical trials through enrichment.
- Identify key variables derived from the hypotheses generated, which serve as guidance to identify novel therapeutic targets and provide exclusion/inclusion criteria for clinical trials.

METHODOLOGY

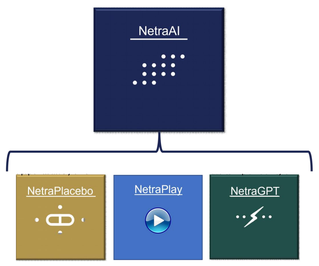
NetraMark Workflow

Generative Clinical Trial Decision Support

1 Data Preparation & Ingestion



2 NetraMark Analysis



3 Insight Delivery

4 Clinical Trial Implementation

DATASETS:

Oligodendroglioma:

- mRNA expression arrays

Glioma:

- Methylation data

AI APPROACH:

A unique *long-range memory mechanism reminiscent of attention mechanisms in LLMs* is utilized to cut through the monstrous number of combinations of multiple variables, facilitating the generation of hypotheses regarding oncology patient subpopulations in the context of patient disease outcomes.

HYPOTHESIS GENERATION:

Hypotheses produced are a composite of various variables that describe patient disease outcomes to provide explanations for underlying driving mechanisms behind each subpopulation. These hypotheses help identify which patients are explainable to improve OG patient outcomes through a precision medicine approach. These distinctive features allow for accurate and transparent definitions of patient subpopulations that provide a necessary tool for clinical trial success and risk mitigation.

RESULTS

NetraAI Generated Hypothesis about HPV from Oligodendroglioma Transcriptomic Data

NetraAI identified 3 subpopulations of OG patients based on survival and differentially expressed genes

Dataset consisted of: 156 primary OG tumors, 14 primary glioma samples, 9 normal samples

Examining low- versus high-grade OG, we identified two subpopulations:

- 26 low-grade and 21 high-grade OGs characterized by higher *RPS6KB1*
- 6 low-grade and 61 high-grade OGs characterized by higher *LFNG*

Examining *1p/19q* co-deletion OGs, we identifid a third subpoppulation:

- 60 no-codeletion, and 3 co-deletion OGs characterized by higher *HDAC1*

NetraGPT

The NetraGPT Module: LLM Integration with Attractor AI offered the following linking OG and HPV

***HDAC1* (Histone Deacetylase 1)**: involved in chromatin remodeling and gene expression regulation through histone deacetylation, which leads to a closed chromatin structure and transcriptional repression. It is vital role for cell cycle progression and differentiation.
Hypothesis: *HDAC1* may contribute to OG pathogenesis by dysregulating gene expression critical for cell cycle control. HPV oncoproteins E6 and E7 may alter *HDAC1* activity, promoting tumorigenesis by maintaining cells in a proliferative state.

***LNFG* (Lunatic Fringe):** glycosyltransferase that modulates the Notch signaling pathway, which is crucial for cell differentiation, proliferation, and apoptosis. Aberrations in Notch signaling are associated with various cancers.
Hypothesis: *LFNG* may interact with HPV infection, potentially enhancing environments conducive to oncogenesis through dysregulated Notch signaling. *LFNG* mutations or altered expression could synergize with HPV infection to disrupt normal cell cycle checks, promoting OG development.

***RPS6KB1* (Ribosomal Protein S6 Kinase B1)**: serine/threonine kinase involved in the PI3K/Akt/mTOR pathway, which regulates cell growth, survival, and metabolism. It is often overexpressed in various cancers and associated with aggressive tumor behavior.
Hypothesis: *RPS6KB1* may facilitate OG progression when modulated by HPV infection. HPV oncoproteins may hijack the PI3K/Akt/mTOR signaling cascade through *RPS6KB1* to promote uncontrolled cell growth and survival, providing a direct mechanistic link to tumor development and progression.

Integrative Hypothesis Combining All Elements
HPV could potentially contribute to the molecular etiology of OG by creating a permissive environment for mutations or by altering gene expression patterns directly through interactions with these critical regulatory pathways. This hypothesis provides a conceptual framework for exploring the potential oncogenic mechanisms driven by the combination of HPV infection and the alteration of specific genes in the development of OG. Further research would be needed to validate these hypothesized mechanisms.

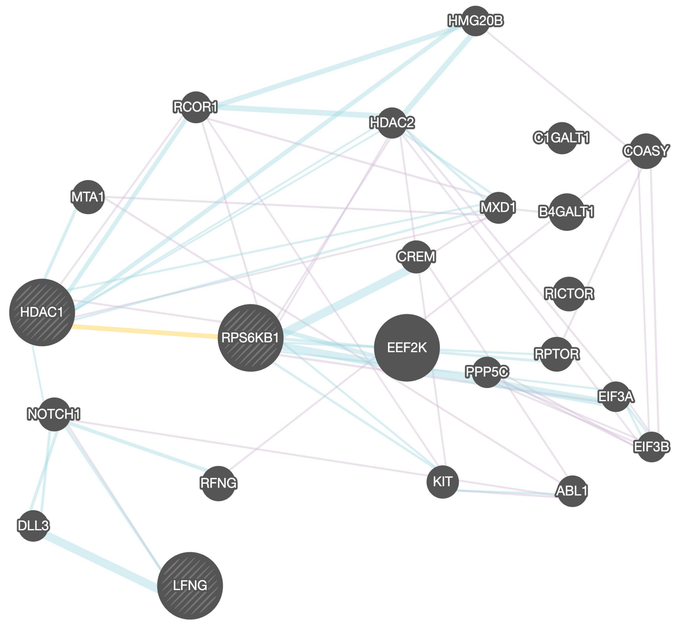


Figure 1. Protein-protein interaction network of *RPS6KB1*, *LFNG*, and *HDAC1*. Blue represents shared pathway, purple represents co-expression, and orange represents physical interaction. Created with GeneMania.

Validating HPV Link on Glioma Methylation Data

NetraAI extrapolated the HPV-glioma link using methylation data to identify 5 differentially methylated genes in IDH-mutated patients

Two datasets:

- 293 lower-grade glioma patients
- 1122 pan-glioma (grade II-IV) patients

Combined to form a comprehensive dataset consisting of 514 glioma patients with *IDH* mutation with and without *1p/19q* co-deletion.

NetraAI has the potential to learn beyond the labels given, while also explaining the driving factors identified. Data was labeled as wild-type or mutated. Each subpopulation identified is explainable by a combination of methylation measures that provide a precise characterization without further prompting.(**Figure 2**)

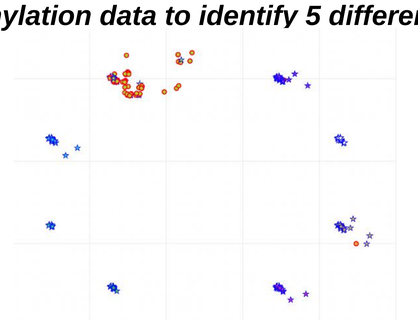


Figure 2. NetraAI is able to learn about subpopulations of patients that go beyond the dependent variables. Here you can see NetraAI organizing patients into various wild-type subpopulations.

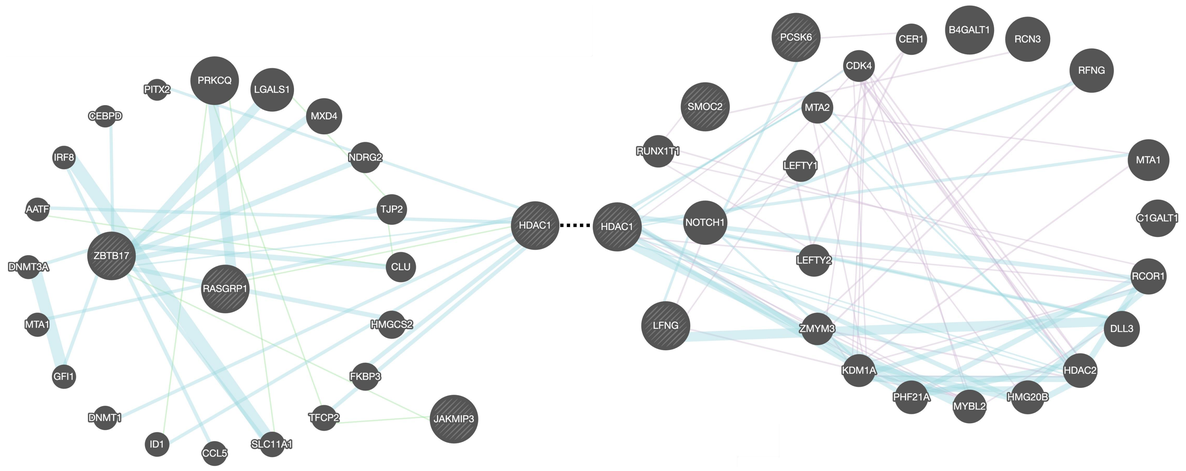


Figure 3. NetraAI identified extrapolated HPV association to a new glioma methylation dataset.

NetraAI identified 5 differentially methylated genes connected to HPV **(Figure 3):**

- *JAKMIP3* → *E6/E7*
- *PCSK6* → *MKI67*
- *ZBTB17* → *MKI67*
- *RASGRP1* → *CCND1*
- *SMOC2* → *HER2/Neu*

Our findings from the first dataset, which was transcriptomic data from a set of OG patients, led us to believe that HPV could be playing a role in cancer initiation and progression. NetraAI generated this hypothesis due to the enrichment of the HPV pathway from differentially expressed genes, (*HDAC1, LFNG, RPS6KB1*).

We applied NetraAI to an independent methylation glioma dataset to explore if any of the generated hypotheses about the patient populations coincided with any genetic players that would implicate HPV.

A novel set of candidates were found to be differentially methylated and tied to know genes affected by HPV, namely *HDAC1*, *MKI67*, *CCND1*, and HER2/Neu as well as HPV oncoprotein *E6/7*. **Figure 3** demonstrates how these genes may potentially cause alterations in the molecular machinery that drives certain subtypes of brain cancers.

CONCLUSIONS & SIGNIFICANCE

SUMMARY OF KEY FINDINGS:

1. Identifying a link between OG and HPV

- 3 genes and their associated pathways (*HDAC1, LFNG,* and *RPS6KB1) were* identified as potential indicators of HPV involvement

2. Validating the link between gliomas and HPV

- 5 differentially methylated genes were found in the glioma dataset (*JAKMIP3, PCSK6, ZBTB17, RASGRP1,* and *SMOC2),* which were found to be linked to established HPV mechanisms

CLINICAL TRIAL SIGNIFICANCE OF METHODOLOGY & FINDINGS:

This research underscores the powerful synergy of Attractor AI and LLMs in dissecting patient population outcomes in clinical trials. Identifying causal variables ensures optimized participant selection, fortifying trial efficacy. By providing clear decompositions of patient populations ***into explainable and unexplainable subpopulations***, we can derive generalizable insights that can help evaluate the chance of success of future trials from past trial data, and simultaneously provide exclusion and inclusion criteria to enhance endpoints.

FUTURE USE AND APPLICATIONS:

Using variables derived from *explainable subpopulations,* as identified through this hypothesis generation technology, to impact clinical trial enrichment with actionable inclusion/exclusion enrichment criteria and to provide novel drug targets.

REFERENCES

1. Kamoun, A. *et al.* Integrated multi-omics analysis of oligodendroglial tumours identifies three subgroups of 1p/19q co-deleted gliomas. *Nat Commun* **7**, (2016).
2. Vidone, M. *et al.* Evidence of association of human papillomavirus with prognosis worsening in glioblastoma multiforme. *Neuro Oncol* **16**, 298 (2014).
3. Yeo-Teh, N. S. L., Ito, Y. & Jha, S. High-Risk Human Papillomavirus Oncogenes E6 and E7 Target Key Cellular Pathways to Achieve Oncogenesis. *Int J Mol Sci* **19**, (2018).
4. Bazzoni, R. & Bentivegna, A. Role of Notch Signaling Pathway in Glioblastoma Pathogenesis. *Cancers (Basel)* **11**, (2019).
5. Yang, S. *et al.* HPV-related methylation-based reclassification and risk stratification of cervical cancer. *Mol Oncol* **14**, 2124 (2020).
6. Dil, B. *et al.* Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* **372**, 2481–2498 (2015).
7. Ceccarelli, M. *et al.* Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell* **164**, 550–563 (2016).
8. Liebermann, D. A., Hoffman, B. & Vesely, D. Cell Cycle p53 Induced Growth Arrest versus Apoptosis and Its Modulation by Survival Cytokines Extra View p53 Induced Growth Arrest versus Apoptosis and Its Modulation by Survival Cytokines. *Cell Cycle* **6**, (2007).
9. Chen, W. S. *et al.* CDKN2A copy number loss in HPV- and HPV+ head and neck cancer to indicate poor prognosis: An integrated genomic and clinical TCGA analysis. **35**, 6060–6060 (2017).
10. Moravabdi, D. *et al.* The human papillomavirus type 16 E7 oncoprotein targets Myc-interacting zinc-finger protein-1. *Virology* **422**, 242 (2012).
11. Zeng, A. *et al.* IDH1/2 mutation status combined with Ki-67 labeling index defines distinct prognostic groups in glioma. *Oncotarget* **6**, 30232 (2015).
12. Novotný, J. *et al.* Analysis of HPV-Positive and HPV-Negative Head and Neck Squamous Cell Carcinomas and Paired Normal Mucosae Reveals Cyclin D1 Deregulation and Compensatory Effect of Cyclin D2. *Cancers (Basel)* **12**, 792 (2020).
13. Fernandes, A., Bianchi, G., Feltri, A. P., Pérez, M. & Corrent, M. Presence of human papillomavirus in breast cancer and its association with prognostic factors. *Ecancermedicalscience* **9**, (2015).