Hypoxia modulates tumor progression by controlling cell cycle proliferation and immunogenicity at the same time

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INTRODUCTION

Neuroblastoma is a pediatric cancer of the peripheral nervous system. Due to abnormal cell division rates, cancer cells increase oxygen consumption and decrease its availability at the tumor microenvironment. In neuroblastoma, hypoxia is thought to induce dedifferentiation, a phenomenon thought to drive tumor aggressiveness (Jogi 2002). Therefore, great interest is involved in studying mechanisms underlying hypoxia-driven aggressiveness in neuroblastoma.

The biological mechanisms mediating tumor aggressiveness in neuroblastoma are not completely understood. Pediatric cancers are thought to be driven by epigenetics rather than tumor mutation. Our group has investigated an epigenetic mechanism mediated by TET1 enzyme and deposition of 5-hydroxymethyl-cytosine (5-hmC), a marker of gene expression, identified in neuroblastoma cells exposed to hypoxia (Mariani 2014; Hains 2022). The hypoxia-induced TET1 deposition of 5-hmC was suggested to characterize a cell line transition or plasticity mechanism, triggered by hypoxia and impacting the two main neuroblastoma cell populations: ADRN and MES cells. Specifically, it was hypothesized that hypoxia mediates ADRN to MES (AMT), but not MES to ADRN transition (Chaves 2023, in preparation).

ADRN cells have neuronal-like features, are faster-growing and comprise the bulk of tumors, whereas MES cells are stem-like and thought to associate with resistance to chemotherapy and immunotherapy. It was suggested that hypoxia causes a change in the biological identity of the ADRN cell population using 5-hmC deposition, transitioning to a more MES state maintained under hypoxia (Chaves 2023, in preparation). It is accepted that MES cells are responsible for mechanisms of minimal residual disease and relapse of neuroblastoma (cite) after chemotherapy. Therefore, hypoxia represents an important mechanism mediating cell transition that leads to metastasis.

The MES cellular state induced by hypoxia caused global and local 5-hmC depositions on MES genes of the proinflammatory TNFA pathway, notably the STC1 gene. Surprisingly, this change paradoxically correlated with both improved overall survival and worse histology (TARGET dataset 149 samples). This information was paradoxical because the MES genes and the highlighted 5-hmC deposition peaks in the example of STC1 gene, present in the TNFA pathway had such correlation with positive and negative outcomes. This has caused us to be interested in investigating the correlation between HIF1A and outcome in different neuroblastoma (the Discovery and Validation) cohorts.

Our findings correlate the hypoxia state with the MES cell state indicative of an inflammatory state, showing that the inflammatory state is reached with the participation of hypoxia and/or HIF1A and the other hypoxia transcription factors. Furthermore, it was suggested that hypoxia induces TNFA signaling via NFKB (Chaves 2023, in preparation), an important process for inflammation (Liu 2017).

Hypothesizing the existence of a time-dependent relationship between hypoxia/MES gene set, it may be possible to understand the relationship between MES-ness and hypoxia over time, as a function of risk (the relationship of time and risk are not clear in this statement: check). The MES cell state, while highly related to the immunological phenomenon of ADCC triggered by dinutuximab according to Mabe 2022, renders MES cells unable to express GD2, important in dinutuximab immunotherapy.

At the same time, HIF1A is thought to be associated with T-cell exhaustion (Baldominos 2022) and fundamental for cell-cycle arrest under hypoxia (Goda, 2003). It was suggested that cancer cells in the quiescent, non-proliferative state, have reduced antigen in the cell surface, a phenotype controlled by HIF1A (Baldominos 2022). Tumors that loose targeted antigens do not need any other means to escape from T cell attack (Baldominos 2022). Emens et al., (2021) and Baldominos et al., (2022) suggested cell proliferation, a surrogate for HIF1A inhibition and cellular differentiation, to be a marker of patients that responded to ICB. Furthermore, an interplay between c-Myc and HIF1A that maintains cell-cycle arrest (Druker 2021), via increased p21 levels (Koshiji 200X), where HIF1A controls the highly demanding need for energy for the cell cycle to happen (Druker 2021). Therefore, from this information, it follows that hypoxia and HIF1A have a deep impact on immunological mechanisms in neuroblastoma, contributing to tumor progression.

Altered proliferation, increased DNA damage repair mechanisms and reduced apoptosis are hallmarks of anticancer drug resistance (Cree and Charlton 2017). Cell adhesion also has a role in chemotherapy resistance (Alimbetov 2018). Consider the Von Groningen 2017 paper for the MES cell enrichment upon chemotherapy treatment and potential involvement with hypoxia.

These findings caused us to propose a model where genes modified by hypoxia have a role on cell wall permeability of blood vessels as part of the inflammatory process, potentially influenced by time. This is a hypothesis and we have not yet conducted an experiment to follow-up on this hypothesis.

2. OBJECTIVES

2.1. General

Our goal is to develop precision oncology algorithms to quantify phenotypes that drive progression and aggressiveness of neuroblastoma tumors, using gene expression data from cell lines isolated from high risk neuroblastoma patients.

2.2. Specific

To investigate the nature of hypoxia signaling in neuroblastoma cells: positive and negative outcomes in the discovery and validation neuroblastoma cohorts, by means of the TET1 induction of 5-hmC deposition triggered by hypoxia in the ADRN to MES transition in neuroblastoma . Correlate neuroblastoma phenotypes hypoxia, HIF1A expression, T-cell exhaustion, to the high risk variable, survival (OS and/or EFS) and risk Hazards Ratio (Cox analysis). Write a machine learning algorithm to evaluate the effect of hypoxia in survival of high risk neuroblastoma patients profiled by 5-hmC deposition in their TE elements in cfDNA.

3. MATERIALS AND METHODS

General pipeline for the machine learning algorithm development was based on figure 1 of Rabiei et al. (2022), Nemade and Fegade (2023) and Khalid et al. (2021). For an integration of deep learning on cancer diagnosis, prognosis and treatment selection, please see Tran et al. (2021). Another highly cited feature selection paper was Akay (2009).

3.1. Phenotype quantification

Eight gene sets were used to characterize the impact of hypoxia on high risk, survival, hazard ratio and cumulative hazard ratio.. , to quantify the correlation between gene set signatures in tumors.

These algorithms were developed in precision oncology to identify processes driving progression and aggressiveness of neuroblastoma tumors, using gene expression data from cell lines isolated from high risk neuroblastoma tumors.

3.2. Phenotypes contributing to the risk category

We will determine the most important variables ot predict high risk in neuroblastoma (Sato et al. 2019). Although the down regulated genes had a significant impact on the separability of the low risk and high risk categories, the 635 gene set did not improve that ability significantly (Figure 1). However, this gene set was significant in the survival analysis of the high risk group after the multiple test correction (Figure 6). The 635 gene set did not improve the sensitivity of the method (Table X), XX % with and XX % without this group as a selected feature.

3.3. Principal Component Analysis

Impact of feature on label outcome was evaluates using the Scree Plot of Principal Component Analysis. Feature selection was revised by Chellappa and Turaga (2022). For high profile paper about risk prediction, see Placido et al. (2023). For methods and feature selection, please see Fakoor et al. (2013). Feature selection can help in the design of gene expression panels for early cancer detection. Recent advances have made it possible to use machine learning and feature selection for detection and early detection of imperceptible cancers such as cancers with elusive symptomes or tumors that have challenging diagnostic criteria Metcalf (2024). Our aim is for this framework to be used as a feature selection tool for oncologic panels for diagnostic selection criteria as in Costa et al. (2023).

3.4. Correlation matrix to eliminate redundant features:

ADRN Norm vs Hypo Down (635) gene set was removed because the Scree plot showed a high impact of the other decreasing proliferation gene sets. Therefore in this part several pipelines can be proposed on how to choose a threshold of the correlation to remove features that do not increase specificity of the label of the sample.

3.4. Development of machine learning framework for risk classification and diagnosis

Development of a framework may help the implementation of liquid biopsy protocols for management and therapeutics of oncologic patients. To determine parameters contributing to neuroblastoma risk classification, we constructed a platform for visualization of phenotypes driving tumor progression using R version 4.1.1. (http://www.r-project.org) and the Shiny and Caret packages. The first step for the platform construction was to establish a algorithms for supervised learning and classifiers as well as their hyperparameters. Algorithms tested included Logistic Regression, Random Forest, Neural Network, Supporting Vector Machines and Deep Learning were used to construct a non-linear classification model.

Variable importance, correlation plots and PCA components will be visualized using the Shiny package in R.

4. RESULTS

4.1. Principal Component Analysis of features contributing to survival and high risk labels

In this part we used PCA to determine which features are most relevant to risk and or survival. Which features contribute the most to the risk category and the MYCN category. After that, we will plot some correlations between the features, for example inflammation and hypoxia. Then there can be a plot comparing the scores of the phenotype comparing the high and non-high-risk categories. Finally, we can draw a couple of survival analysis to compare the scores of the phenotypes. It is surprising that in the plot of Figure 1, we did not have an effect of hypoxia down-regulation in determining an increase in the specificity of the machine learning algorithm to separate the low risk and high risk categories (Figure 1).

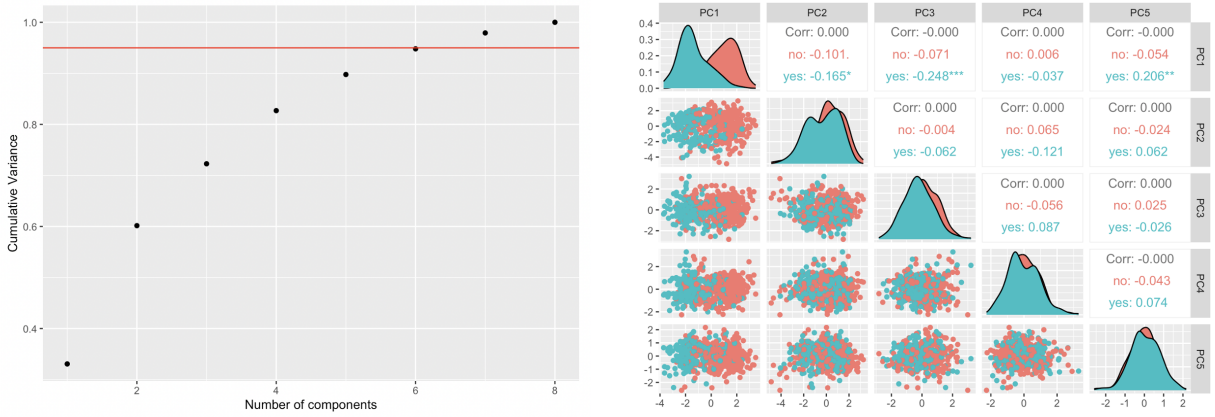

Figure 1: Cumulative variance and combined principal components to evaluate the role of 8 gene set phenotypes in differentiating the neuroblastoma high risk group.

4.2. High risk neuroblastoma is characterized by increased quiescence and low inflammatory activities correlated with hypoxia markers

High risk classification of neuroblastoma represents the better prognostication system available for the disease. Patients with less than 50% 5 years event-free survival are classified as high risk patients (Cohn et al., 2009). To evaluate the correlation of hypoxia phenotype scoring with high-risk neuroblastoma, we evaluated the hypoxia gene-sets comparing the low and high risk groups. High risk patients showed a trend to have increased scores for hypoxia phenotypes that decrease cellular proliferation evaluated in the Discovery cohort (Figure 2).

Considering the dual phenotypic effects of hypoxia, one in the genes that are activated by the hypoxia signal and the other on genes that are down-regulaeted, we evaluated the correlation of inflammation phenotypes with the high-risk variable. The high-risk group presented decreased inflammatory response events than the low risk group (Figure 2). Together these results show a trend in which hypoxia modulates genes by activation and inhibition of gene expression (Figure 2). Hypoxia-activated genes seem to activate inflammation whereas hypoxia-inhibited genes seem to decrease cell-cycle proliferation (Figure 2).

Now, I will plot the combined plot:

Now, I will plot ADRN Norm vs Hypo Down (635) plot:

4.3. Predictive accuracy for each neuroblastoma high risk classifier

Table X shows the predictive accuracy and area under the curve of each classifier using different types of machine learning algorithms Sato et al. (2019). We started with Linear regression model and found a Receiver Operating Characteristic (ROC) Curve of 91%. Sensitivity and Specificity were 88 and 78% respectively when createDataPartition(df3_nb$high_risk, times = 1, p = 0.67, list = FALSE).

Table X.: ROC, Sensitivity and Specificity for the Logistic Regression Model. ROC Sens Spec
0.919246 0.8895238 0.777381

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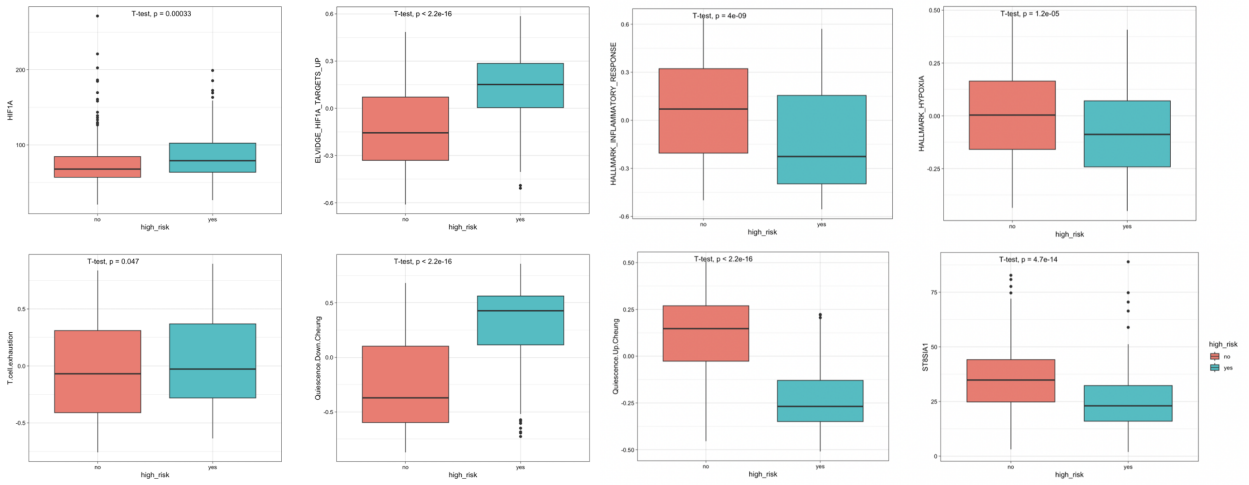

Figure 2: Correlation between cell cycle phenotypes impacted by hypoxia and the high risk variable. A) HIF1A and B) HIF1A gene targets and C) T-cell exhaustion and D) quiescence as defined in Cheung (2019) were all associated with higher scores in high risk patients in the discovery cohort with similar trends observed in the validation cohort. Correlation between inflammatory phenotypes impacted by hypoxia and the high risk variable. A) Hallmark inflammatory response, B) hallmark hypoxia, C) quiescence as defined in Cheung 2019 and D) ST8A1 were all phenotypes associated with higher scores in high risk patients. Data shown is for the discovery cohort and similar trends were observed in the validation cohort.

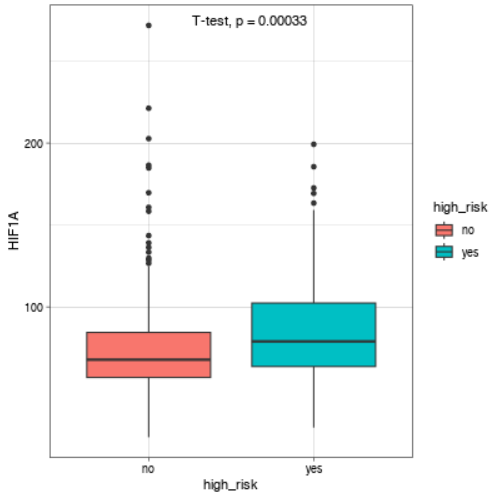

Figure 3: Correlation between cell cycle phenotypes impacted by hypoxia and the high risk variable. A) HIF1A and B) HIF1A gene targets and C) T-cell exhaustion and D) quiescence as defined in Cheung (2019) were all associated with higher scores in high risk patients in the discovery cohort with similar trends observed in the validation cohort.

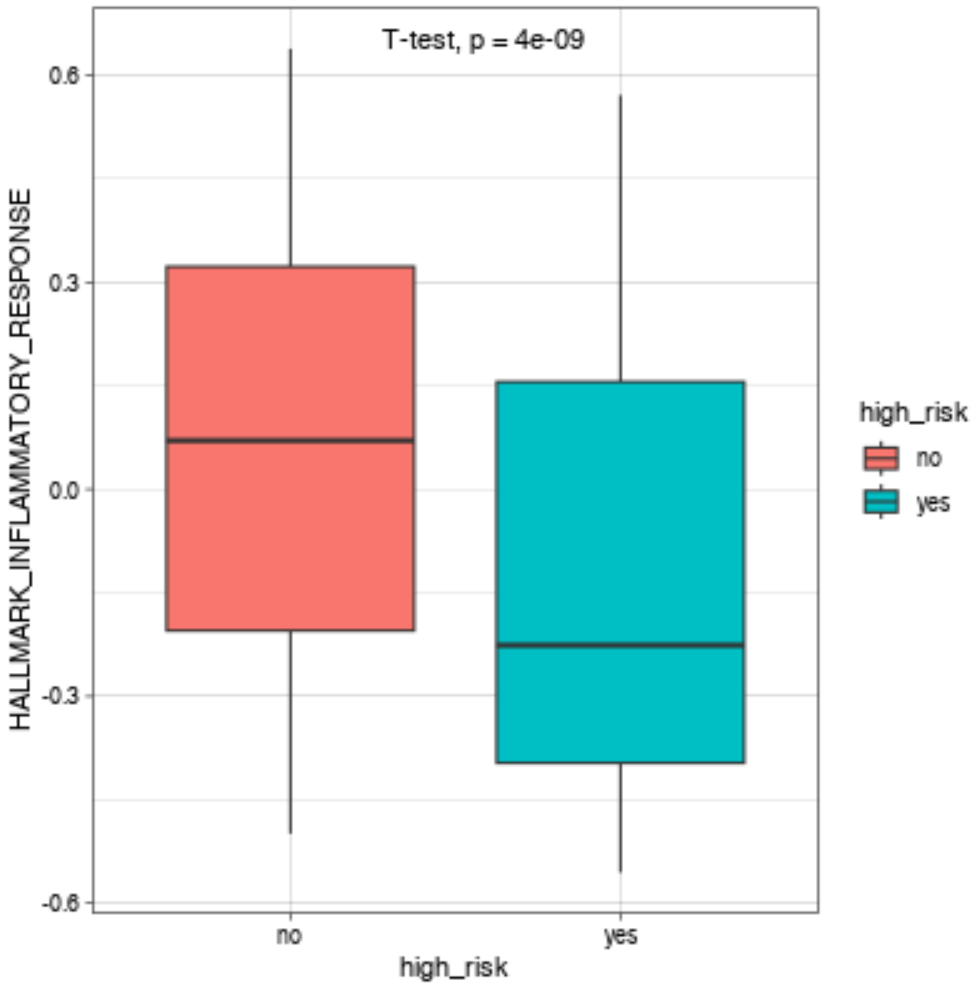

Figure 4: Inflammation.

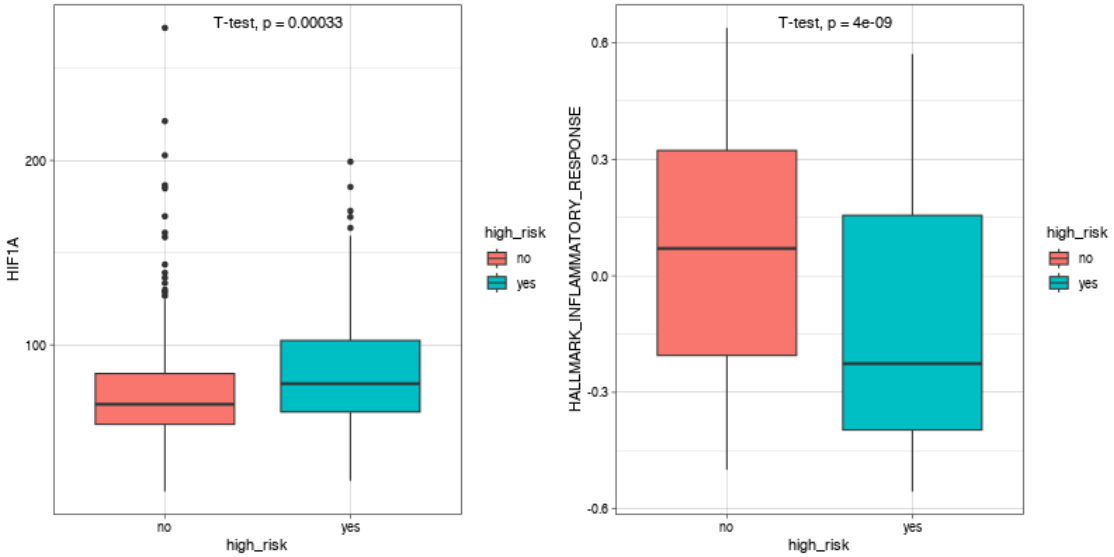

Figure 5: Inflammation.

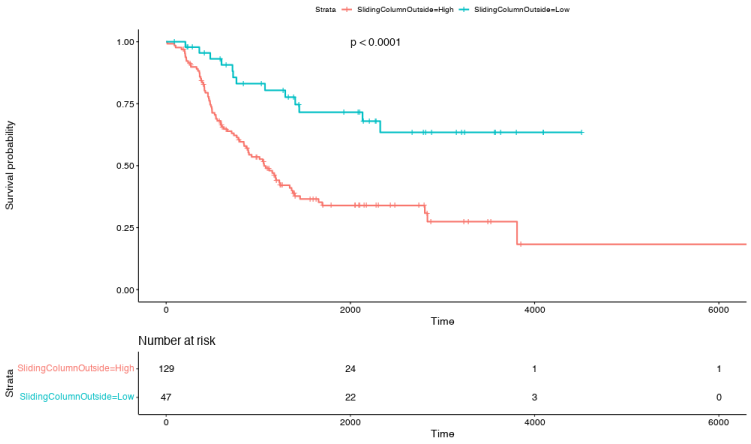

Figure 6: Survival analysis of ADRN Norm vs Hypo Down (635) after multiple test correction.

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