The Prognostic Significance of Glucose-6-Phosphate Dehydrogenase as a Biomarker in Head and Neck Squamous Cell Carcinomas treated with Conventional Chemoradiation.

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Introduction

The prognosis of patients with head and neck squamous cell carcinoma (HNSCC) treated with chemoradiation can be predicted using p16 as a surrogate biomarker of Human Papilloma Virus (HPV) status, but a subset of patients continues to do poorly despite a positive or negative p16 status. This project attempted to identify another biomarker, glucose-6-phosphate dehydrogenase (G6PD) as a marker for prognosis in HNSCC patients.

Glucose 6 phosphate dehydrogenase (G6PD) has been suggested to play an important role in tumor cell proliferation and therapeutic resistance due to G6PD's direct involvement in a cell's nucleotide synthesis and reduction of reactive oxygen species (ROS)[1]. G6PD catalyzes the first step of the oxidative pentose phosphate pathway (PPP), which produces both ribulose 5 phosphate used for nucleotide synthesis, and nicotinamide adenine dinucleotide phosphate (NADPH) used to protect cells from ROS[1,2].

Due to the critical role of G6PD in tumor cell proliferation and therapeutic resistance, and limited data on G6PD expression in HNSCC, this project aimed to investigate the utility of G6PD for use as a potential prognostic biomarker in head and neck squamous cell carcinomas (HNSCC) to further guide treatment plans in this subset of patients.

Aims and Objectives

Aim 1: Acquire histological blocks from the Royal Oak Pathology Archive and create two tissue microarrays (TMA).

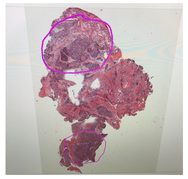
Aim 2: Immunohistochemically stain the TMAs for G6PD and analyze the level of G6PD expression.

Aim 3: Correlate G6PD staining analysis with clinical outcome parameters such as local control, disease-free survival and overall survival.

Methods

1. All clinical research was conducted through IRB protocol through Beaumont. Patient's were identified from a radiation oncology database, and a list of 65 suitable patients and their pathology numbers were presented to pathology to retrieve blocks.

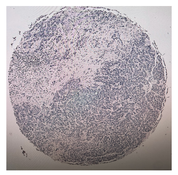
2. The area of tumor within each sample was identified under microscopy. Two core samples taken from the tumor area of each sample was used to generate a tissue micro array containing a duplicate set of core samples from each patient's pathological sample.



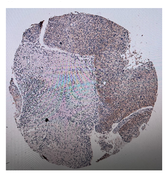
2. Thin slices from each TMA were then deparaffinized and ran through the following immunohistochemistry protocol:

- Warmed tissue slides in hot buffer to expose antigen sites within tissue.
- Incubated slides in 3% H2O2 to block endogenous tissue peroxidase.
- Incubated slides in CAS block to prevent non-specific antibody binding.
- Incubated slides in anti-rabbit G6PD antibody.
- Incubated in horseradish peroxidase (HRP) anti-rabbit secondary antibody to bind to anti-rabbit G6PD.
- Incubated in DAB. DAB oxidation is catalyzed by HRP to form a light brown precipitate.

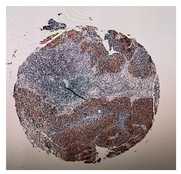
3. The level of expression of G6PD in each core sample was then visualized using 10x microscopy. Scoring on the intensity of the expression was done on a scale from 0-4 with 0 being no expression and 4 being high expression.



Core sample with low intensity of G6PD expression.



Core sample with intermediate intensity of G6PD expression.



Core sample high intensity of G6PD expression.

4. The intensity score was then multiplied by the percent area within the core exhibiting expression to generate a core score, and then the percent area within only the tumor portion of the core to generate a tumor score.

5. Samples were then classified as either high G6PD expression or low G6PD expression with the median as the cut off point in the following different analysis techniques:

- Calculating the average core score between each patient's duplicate samples.
- Taking the highest core score between each patient's duplicate samples.
- Calculating the average tumor score between each patient's duplicate samples.
- Taking the highest tumor score between each patient's duplicate samples.

6. Each patient's clinical outcome of overall survival time, time to local recurrence, and time to distant metastasis was found through retrospective chart review. These outcomes were then correlated with the differing analysis to determine G6PD expression to generate five Kaplan Meir survival plots for each respective clinical outcome using JMP 11.20 SAS software.

Results

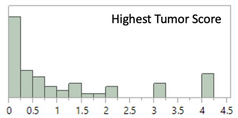


Figure 1 Distribution of tumor scores using the highest graded score of the two duplicate samples.

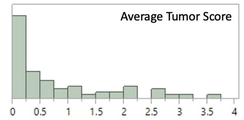


Figure 2 Distribution of tumor scores using the average graded score of the two duplicate samples.

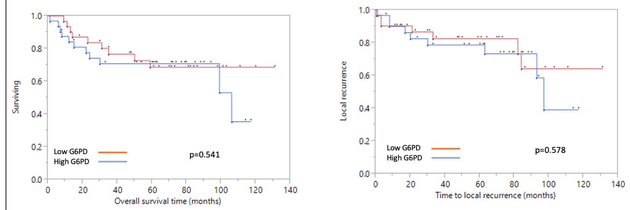


Figure 3 Analysis using median highest tumor score. Low = 0 to 0.3 High = 0.4 to 4.0. An event occurring is represented on the K-M plots by a drop in the graph. The dotted lines represent a patient who did not have an event occur as of the last recorded follow up for that patient. All three KM-plots were statistically insignificant.

G6PD score cutoff score	Overall survival time p- value	Time to local recurrence p-value	Time to distant metastasis p-value
Median highest tumor score Low = 0 to 0.3 High = 0.4 to 4.0	0.541	0.578	0.806
Three cut-offs of highest tumor score Low = 0 to 0.225 Intermediate = 0.3 to 1.8 High 2.0 to 4.0	0.457	0.417	0.285
Median average tumor score Low = 0 to 0.25 High = 0.3 to 3.5	0.511	0.264	0.578
Median highest core score Low = 0 to 0.3 High = 0.4 to 4.0	0.279	0.132	0.967
Median average core score Low = 0 to 0.2 High = 0.3 to 3.35	0.784	0.562	0.823

Table 1 Analysis for each cutoff parameter used yielded statistically insignificant results as seen in the p-values shown in the table.

Conclusions

The level of G6PD expression in each HNSCC tumor core sample did not correlate with clinical outcome or with p16 status which does not support the use of G6PD as a prognostic biomarker in HNSCC patients treated with conventional chemoradiation.

Cancer cells frequently are found to rewire metabolic pathways to favor enhanced growth, proliferation, and survival. The shift from aerobic to anaerobic metabolism describe by the Warburg effect also creates an increased activity in the PPP to protect cells from resulting ROS formation[9]. The interplay between the anaerobic glycolytic pathway and PPP found in tumor cells presents an array of potential metabolic biomarkers with potential prognostic value.

While the results from this project suggest G6PD has limited to no prognostic value in HNSCC patients, further investigation into other potential metabolic biomarkers is warranted to continue furthering more personalized treatment plans for better clinical outcomes in patients suffering from malignancy.

References

1. Zhang C, Zhang Z, Zhu Y, Qin S. Glucose-6-phosphate dehydrogenase: a biomarker and potential therapeutic target for cancer. *Anticancer Agents Med Chem*. 2014;14(2):280-289. http://www.ncbi.nlm.nih.gov/pubmed/24066844. Accessed December 29, 2018.

2. Tian WN, Braunstein LD, Pang J, et al. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. *J Biol Chem*. 1998;273(17):10609-10617. doi:10.1074/JBC.273.17.10609

3. Ghanbari Movahed Z, Rastegari-Pouyani M, Mohammadi M hossein, Mansouri K. Cancer cells change their glucose metabolism to overcome increased ROS: One step from cancer cell to cancer stem cell? *Biomed Pharmacotherapy*. 2019;112:108690. doi:10.1016/J.BIOPHA.2019.108690

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