Immunotherapy meets epigenetics: Gp96-Ig cellular vaccine genetic signature is modulated by the inhibition of histone modifications

George Fromm[1], Sara Selitsky[2], Neal Schilling[1], Jason Rose[1] and Taylor H. Schreiber[1]

[1]Heat Biologics, Inc., Durham NC, [2]University of North Carolina Chapel Hill, Chapel Hill NC

Abstract

A number of novel cancer treatment strategies have been identified over the last decade with hopes of eradicating a diverse range of cancers, however the molecular mechanisms behind patient immune responses and tumor microenvironment signaling are complex, and have often hindered the translation of these ideas into successful clinical outcomes. Two modalities at the forefront of these potential blockbuster strategies are combinatorial immunotherapy and epigenetic manipulation. These approaches are supported by compelling clinical data, but have been encumbered by a number of factors including: adverse event profiles, toxicity due to non-specific small molecule interactions, limited overlap in patient/therapy antigen presentation, and exorbitant cost of biological treatments.

We have engineered allogeneic cellular vaccines tailored to specific cancers, that secrete heat-shock protein gp96-Ig and achieve high-frequency CD8+ T cell responses to tumor antigens that overlap between patients and our vaccine.

To determine this overlap across the entire human transcriptome, we carried out RNA-sequencing of our bladder cancer vaccine cell line Vesigenurtacel-L or HS410, along with several patient bladder biopsy samples, and performed novel cluster/correlation analysis on differentially expressed (DE) genes with over 400 existing bladder tumor datasets that are part of The Cancer Genome Atlas (TCGA) consortium.

This clustering generated interesting candidate gene targets, or biomarkers, that are specific to different bladder cancer subtypes, which may allow us to diagnose disease recurrence or predict whether patients will respond to our vaccine.

When put to the test, our genomic prediction of patient response was highly accurate and showed distinct trends for a patient that responded to our treatment when compared to a patient that did not. This response was confirmed by histology and gene expression analysis showing significant infiltration of CD8+ cytotoxic T cells in the tumor microenvironment of the 'responder', with no increase in CD4+ Helper T cells.

Our analysis also identified epigenetic regulators as a key group of genes significantly dysregulated in bladder cancer, and importantly we are able to influence the potency of our vaccine and alter tumor associated gene expression by modulating some of these epigenetic factors.

We are hopeful that these advances will allow us to develop next-generation vaccines capable of targeting all sub-types of a particular cancer, leading to maximum clinical response in patients.

Experimental Design

Samples

TCGA Normal Bladder, N=19
TCGA Bladder Tumor, N=408
HS410 Vaccine Cells, N=2
Patient 4 Week 0 (tumor)
Patient 4 Week 7 after treatment
Patient 5 Week 0 (tumor)
Patient 5 Week 7 after treatment
Patient 5 Week 21 after treatment

Novel Genomic Analysis →

Goals

-Identify DE genes and pathways enriched in bladder cancer
-Correlate DE genes between TCGA tumors, vaccine and patients
-Develop genomic tools to diagnose patient tumor subtypes and predict responders
-Manipulate current vaccine to increase its potency and maximize the number of responders and clinical success

Bladder Cancer/HS410 Vaccine Genomics

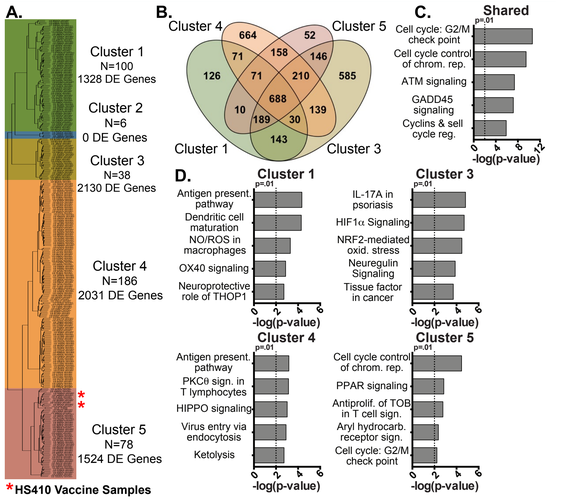


Figure 1. Clustering of bladder tumors reveals sets of shared and unique DE genes. (A) Dendogram: hierarchical clustering of 409 TCGA bladder tumors and 2 HS410 RNA-seq datasets. (B) Venn diagram showing shared and unique DE genes between clusters. (C)(D) Ingenuity Pathway Analysis (IPA) of enriched molecular pathways between shared and unique DE genes.

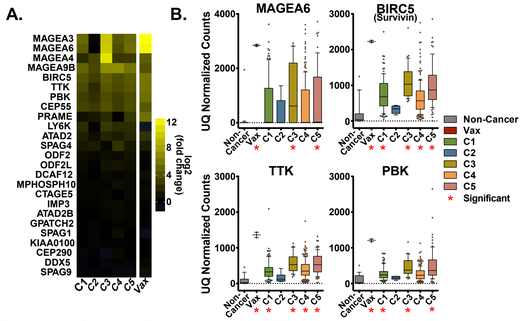
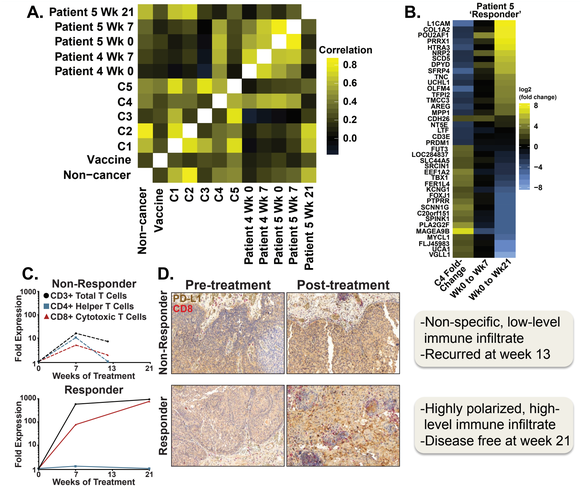


Figure 2. Cancer Testis Antigen (CTA) expression forms a genomic signature between clusters. (A) Heat map of select CTAs in clusters generated from 407 TCGA bladder tumors. (B) Upper-quartile (UC) normalized counts at CTAs show significant up-regulation compared to non-cancer.

Predicting Clinical Responders



-Non-specific, low-level immune infiltrate
-Recurred at week 13

-Highly polarized, high-level immune infiltrate
-Disease free at week 21

Figure 3. Genomic prediction of responders followed by histological analysis of actual response. (A) Spearman correlation matrix between TCGA, our HS410 vaccine and bladder biopsies from 2 patients at week 0 (tumor) and weeks 7 and 21 after tumor resection and start of HS410 therapy. ***Patient samples correlate best with cluster 4.** (B) Heatmap depicting the fold-change in the 20 most up- and 20 most- down regulated genes in cluster 4, showing anti-correlation with patient 5 from weeks 7 to 21, indicating a successful genetic response. (C) qRT-PCR showing an increase in CD3+/CD8+ T cells in responding (#5) vs. non-responding (#4) patient. (D) IHC showing an increase in CD8+ T cells in the responding patient (#5).

Epigenetic Traits Vary Based on Vaccine Response

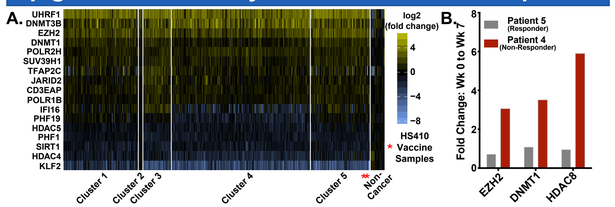


Figure 4. DE genes in bladder cancer are enriched with epigenetic regulators. (A) Heatmap depicting +/- fold-change values at epigenetic regulators. (B) Fold-change in RNA-seq values from week 0 (tumor) to week 7 after vaccine in patients 5 (responder) and 4 (non-responder) as in Fig. 3. Increase in epigigenetic gene expression in patient 4 is indicative of patient 4 regressing back to a cancerous state.

Increasing Vaccine Potency

Methods of HDAC Inhibition in HS410 Cells:

Epigenetic Targets	dsRNAi	Small Molecule Inhibitors (SMi)
HDAC1	dsHDAC1	**HDAC1:** CI994(1μM), SAHA(100nM)
HDAC3	dsHDAC3	**HDAC3:** RGFP966(500nM), SAHA(100nM)
HDAC8	dsHDAC8	**HDAC8:** PCI-34051(15μM)

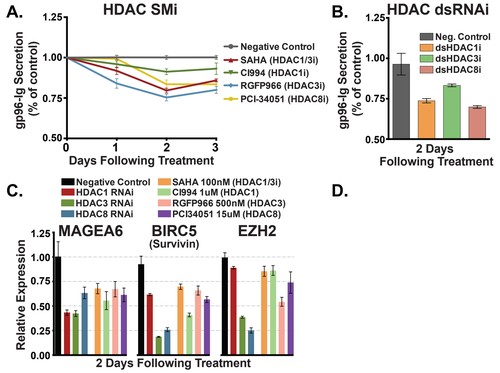


Figure 5. HDAC inhibition/knockdown attenuates gp96-Ig secretion and cancer specific gene expression. (A) HDAC specific small molecule inhibitors (SMi) and (B) dsRNAi decrease Gp96-Ig secretion as detected by ELISA. (C) HDAC SMi/dsRNAi lower the expression of genes normally found elevated in cancer, like CTAs (MAGEA6 & BIRC5) and epigenetic regulators (EZH2). (D) HDAC SMi/dsRNAi results in the inverse expression of genes associated with cluster 4.

Key Concepts

-We provide a comprehensive genomic analysis of the transcriptome in bladder cancer.
-Our unique clustering of 408 bladder cancer RNA-seq datasets allows us to identify genes and pathways that are both shared and unique between cancer subtypes and our bladder cancer vaccine Vesigenurtacel-L or HS410 .
-This clustering generated candidate gene targets, or biomarkers, which may allow us to diagnose disease recurrence or predict whether patients will respond to our vaccine.
-Our analysis also identified epigenetic regulators as a key group of genes significantly dysregulated in bladder cancer.
-Lastly, we provide exciting evidence that the potency of our vaccine can be influenced through epigenetic manipulation, providing a tool for fine-tuning cancer-specific gene expression to produce an advanced vaccine capable of targeting more patients.

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Contact: gfromm@heatbio.com