Determination of tumor PSMA expression in prostate cancer from blood using a novel epigenomic liquid biopsy platform

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BACKGROUND

- PSMA (Prostate-Specific Membrane Antigen), a highly expressed cell surface protein in prostate cancer, has emerged as a pivotal biomarker for both diagnosis and therapeutic targeting in patients with advanced disease.
- 177Lu-PSMA-617 is an FDA-approved radiopharmaceutical therapy directed against PSMA for men with metastatic castration-resistant prostate cancer (mCRPC).
- Treatment eligibility requires a PSMA PET scan to confirm tumor PSMA expression.
- With therapeutic strategies in development targeting an array of cell surface proteins, there is an emerging unmet need to quantify tumor drug target expression minimally invasively.
- We performed comprehensive epigenomic profiling on 1mL of plasma[1] and demonstrated an accurate, minimally invasive readout of tumor PSMA expression in mCRPC.

METHODS

- We collected plasma at the time of PSMA PET scan from 50 men with mCRPC and analyzed the PET images to quantify PSMA PET SUV_{mean}.
- We profiled genome-wide epigenomic signals across histone modifications associated with active enhancers, promoters and DNA methylation on these plasma samples and healthy controls.
- We performed a genome wide analysis of differential epigenomic signal between healthy volunteers and mCRPC patients to confirm detection of known prostate cancer biology.
- We performed a genome wide analysis of epigenomic signal that correlates with PSMA PET SUV_{mean} measurements to identify genes associated with PSMA PET SUV_{mean} in mCPRC patients.
- Plasma samples with detectable circulating tumor DNA (ctDNA, ≥3%) were used to train a multi-analyte model on *FOLH1* (PSMA) epigenomic signal to predict PSMA PET SUV_{mean}. Performance was assessed in a leave-one-out cross-validation (LOOCV) schema and in an independent validation cohort.

RESULTS

Figure 1. Comprehensive epigenomic platform offers dynamic resolution into target and pathway biology from 1mL of plasma

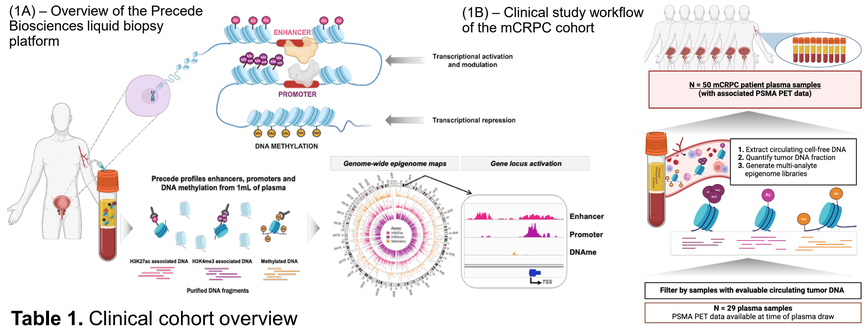
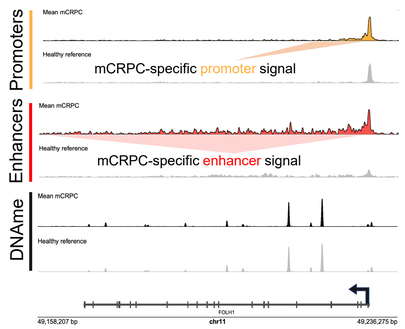


Table 1. Clinical cohort overview

COHORT DESCRIPTION	Total N = 29
Patient characteristics	
Age in years, median(range)	72.3 (53.2–85.7)
Race, number (%)	
White	27 (93.1%)
Black or AA	1 (3.5%)
Hispanic	1 (3.5%)
Disease characteristics	
Stage at Diagnosis, number (%)	
Localized	20 (69.0%)
Metastatic	9 (31.0%)
Sites of Disease on PSMA PET, number (%)	
Lymph Node	15 (51.7%)
Bone	23 (79.3%)
Lung	4 (13.8%)
Liver	6 (20.7%)
Other	4 (13.8%)
# of Prior Line of Systemic Therapies for Metastatic Disease, median (range)	4 (2–7)
PSA at Plasma in ng/mL, median (range)	162.3 (0–1435)
PSMA PET SUVmean, median (range)	6.2 (2.8–16.8)
Days from PSMA PET scan to plasma collection, median (range)	62 (-62–133)

(A) Cell free (cf)DNA derived from tumors exists in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against H3K27ac marking active enhancers, H3K4me3 marking active promoters, and DNA methylation are used to enrich for associated DNA fragments from 1mL plasma and sequenced to define genome-wide epigenomic maps that capture the underlying transcriptional state of the tumor cells. **(B)** Clinical study overview of mCRPC patient plasma samples evaluated in the current study.

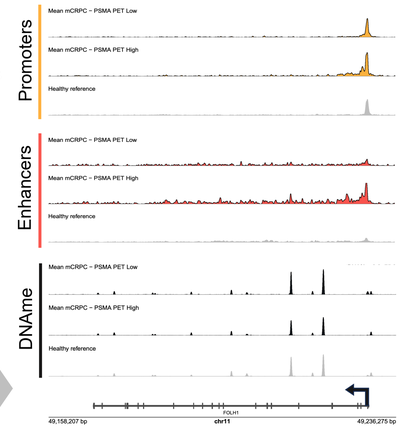
Figure 3. The *FOLH1* locus has robust enhancer and promoter signal in mCRPC patients compared to healthy volunteers



Epigenomic signal at the *FOLH1* locus shows increased active promoter and active enhancer marks in mCRPC patients compared to healthy volunteers. Enhancer, promoter, and DNA methylation signal in mCRPC patients was normalized, smoothed, and averaged together (within analyte) with the mean signal from a cohort of male healthy volunteers.

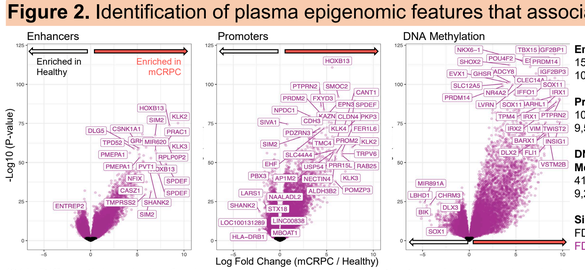
Group	N	Mean ctDNA%	Mean PSMA PET SUV_{mean}
mCRPC – PSMA PET Low	14	32 (3-75)	4.7 (2.8-6.1)
mCRPC – PSMA PET High	15	37 (5-78)	9.2 (6.2-16.8)

Figure 4. The *FOLH1* locus has higher enhancer and promoter signal in patients with higher PSMA PET SUV_{mean}

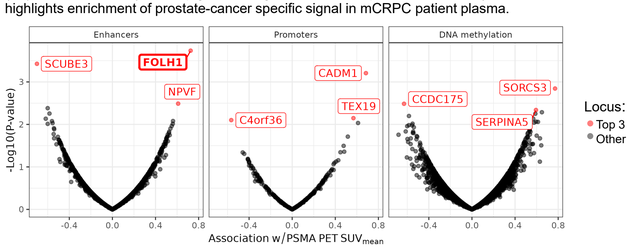


Epigenomic signal at the *FOLH1* locus shows increased active promoter and active enhancer marks in mCRPC patients with high PSMA PET SUV_{mean} (≥median, N=15) compared to mCRPC patients with low PSMA PET SUV_{mean} (<median, N=14). Enhancer, promoter, and DNA methylation signal in mCRPC patients with either high or low PSMA PET SUV_{mean} was normalized, smoothed, and averaged together (within analyte) with the mean signal from a cohort of male healthy volunteers.

Figure 2. Identification of plasma epigenomic features that associate with mCRPC and PSMA-PET signal



(2A): Differential analysis of epigenomic signals in mCRPC patients vs. healthy male volunteers highlights enrichment of prostate-cancer specific signal in mCRPC patient plasma.



(2B) – Genome wide analysis of epigenomic signal associated with PSMA PET SUV_{mean} confirms *FOLH1* enhancer signal as a top correlate.

(A) Differential analysis of normalized epigenomic signal between mCRPC patients (N=29) and healthy male volunteers (N=51) reveals enrichment of multiple prostate-cancer specific signals including *HOXB13, KLK2, KLK3, and SPDEF* in mCRPC patient plasma. Fold enrichment and significance was calculated using DESeq with Benjamini-Hochberg p-value correction. Points represent individual peaks, are colored by significance (Purple=FDR<0.05, Black=FDR>0.05), and "Up"/ "Down" labels indicate the number of statistically-significant loci for enhancers, promoters, and DNA methylation that are upregulated (Up) or downregulated (Down) in mCRPC patients vs healthy volunteers. Labels represent the nearest TSS to statistically significant peaks of interest.

(B) A genome wide analysis correlating epigenomic signal to PSMA PET SUV_{mean} quantifications in mCRPC patients (ctDNA% ≥3, N=29) identifies enhancer signal at the *FOLH1* locus as the top association. For each analyte, robust mCRPC-specific epigenomic features were identified and normalized to reduce technical variability and dependence on ctDNA fraction. Each feature was then tested for its association with PSMA PET SUV_{mean} via linear regression. The x-axis values represent the slope of the association between the z-score of the epigenomic feature and the z-score of PSMA PET SUV_{mean}, and the y-axis is the statistical significance of that association. The top three features for each analyte are labeled with their closest gene (TSS).

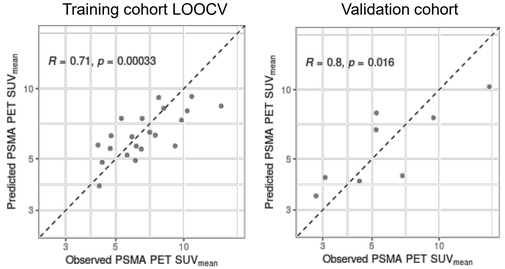
Figure 5. Enhancer and promoter signal at *FOLH1* locus predicts PSMA PET SUV_{mean} in both cross-validation and in validation cohort

Validation schema

1. Use all samples in training cohort.
2. Empirically determine regions of robust *FOLH1* enhancer/promoter signal.
3. Train ML model to predict PSMA PET SUV_{mean} from enhancer/promoter signal.
4. Predict validation cohort.

LOOCV schema

1. Leave out one sample out of 21 training samples.
2. Empirically determine regions of robust *FOLH1* enhancer/promoter signal.
3. Train ML model to predict PSMA PET SUV_{mean} from enhancer/promoter signal.
4. Predict left-out sample.
5. Repeat 21 times.



Cohort	N	Mean ctDNA%	Mean PSMA PET SUV_{mean}
Training	21	33.3 (3.9-65.4)	7.1 (4.2-14.7)
Validation	8	37.8 (3.3-78.7)	6.7 (2.8-16.8)

Enhancer and promoter signal at the *FOLH1* locus were used to train a machine learning (ML) model to predict PSMA PET SUV_{mean}. Samples were first split into training and validation cohorts, which were matched for ctDNA% and PSMA PET SUV_{mean} distributions. For model training, training cohort samples (ctDNA% ≥3) were used to identify robust, mCRPC-specific enhancer/promoter regions at the *FOLH1* locus. Signal at these regions were then used train a model to predict the corresponding PSMA PET SUV_{mean} quantifications. Performance was assessed via Pearson correlation in both a leave-one-out (LOO) cross-validation (CV) setting within the training cohort, as well as the held-out validation cohort using a final model trained on all data from the training cohort.

CONCLUSIONS

- This retrospective study profiled 50 patients with metastatic castration resistant prostate cancer who were treated with 177Lu-PSMA-617 based on PSMA PET evaluation.
- Evaluating PSMA levels from blood is crucial for optimizing patient selection and treatment efficacy in the growing landscape of PSMA-targeted therapies, the current analysis suggests that comprehensive epigenomic cfDNA profiling provides an accurate surrogate of tumor PSMA expression in men with mCRPC.
- With myriad drugs in development targeting a wide array of cancer-enriched cell surface proteins, these results highlight the potential of epigenomic cfDNA profiling as a real-time, expedient and non-invasive readout of tumor drug target expression.
- Future work will be aimed at increasing the accuracy and sensitivity of our PSMA predictive model, as well as examining the association of these plasma epigenomic signal-based PSMA PET predictions with response to 177Lu-PSMA-617 and other PSMA-targeted therapies.

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References: 1. Baca S et al., *Nature Medicine* (2023)