Molecular and Immunologic Correlates of High PSMA/FOLH1 RNA expression in Prostate Cancer

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BACKGROUND

- The *FOLH1* gene encodes prostate-specific membrane antigen (PSMA), a transmembrane glycoprotein that is highly expressed in most prostate cancer cells.
- PSMA expression can increase in metastatic castration resistant prostate cancer (mCPRC) but can be lost with the emergence of treatment-induced AR negative or neuroendocrine prostate cancer.
- PSMA is now a target for diagnostic imaging and treatment in prostate cancer. ^{177}Lu-PSMA-617, a β-emitting PSMA targeted radioligand, has demonstrated improved survival for patients with mCRPC. Additionally, other novel therapeutics targeting PSMA including alternative radioligands, antibody drug conjugates, and bispecific antibodies are currently under development.
- Characterizing the molecular profile of high and low PSMA/*FOLH1* expressing tumors will be critical to optimizing patients selection for PSMA targeted treatments alone and in combination with other therapeutic strategies.

OBJECTIVES

- Primary objectives:
  - Evaluate *FOLH1* RNA expression across tumor sites in patients with prostate cancer.
- Secondary objectives:
  - Evaluate commonly occurring DNA alterations in tumors with high and low *FOLH1* expression.
  - Evaluate *FOLH1* expression in tumor with high and low AR and NEPC signature scores.
  - Evaluate overall survival in patients with high and low *FOLH1* expression.

METHODS

- NextGen sequencing of DNA (592-gene/whole exome) and RNA (whole transcriptome) was performed for prostate cancer patient specimens (n=7,558) through Caris Life Sciences (Phoenix, AZ).
- *FOLH1*-High/Low expression were defined as percentile of RNA transcripts per million (TPM):

  Q1: < 25th (Low), Q2: 25th - 50th ,

  Q3: 50th - 75th , Q4: ≥75th (High)
- All the analysis is performed on adenocarcinoma cases as defined by the pathology report.
- Androgen receptor (AR), neuroendocrine (NEPC), and T-cell inflamed RNA signature scores were calculated. *FOLH1* was removed from the AR signature gene list.
- Tumor cell PD-L1+ expression (≥2+, ≥5%; SP142) was assessed by IHC.
- Kaplan-Meier estimates for real-world overall survival (OS) were calculated from time of diagnosis to last contact (death).
- q-value (adjusted p-value) is annotated by *, q < 0.05; **, q < 0.01; ***, q < 0.001; ****, q < 0.0001

Table 1: Baseline demographics.

Cohort Characteristics	N (%)
Samples	7558 (100%)
Median Age (range)	68.0 (35- >89)
Histology	**N (%)**
Prostatic adenocarcinoma	7,020 (92.9%)
Neuroendocrine prostate cancer	22 (0.29%)
Mixed neuroendocrine tumors	40 (0.53%)
Specimen site	**N (%)**
Prostate	4495 (62.5 %)
Metastasis	2697 (37.5 %)
Lymph node	832 (11.6 %)
Bone	568 (7.9 %)
Liver	359 (5.0 %)
Urinary tract	340 (4.7 %)
Lung	116 (1.6 %)
Gastrointestinal	53 (0.7 %)
CNS	49 (0.7 %)
Soft tissue	30 (0.4 %)
Adrenal gland	22 (0.3 %)
Mixed/other sites	238 (4.6 %)

RESULTS

Figure 1. *FOLH1* gene expression in prostate cancer. (A) *FOLH1* expression in prostate cancer, by histological subtype; adenocarcinoma, mixed neuroendocrine tumors, and neuroendocrine tumors. (B) *FOLH1* gene expression (log2 TPM+1) in primary prostate versus metastatic prostate samples in the Caris cohort (Adenocarcinoma cases only)`. Middle line represents the median. ****, q < 0.0001. (C) *FOLH1* gene expression [(log 2 TPM)+1] in all specimen site of prostatic adenocarcinoma in the Caris cohort, from highest to lowest expression. *, q < 0.05; ****, q < 0.0001 relative to primary prostate. The dotted horizonal line is the median *FOLH1* expression value in prostate site.

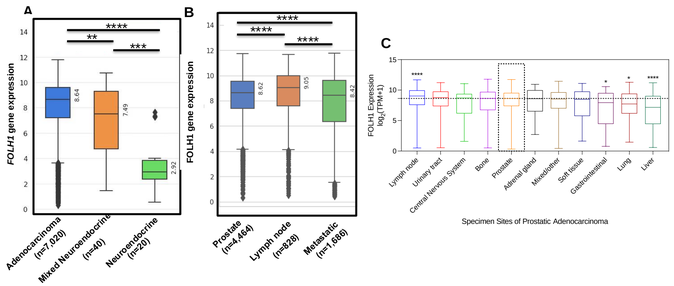


Figure 3. Association of *FOLH1* expression with androgen receptor (AR) signaling and neuroendocrine prostate cancer (NEPC) signatures scores. Spearman rank correlation of *FOLH1* gene expression (TPM) and AR signaling scores or NEPC scores in primary prostate **(A-B)**, lymph node **(C-D)**, other metastatic sites **(E-F)**. Insets show median AR signaling and NEPC scores in *FOLH1*-High and *FOLH1*-Low groups. Q1-Q4 are FOLH1 expression quartiles as stated in methods. **, q < 0.01; ***, q < 0.001; ****, q < 0.0001. TPM = transcripts per million.

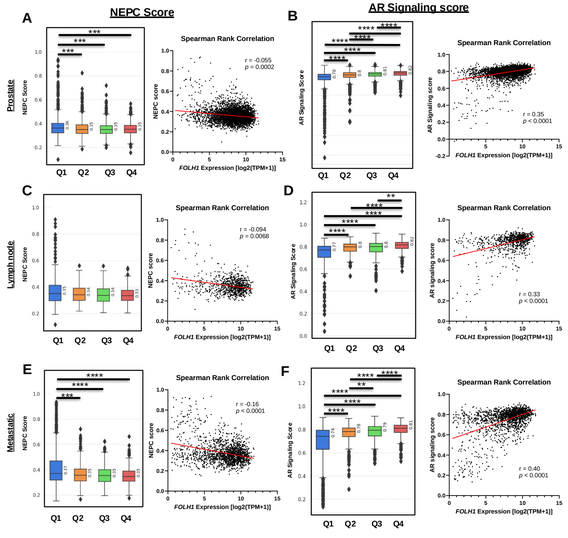


Figure 5. FOLH1-associated tumor immune-cell microenvironment. QuanTIseq immune cell infiltration in *FOLH1*-Low (Q1) and *FOLH1*-High (Q4) in primary and metastatic sites. LN = lymph node.

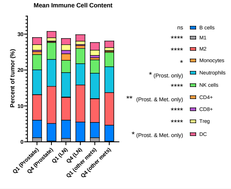


Figure 2. *FOLH1*-associated genomic landscape. Next-generation sequencing (NGS) was used to identify pathogenic/likely-pathogenic mutations, while fusions were identified from whole transcriptome sequencing (WTS) data. Prostate samples were stratified by quartiles of FOLH1 gene expression (Q1 = low; Q4 = high). The analysis was performed in primary prostate samples **(A)**, lymph node metastases **(B)**, and all other metastatic sites **(C)**. Significance was determined using Chi-square or Fisher's exact tests. *, q < 0.05; **, q < 0.01; ***, q < 0.001; ****, q < 0.0001.

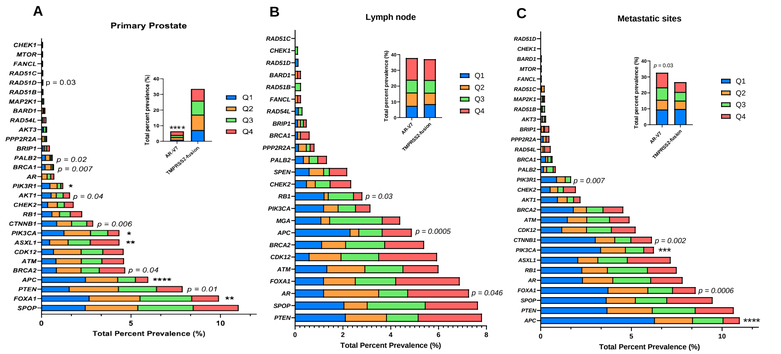


Figure 4. *FOLH1*-associated tumor microenvironment. (A) Percentage of tumors that were dMMR/MSI-High, TMB-High, and PD-L1(+) in *FOLH1-Low* (Q1) and *FOLH1-High* (Q4) in primary prostate, lymph node metastases, and other metastatic sites. **(B)** T cell inflamed scores in primary prostate, lymph node metastases, and other metastases, stratified by *FOLH1* expression quartiles (Q4 = *FOLH1*-High and Q1 = *FOLH1*-Low) (Q1). *, q < 0.05; **, q < 0.01; ***, q < 0.001; ****, q < 0.0001.

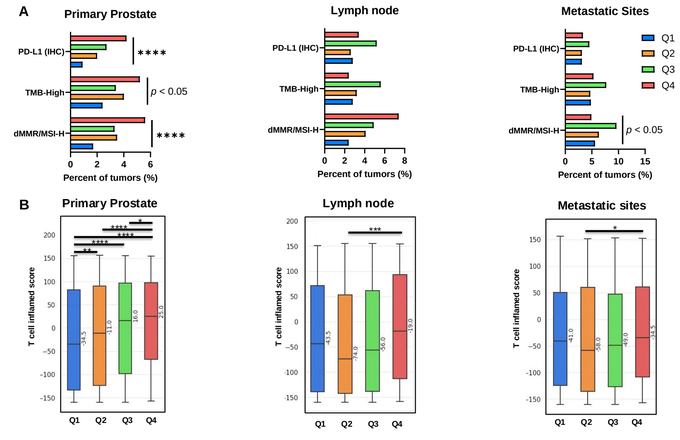
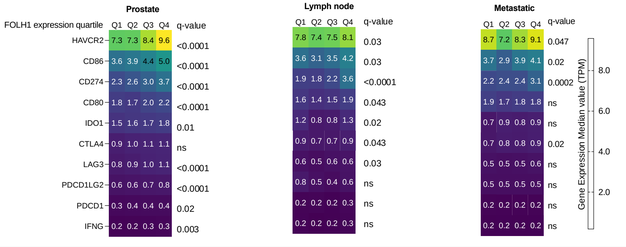
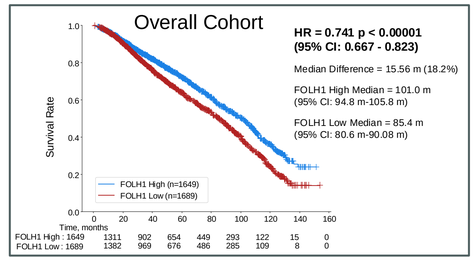


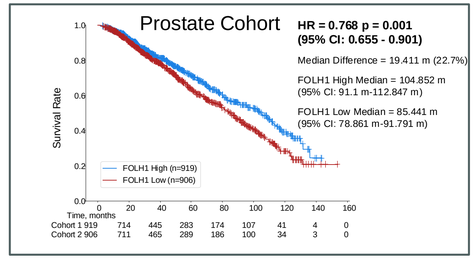
Figure 6: Immune checkpoint Gene expression by *FOLH1 expression* in primary, lymph node metastases, and metastatic sites.

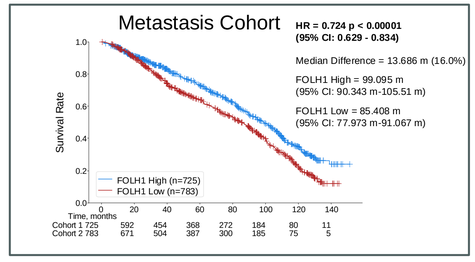


RESULTS

Figure 6: Overall survival among *FOLH1* high (quartile 4) and low groups (quartile 1).







CONCLUSIONS

- This is the largest analysis to date of *FOLH1*-related genomic/transcriptomic features and survival outcomes in prostate cancer.
- High *FOLH1* expression significantly correlated with *AR-V7* alterations and a depletion of *APC*, *FOX1A*, *PIK3CA*, *CTNNB1*, and *PIK3R1* alterations.
- *FOLH1* tumors frequently express high MSI/dMMR and were PD-L1+.
- High FOLH1 expression correlated with increased RNA expression of PD-L1, PD-L2, LAG-3, CD80, CD86, and IFN-γ (all p<0.001).
- High *FOLH1* expression was associated with greater OS overall, and in men with primary and metastasis sequencing.
- Tumors with high *FOLH1* are molecularly and immunologically distinct, providing insights for unique therapeutic strategies in this group.

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