Molecular Characterization of Imaging-Invisible Prostate Tumors

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Background: Up to 13% and 20% of aggressive localized prostate tumors are not visible on PSMA PET or mpMRI, respectively. This invisibility limits poses challenges for appropriate diagnostics, staging, and treatment selection. Since PSMA PET and mpMRI are commonly obtained for most patients with newly diagnosed prostate cancer, understanding how heterogeneity in imaging visibility reflects underlying tumor biology could enable imaging as a biomarker to personalize care for patients. Here, we performed RNA sequencing on localized tumors obtained from patients who underwent mpMRI and PSMA PET prior to prostatectomy.
Methods: A total of 71 grade group 2-3 prostate tumors that were at least 1cm in one dimension were identified from 59 patients who underwent mpMRI and PSMA PET prior to prostatectomy at a single institution. Tissue from these tumors were obtained for RNA sequencing, shotgun proteomics, and target DNA sequencing. For RNA analyses, differential expression compared imaging-visible versus imaging-invisible tumors using DESeq2 Wald tests with Benjamini–Hochberg correction (FDR < 0.05) in models that included relevant clinical covariates. Associations with continuous imaging metrics, including PET SUVmax and MRI PIRADS, were tested using multivariable linear and logistic regressions, respectively, on transformed expression. Gene set enrichment was performed on ranked statistics using preranked GSEA (clusterProfiler) with MSigDB Hallmark, KEGG, and immunologic signatures, applying Benjamini–Hochberg adjusted p values for significance.
Results: Bulk RNA sequencing identified transcriptomic signatures associated with MRI visibility and PSMA PET uptake (Figure 1). Gene set enrichment analysis revealed that MRI– tumors (PIRADS 1,2) were negatively enriched for multiple hallmark pathways, including cell cycle regulation, DNA repair, glycolysis, cholesterol and heme metabolism, and interferon responses, relative to MRI+ tumors (PIRADS 3,4,5) (Figure 2). In contrast, higher PSMA was positively associated with enrichment of androgen response, PI3K/AKT/mTOR signaling, MYC targets, TGF-β, WNT/β-catenin, unfolded protein response, and protein secretion pathways, while inflammatory response was enriched in tumors with lower PSMA. These findings demonstrate that molecular features captured by transcriptomic profiling align with imaging-defined tumor visibility and PSMA uptake, revealing distinct biological programs underlying detection by MRI and PET.
Conclusions: This study applies RNA sequencing to define the molecular features of imaging invisibility in prostate cancer. These findings demonstrate that molecular features captured by transcriptomic profiling align with imaging-defined tumor visibility and PSMA uptake, revealing distinct biological programs underlying detection by MRI and PET. Ongoing integration of DNA sequencing and proteomic analyses will provide further insight, with the goal of improving diagnostic accuracy, staging, and personalized therapeutic strategies.

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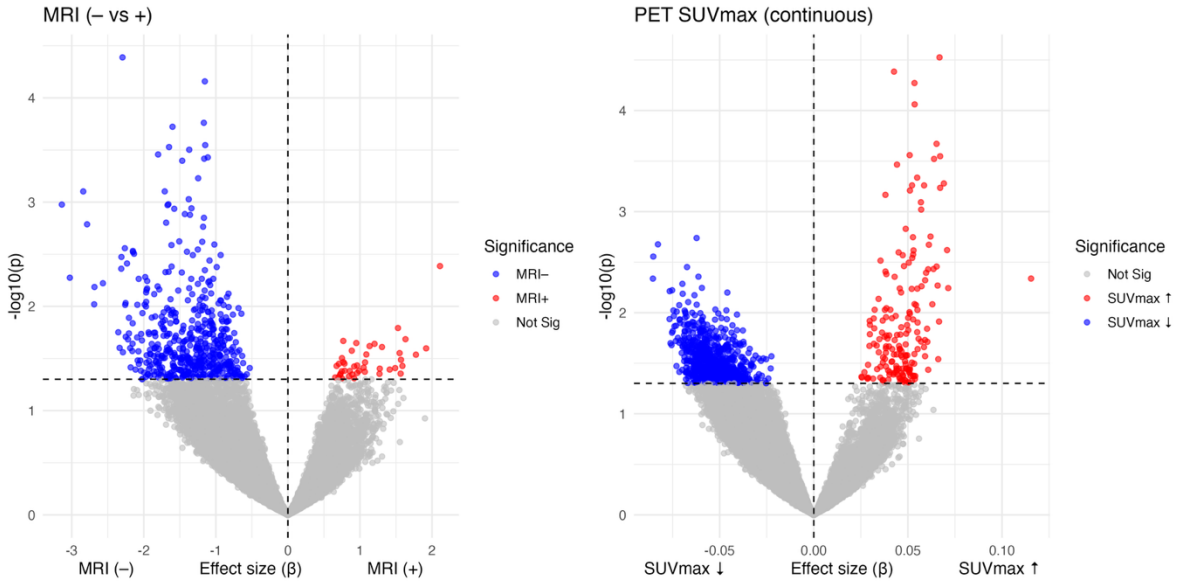

Figure 1. Volcano plots of gene associations across imaging-defined prostate cancer groups.
Volcano plots display gene expression associations with imaging metrics. *Left*: Comparison of tumors classified as MRI– (PIRADS 1,2) or MRI+ (PIRADS 3,4,5). Genes enriched in MRI– tumors are shown in blue, whereas those enriched in MRI+ tumors are shown in red. *Right*: Associations with continuous PSMA PET SUVmax. Genes positively correlated with SUVmax are shown in red, and those negatively correlated are shown in blue. Gray dots denote genes not reaching statistical significance. Dashed horizontal lines indicate the nominal significance threshold ($p < 0.05$), and vertical dashed lines represent $\beta = 0$.

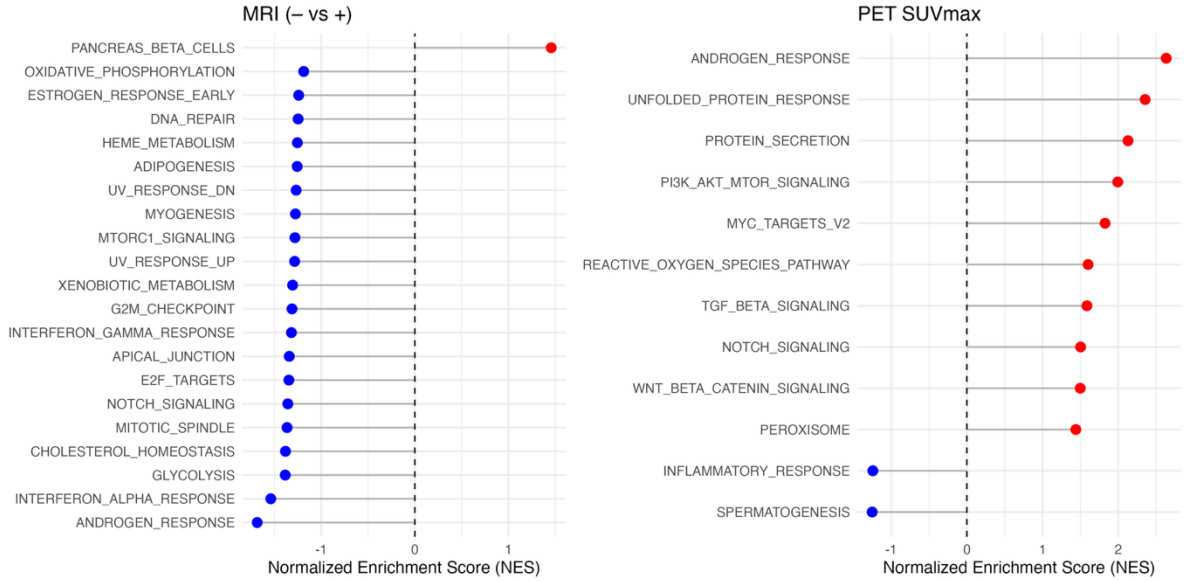

Figure 2. Pathway enrichment analysis of imaging-defined prostate cancer groups.
Normalized enrichment scores (NES) from preranked GSEA are displayed for Hallmark pathways. *Left*: NES for tumors classified as MRI– (PIRADS 1,2) or MRI+ (PIRADS 3,4,5). Negative NES values indicate enrichment in MRI–, whereas positive NES values indicate enrichment in MRI+. *Right*: NES for associations with continuous PSMA PET SUVmax. Negative NES values indicate enrichment in SUVmax-low tumors, whereas positive NES values indicate enrichment in SUVmax-high tumors.