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TITLE: Radiogenomic Characterization of Prostate Cancer: Distinguishing Aggressive From Indolent Disease

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CONTRACTING ORGANIZATION: University of Michigan, Ann Arbor, MI

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None

14. ABSTRACT: Over the past several years, there has been growing utilization of multi-parametric magnetic resonance imaging (mpMRI) to detect aggressive or high-grade PCa. Even though close to 20 % of aggressive PCa are missed by mpMRI, this imaging modality is currently being used to guide treatment decisions, such as for active surveillance and delineating areas for focal therapy. The goal of this project is to improve the detection of aggressive (Gleason ≥7) PCa by combining mpMRI and urinary biomarkers. Building on our prior work on using PCA3 and TMPRSS2:ERG to detect aggressive PCa, we have developed a novel urine-based targeted next generation sequencing (NGS) assay to detect PCa. We hypothesize that aggressive PCa harbors unique molecular alterations that impact detection by mpMRI or a urine-based sequencing assay. To test this hypothesis, we propose the following Specific Aims: 1): To assess the accuracy of a novel urine-based NGS assay for the detection of high-grade PCa. We will perform targeted DNA/RNA NGS on already collected pre-biopsy post-DRE urine specimens in patients who underwent radical prostatectomy (RP) at the University of Michigan (U-M). The molecular profile of patients with high-grade (cases) versus low-grade (controls) PCa will be compared. 2): To comprehensively characterize the genomic and transcriptomic alterations associated with cancer visibility on mpMRI. We will collect formalin-fixed paraffin-embedded (FFPE) radical prostatectomy (RP) specimens with multiple foci of cancer in men who had mpMRI prior to RP, with an emphasis on those with multiple Gleason grades. Where available, corresponding matched pelvic lymph node specimens with metastasis will also be collected. Targeted multiplexed PCR-based NGS will be performed to characterize and compare the molecular profiles of visible and invisible PCa foci on mpMRI. 3): Determine and compare tissue versus a urine-based prognostic assays to predict upgrading at RP. Targeted DNA/RNA NGS will be performed on post-DRE urine and biopsy tissue obtained prior to RP. We will assess and compare the performance of the novel urine-based assay with tissue-based prognostic assays to predict Gleason upgrading at RP.

15. SUBJECT TERMS

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1. INTRODUCTION:

The goal of this project is to improve the detection of aggressive (Gleason ≥7) prostate cancer by combining multiparametric MRI and urinary biomarkers.

2. KEYWORDS:

Prostate cancer, urinary biomarkers, MRI, aggressive, genomics, transcriptomics

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Training and educational development in prostate cancer research	**1 – 48 Months**

Research-Specific Tasks:

Specific Aim 1: To assess the accuracy of a novel urine-based NGS assay for the detection of high-grade PCa.	0 – 20 months
Major Task 1: Test the capacity of a novel urine-based NGS assay to detect aggressive PCa at first biopsy.	0 – 14 months
Major Task 2: Determine the differential performance characteristics of a novel urine-based NGS assay to detect aggressive PCa in the setting of a – vs. + prostate mpMRI.	10 – 20 months
Specific Aim 2: To comprehensively characterize the genomic and transcriptomic alterations associated with cancer visibility on mpMRI	
Major Task 3: Interrogate specific molecular changes associated with high-grade PCa visibility on mpMRI.	16 – 33 months
Major Task 4: Elucidate the molecular profile of low-grade fusion biopsy cores obtained from PIRADS 4 and 5 lesions and correlate these findings with RP pathology.	28 – 38 months
Specific Aim 3: Determine and compare tissue versus a urine-based	

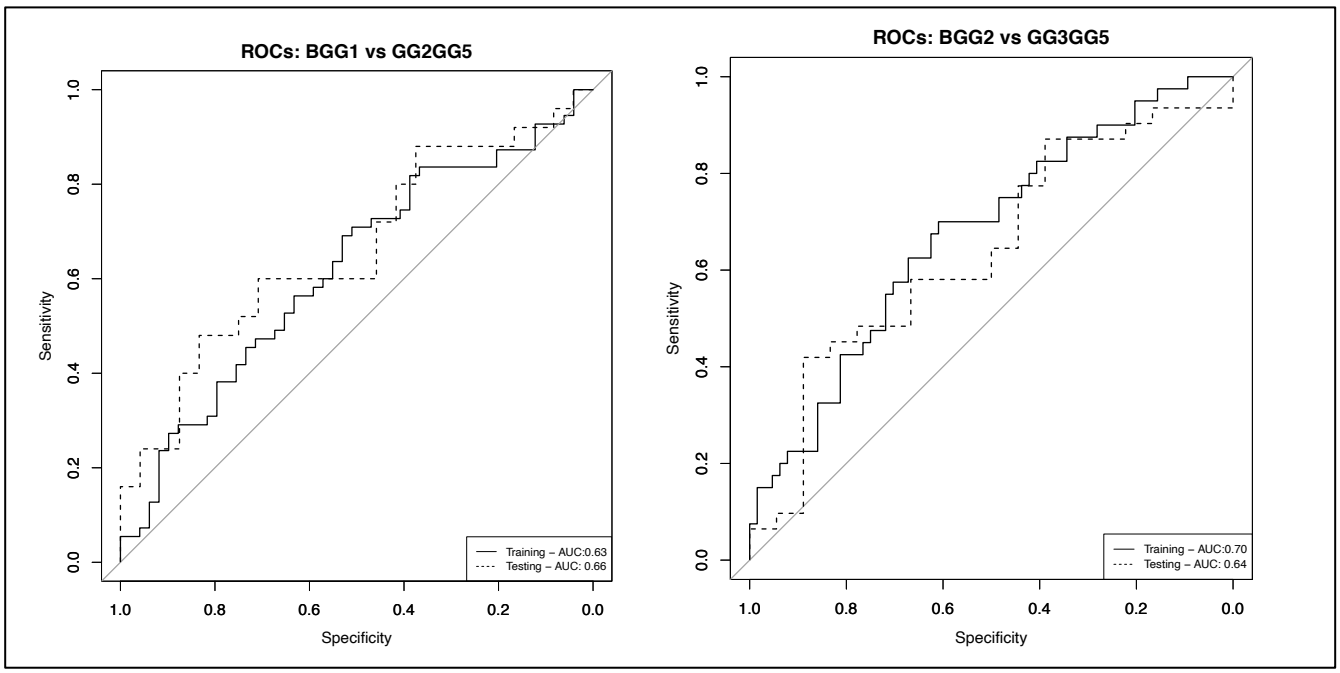
prognostic assays to predict Gleason upgrading at RP.	
Major Task 5: Assess tissue-based and novel urine-based prognostic scores in biopsy core and urine respectively in patients with Gleason upgrading	34 – 48 months

What was accomplished under these goals?

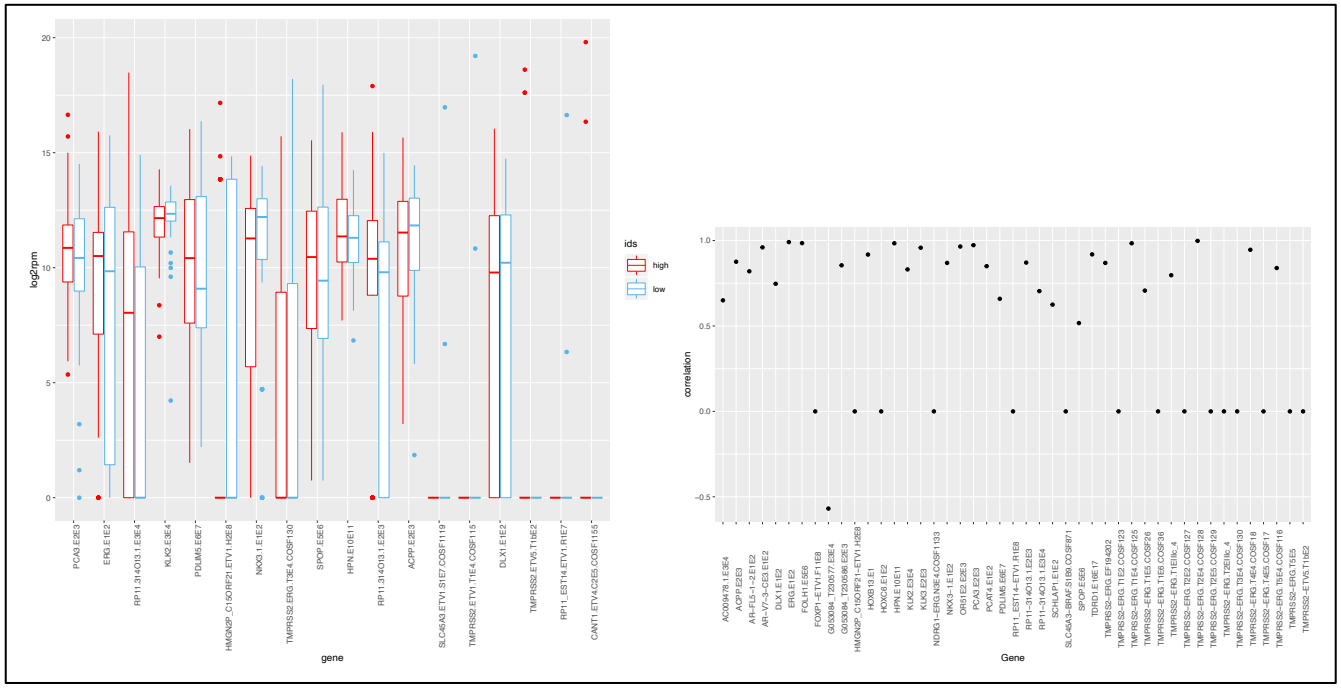
Major Task 1: Training and educational development in prostate cancer research: I have continued to attend basic science seminar series and research meetings. I attend mentorship meetings with my mentors. I completed an R01 bootcamp at the University of Michigan which culminated in the submission of R01 grant application that was reviewed in June 2020 but was triaged. I am in the process of revising the grant application based on the reviewer comments for planned submission in February 2021. I have attended the American Urological Association Annual meeting, Society of Urologic Oncology annual meeting, and Prostate Cancer Foundation annual meeting. I have presented initial results from this work at some of these meetings.

Specific Aim 1: To assess the accuracy of a novel urine-based NGS assay for the detection of high-grade PCa.

- **Major Task 1:** Test the capacity of a novel urine-based NGS assay to detect aggressive PCa at first biopsy. In the prior progress report, we reported that targeted RNA next generation sequencing (NGS) of urine obtained from patients prior to first biopsy has been completed in 170 patients. Initial analyses to develop multiplex models to predict Grade Group (GG2-5) as well as GG3-5 prostate cancers revealed training AUC of 0.63 and 0.70 respectively.



We explored options for the potential improvement in the AUC. We found wide variation in the distribution of the transcripts, with some transcripts having poor detection (**Left panel)**. We believe this impacted the performance (measured by AUC) of the resulting models as shown above. Thus, we used a different RNA extraction method from urine in a subset of samples, the Zymo method which requires more urine volume for RNA extraction but takes more time and more hands-on (a manuscript using the Zymo method for RNA extraction is currently under review). We observed high correlation of most transcripts (r = 0.75 – 1.0) between both RNA extraction methods. However, we also observed poor correlation ins omse transcripts between the two methods (**Right panel**).

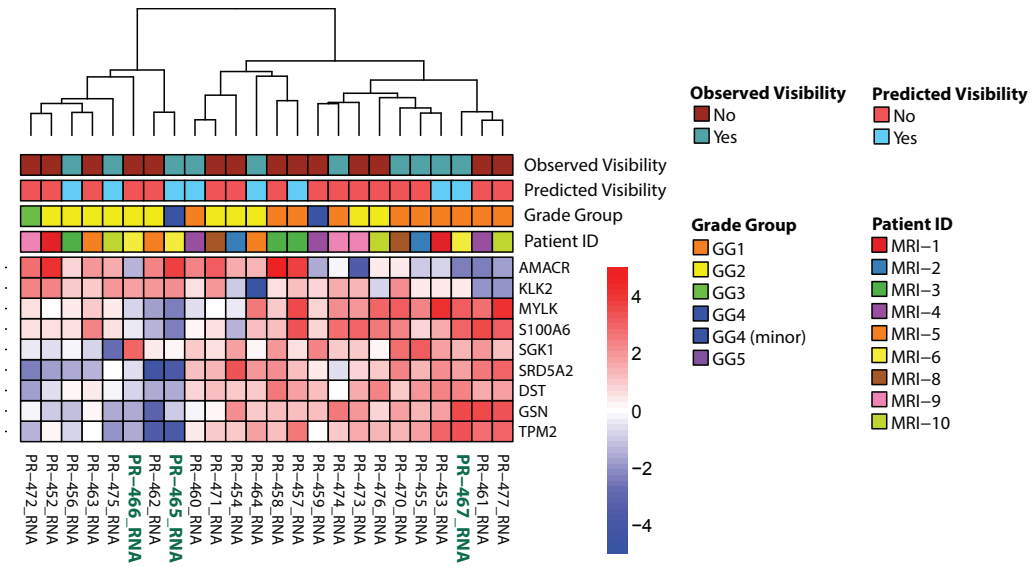


- Ongoing experimental and analytic effort is geared towards
    - Optimizing RNA extraction in a subset of urine samples with low transcripts detection/expression
    - Limiting the transcripts for model development to those with high correlation between both urine RNA extraction approaches
    - Introducing clinical variables for predicting aggressive disease in model development.

- **Major Task 2: Determine the differential performance characteristics of a novel urine-based NGS assay to detect aggressive PCa in the setting of a – vs. + prostate mpMRI.**
  Next generation sequencing and bioinformatics analysis to evaluate the performance of the novel urine based assay independent of mpMRI is currently ongoing. The planned experiments was severely hampered by the COVID-19 pandemic.

Specific Aim 2: To comprehensively characterize the genomic and transcriptomic alterations associated with cancer visibility on mpMRI

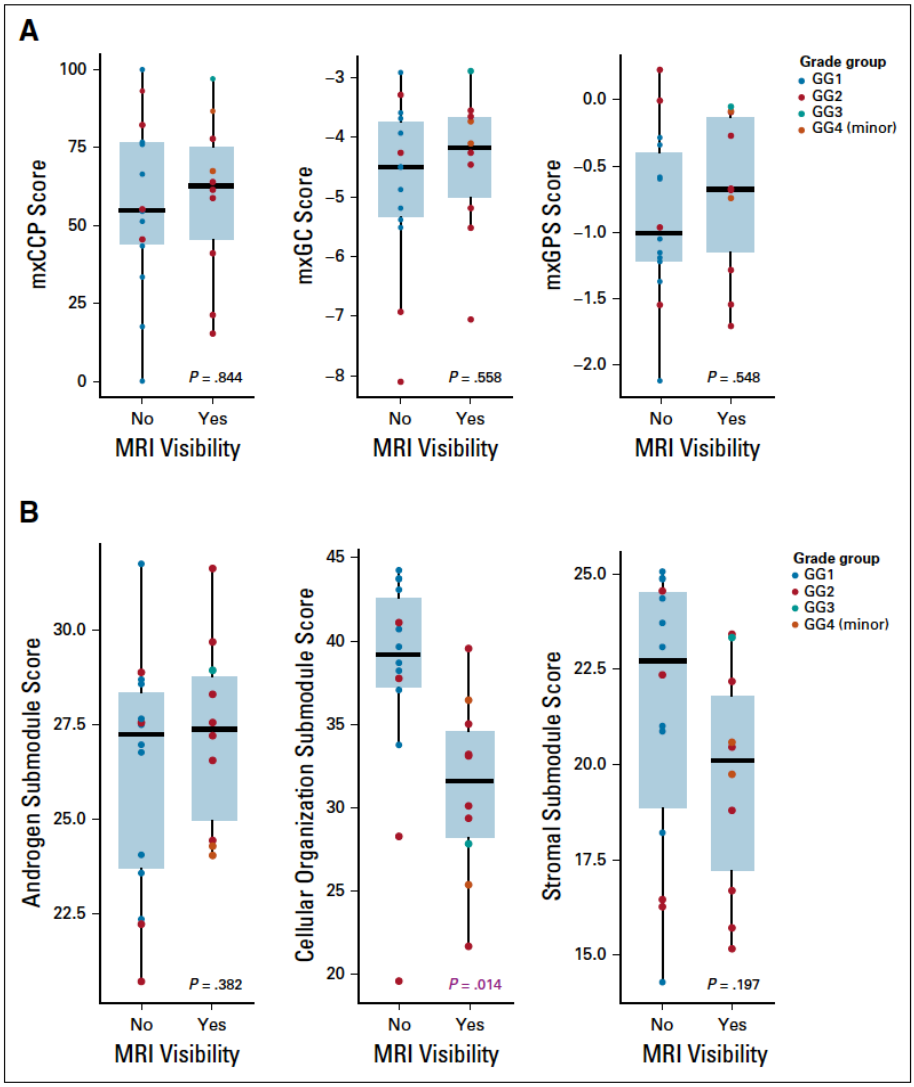
- **Major Task 3: Interrogate specific molecular changes associated with high-grade PCa visibility on mpMRI.**
  We performed targeted NGS in 26 samples of mpMRI visible and invisible prostate cancer. Bioinformatics analysis to compare the molecular profile of mpMRI visible versus invisible lesions indicates that under expression of cellular organization and structure underlies mpMRI invisibility (see publication, **Appendix A)**.



Development a 9-gene signature to predict mpMRI visible tumors. Annotated heatmap of differentially expressed genes between mpMRI visible and invisible PCa which were identified with the EdgeR package using a Benjamini-Hochberg procedure (BH) adjusted false discovery rate (FDR) cutoff of < 0.05. Targets and samples were clustered using hierarchical clustering based on Euclidian distances. Comparisons of observed (first row) vs. predicted (second row) mpMRI visibility using the 9-gene signature is shown.

- We also compared derived commercially available tissue-based prognostic biomarker assays (Myriad Prolaris cell cycle progression (mxCCP), OncotypeDX genomic prostate score (mxGPS), and Decipher genomic classifier (mxGC) between mpMRI visible and invisible lesions and found no significant difference in the scores.

- We are currently exploring the possibility of analyzing additional patient samples using spatial transcriptomic profiling and whole transcriptomic bulk profiling.



Derivation and comparison of expression-based prognostic scores between mpMRI visible and invisible lesions. A) Boxplots of derived Myriad Prolaris cell cycle progression (mxCCP), OncotypeDX genomic prostate score (mxGPS), and Decipher genomic classifier (mxGC) stratified by mpMRI visibility status in our preliminary cohort (n= 10 patients, 26 cancer foci). Points represent individual cancer focus colored according to ISUP Grade Group. Unpaired t-tests were used to test for significant differences in mean score. There was no statistically significant difference between the derived prognostic scores of mpMRI-visible and -invisible lesions ($p > 0.05$). **B)** Comparisons of derived mxGPS submodules stratified by mpMRI visibility status. Boxplots of derived mxGPS Androgen, Cellular Organization, and Stromal submodules stratified by mpMRI visibility status are shown. Unpaired t-tests were used to compare mean sub-component scores. Only the Cellular Organization submodule had a significant difference in mean expression ($p = 0.014$).

- **Major Task 4: Elucidate the molecular profile of low-grade fusion biopsy cores obtained from PIRADS 4 and 5 lesions and correlate these findings with RP pathology.**
  This analysis is scheduled to be performed in the 3rd year of the award.

- **Major Task 5: Assess tissue-based and novel urine-based prognostic scores in biopsy core and urine respectively in patients with Gleason upgrading**
  This analysis is scheduled to be performed in the 4th year of the award.

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
The initial paper from Aim 1 was published in the Journal of Clinical Oncology Precision Oncology.

What do you plan to do during the next reporting period to accomplish the goals?

Major Task 1: Optimize experimental and analytic efforts

- Optimizing RNA extraction in a subset of urine samples with low transcripts detection/expression
- Limiting the transcripts for model development to those with high correlation between both urine RNA extraction approaches
- Introducing clinical variables for predicting aggressive disease in model development.

Major Task 2: Determine the differential performance characteristics of our novel urine-based NGS assay to detect aggressive PCa in the setting of a – vs. + prostate mpMRI. This was hampered by the COVID-19 pandemic and closure of labs for nearly 6 months. We hope to complete this in the next reporting period.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
The finding that mpMRI invisible prostate cancer are just as important biologically as visible ones indicates that we should not use mpMRI alone for determining what patients should undergo focal therapy or active surveillance.

The potential impact of the ongoing analyses will delineate the utility of using a urine test to supplement mpMRI for detecting clinically significant prostate cancer.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
See Major task 1 above.

Actual or anticipated problems or delays and actions or plans to resolve them
This project was severely impacted by lab closures and temporary furloughs due to the COVID-19 pandemic.

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Not applicable

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Not applicable

Significant changes in use of biohazards and/or select agents
Not applicable

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Salami SS, Kaplan JB, Nallandhinghal S, Takhar M, Tosoian JJ, Lee M, Yoon J, Hovelson DH, Plouffe KR, Kaffenberger SD, Schaeffer EM, Karnes R, Lotan TL, Morgan TM, George, AK, Montgomery JS, Davenport MS, You S, Tomlins SA, Curci NE, Kim HL, Spratt DE, Udager AM, Palapattu GS. Biologic Significance of MRI Invisibility in Localized Prostate Cancer (JCO Precision oncology, 2019, in press, acknowledgement of federal support – yes)

  Books or other non-periodical, one-time publications.
  Nothing to report

  Other publications, conference papers and presentations.

Salami S, Kaplan J, Nallandhighal S, Takhar M, Tosoian J, Lee M, Yoon J, Hovelson D, Plouffe K, Kaffenberger S, George A, Montgomerey J, Davenport M, Udager A, Palapattu: Radiogenomic Characterization of Multifocal Prostate Cancer, Journal of Clinical Oncology, 37, 126, 2019. GU-ASCO Annual meeting, 2019*

Salami S, Kaplan J, Nallandhingal S, Takhar M, Tosoian J, Lee M, Yoon J, Hovelson D, Plouffe K, Kaffenberge S, Palapattu G: Radiogenomic Dissection of Multifocal Prostate Cancer, The Journal of Urology, 201, 2019. AUA Annual meeting, 2019*

Tosoian JJ, Trock BJ, Morgan TM, **Salami SS**, Tomlins SA, Spratt DE, Siddiqui J, Kunju LP, Botbyl R, Chopra Z, Pandian B, Eyrich NW, Longton G, Zheng Y, Palapattu GS, Wei JT, Niknafs YS, Chinnaiyan AM. Use of the MyProstateScore (MPS) Test to Rule Out Clinically-Significant Cancer: Validation of a Straightforward Clinical Testing Approach. J Urol. 2020 Oct 20:101097JU0000000000001430. doi:10.1097/JU.0000000000001430. Online ahead of print. PMID: 33080150

Salami SS, Tosoian JJ, Nallandhighal S, Jones TA Jr, Brockman S, Elkhoury FF, Bazzi S, Plouffe KR, Siddiqui J, Liu CJ, Kunju LP, Morgan TM, Natarajan S, Boonstra PS, Sumida L, Tomlins SA, Udager AM, Sisk AE Jr, Marks LS, Palapattu GS. Serial Molecular Profiling of Low-grade Prostate Cancer to Assess Tumor Upgrading: A Longitudinal Cohort Study. Eur Urol. 2020 Jul 3:S0302-2838(20)30470-X. doi: 10.1016/j.eururo.2020.06.041. Online ahead of print. PMID: 32631746

Tosoian JJ, Birer SR, Jeffrey Karnes R, Zhang J, Davicioni E, Klein EE, Freedland SJ, Weinmann S, Trock BJ, Dess RT, Zhao SG, Jackson WC, Yamoah K, Pra AD, Mahal BA, Morgan TM, Mehra R, Kaffenberger S, **Salami SS**, Kane C, Pollack A, Den RB, Berlin A, Schaeffer EM, Nguyen PL, Feng FY, Spratt DE. Performance of clinicopathologic models in men with high risk localized prostate cancer: impact of a 22-gene genomic classifier. Prostate Cancer Prostatic Dis. 2020 Dec;23(4):646-653. doi: 10.1038/s41391-020-0226-2. Epub 2020 Mar 30. PMID: 32231245

- **Website(s) or other Internet site(s)**
  https://www.urotoday.com/categories-media/1748-centers-of-excellence/advanced-prostate-cancer-coe/1248-use-of-mri-to-risk-stratify-patients-with-prostate-cancer-simpa-salami.html
  This was an interview with urotoday to discuss the Biologic Significance of Magnetic Resonance Imaging Invisibility in Localized Prostate Cancer

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Simpa S. Salami*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7461-7079*
Nearest person month worked:	*6*
Contribution to Project:	*Providing scientific and administrative oversight, data interpretation, manuscript writing*
Funding Support:	*DOD*

Name:	*Sri Nallanghighal, MS*
Project Role:	*Bioinformatician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Mr. Nallandhighal has performed bioinformatic analysis of the data generated in Aim 2.*
Funding Support:	*DOD, NIH SPORE*

Name:	*Kevin Hu*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Mr. Hu has performed bioinformatic analysis of the data generated*
Funding Support:	*UM Department of Pathology Training award*

Name:	*Trinh Pham*
Project Role:	*Laboratory technologist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Pham has performed the laboratory experiments – DNA/RNA extraction from urine and Next generation sequencing*
Funding Support:	*Department of Urology funds*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Current DOD PRA AWARD

PC170717 (Salami) 10/01/2018-09/30/2022 4.80 Calendar Months
Department of Defense Source Country: USA
Annual Directs: Total Award:
Radiogenomic Characterization of Prostate Cancer: Distinguishing Aggressive from Indolent Disease
The successful completion of the proposed project will improve our understanding of the molecular basis of PCa visibility on mpMRI and guide treatment decisions based on mpMRI findings.

Role: PI

NEW AWARDS

Palapattu (PI) 10/24/2017-06/30/2022 0.60 Calendar Months
Joint Institute for Translational and Clinical Research Source Country: USA
Annual Directs: Total Award:
Comprehensive molecular profiling of renal cell carcinoma
The long-term goal of our proposal is to improve the health of men diagnosed with renal cell carcinoma (RCC)
Role: Co-Investigator

P50 CA186786 (Chinnaiyan) 09/01/2019-08/31/2024 1.80 Calendar Months
NIH/NCI Source Country: USA
Annual Directs: Total Award:
SPORE Project 2: Michigan Prostate SPORE
The Prostate SPORE program continues to place premiums on rigorous scientific review of its translational research programs, pairing of basic and clinical investigators, drawing on expertise of scientists from within and from outside the prostate cancer field, and utilizing flexibility to fund promising new research approaches.
Role: Co-Lead

ACTIVE No Cost Extension Awards

16YOUN17 (Salami) 06/27/2016-06/27/2020 0.00 Calendar Months
Prostate Cancer Foundation Source Country: USA
Annual Directs: Total Award:
Molecular Characterization of the Biologically Dominant Nodule in Multifocal Prostate Cancer with N1 disease
Goals: To determine and compare the molecular profile of each cancer focus in multifocal prostate cancer; ii) To characterize the biologically dominant nodule or index tumor in multifocal prostate cancer with lymph node (LN) metastasis; and iii) To evaluate the prognostic accuracy of Oncotype DX™, Prolaris™ and Decipher™ scores in predicting LN metastasis.
Role: PI

PENDING

20-PAF05162 (Salami) 09/01/2020-0831/2025 2.40 Calendar Months

NIH Source Country: USA
Annual Directs: Total Award:
Defining the Biological Trajectory of Gleason 6 Prostate Cancer
Goals: The long-term goal of this project is to further reduce the biological uncertainty associated with surveillance for favorable-risk prostate cancer. Although most men with favorable-risk disease are candidates for surveillance, its use varies widely and ranges from 20 to 90% across individual providers.
Role: PI

What other organizations were involved as partners?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable

QUAD CHARTS: *Not applicable*

9. APPENDICES:

a. Appendix A (Published Manuscript): Biologic Significance of Magnetic Resonance Imaging Invisibility in Localized Prostate Cancer

original report

Biologic Significance of Magnetic Resonance Imaging Invisibility in Localized Prostate Cancer

Simpa S. Salami, MD, MPH[1,2]; Jeremy B. Kaplan[1]; Srinivas Nallandhighal, MS[1]; Mandeep Takhar, MS[3]; Jeffrey J. Tosoian, MD, MPH[1]; Matthew Lee, MD[1]; Junhee Yoon, MS[4]; Daniel H. Hovelson, PhD[1]; Komal R. Plouffe, MS[1]; Samuel D. Kaffenberger, MD[1,2]; Edward M. Schaeffer, MD, PhD[5]; R. Jeffrey Karnes, MD[6]; Tamara L. Lotan, MD[7]; Todd M. Morgan, MD[1,2]; Arvin K. George, MD[1,2]; Jeffrey S. Montgomery, MD, MHSA[1,2]; Matthew S. Davenport, MD[1]; Sungyong You, PhD[4]; Scott A. Tomlins, MD, PhD[1,2]; Nicole E. Curci, MD[1]; Hyung L. Kim, MD[4]; Daniel E. Spratt, MD[2,1]; Aaron M. Udager, MD, PhD[1,2]; and Ganesh S. Palapattu, MD[1,2,8]

abstract

PURPOSE Multiparametric magnetic resonance imaging (mpMRI) is used widely for prostate cancer (PCa) evaluation. Approximately 35% of aggressive tumors, however, are not visible on mpMRI. We sought to identify the molecular alterations associated with mpMRI-invisible tumors and determine whether mpMRI visibility is associated with PCa prognosis.

METHODS Discovery and validation cohorts included patients who underwent mpMRI before radical prostatectomy and were found to harbor both mpMRI-visible (Prostate Imaging and Reporting Data System 3 to 5) and -invisible (Prostate Imaging and Reporting Data System 1 or 2) foci on surgical pathology. Next-generation sequencing was performed to determine differential gene expression between mpMRI-visible and -invisible foci. A genetic signature for tumor mpMRI visibility was derived in the discovery cohort and assessed in an independent validation cohort. Its association with long-term oncologic outcomes was evaluated in a separate testing cohort.

RESULTS The discovery cohort included 10 patients with 26 distinct PCa foci on surgical pathology, of which 12 (46%) were visible and 14 (54%) were invisible on preoperative mpMRI. Next-generation sequencing detected prioritized genetic mutations in 14 (54%) tumor foci (n = 8 mpMRI visible, n = 6 mpMRI invisible). A nine-gene signature (composed largely of cell organization/structure genes) associated with mpMRI visibility was derived (area under the curve = 0.89), and the signature predicted MRI visibility with 75% sensitivity and 100% specificity (area under the curve = 0.88) in the validation cohort. In the testing cohort (n = 375, median follow-up 8 years) there was no significant difference in biochemical recurrence, distant metastasis, or cancer-specific mortality in patients with predicted mpMRI-visible versus -invisible tumors (all $P > .05$).

CONCLUSION Compared with mpMRI-invisible disease, mpMRI-visible tumors are associated with underexpression of cellular organization genes. mpMRI visibility does not seem to be predictive of long-term cancer outcomes, highlighting the need for biopsy strategies that detect mpMRI-invisible tumors.

INTRODUCTION

Distinguishing aggressive from indolent clinically localized prostate cancer (PCa) continues to pose a significant clinical challenge. Recent efforts to overcome this have involved the development and optimization of several diagnostic strategies, including multiparametric magnetic resonance imaging (mpMRI). mpMRI permits visual identification of areas that are suggestive for intermediate to high-grade cancer. The emergence of various MRI/ultrasound fusion biopsy platforms has led to increased detection of aggressive PCa by facilitating targeted biopsy of visible lesions.[1-6] As a result, mpMRI is now widely used in guiding treatment decisions in men with clinically localized disease, especially when selecting patients suitable for active surveillance or potentially focal therapy.[7-10] The prevailing view is that only mpMRI-visible cancers require clinical action. However, use of mpMRI in the evaluation of men with PCa is limited by cancer multifocality and interfocal disease heterogeneity. Individual patients are known to harbor multiple spatially distinct PCa foci with varying clinical, radiographic, and pathologic characteristics.[11-15] Up to 55% of all PCa foci and 35% of clinically significant foci are not visible on mpMRI.[3,16,17] Furthermore, more than 35% of lesions 1 cm or larger are missed by mpMRI.[17] Although some studies have demonstrated that up to 50% of mpMRI-invisible PCa may harbor relevant genomic alterations, the clinical and prognostic significance of mpMRI-invisible PCa remains unknown.[18] An improved understanding of the molecular characteristics and clinical trajectories of mpMRI-visible and -invisible cancers could facilitate more optimal treatment allocation. For example, if mpMRI-invisible foci are found

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on April 22, 2019 and published at ascopubs.org/journal/po on June 12, 2019: DOI https://doi.org/10.1200/PO.19.00054

CONTEXT

Key Objective

What is the molecular basis for prostate cancer visibility on multiparametric magnetic resonance imaging (mpMRI), and do mpMRI-invisible tumors harbor any clinical or biologic significance compared with visible tumors?

Knowledge Generated

mpMRI-visible tumors demonstrated underexpression of genes associated with cellular organization and structure. Using a novel genetic signature for tumor visibility on mpMRI, patients with predicted mpMRI-visible and -invisible tumors did not experience significant differences in biochemical recurrence, distant metastasis, or cancer-specific mortality during follow-up.

Relevance

Prostate cancers that are mpMRI invisible have similar clinical behavior to mpMRI-visible tumors. Negative mpMRI seems insufficient to rule out clinically relevant prostate cancer, and patients at increased risk should be considered for additional testing or systematic prostate biopsy.

to be biologically indolent, those with a known diagnosis of low-grade disease and a negative mpMRI could be directed toward active surveillance. Similarly, those with a single lesion detected on mpMRI could be more confidently directed toward focal therapy, with low concern for missing a clinically relevant lesion. We herein sought to characterize the molecular profile of mpMRI-visible and -invisible PCa foci using next-generation sequencing (NGS). In addition, we test the prognostic significance of our mpMRI-derived genomic signature after radical prostatectomy (RP).

METHODS

Study Design

The study used three independent patient populations: discovery, validation, and testing cohorts. Institutional review board approval was obtained for each cohort. First, we identified patients with clinically localized disease who underwent preoperative mpMRI at the University of Michigan in 2015 to 2016 and were subsequently found to harbor multifocal PCa at RP. We enriched for patients with both mpMRI visible and invisible PCa (Figs 1A and 1B) to constitute the discovery cohort. The validation cohort from Cedars-Sinai Medical Center included patients with either mpMRI-visible or -invisible foci, as previously described.[19] The testing cohort was composed of patients from the Decipher GRID PCa database treated at Johns Hopkins Medical Institute and Mayo Clinic (ClinicalTrials.gov identifier: NCT02609269) who underwent genome-wide expression profiling after RP.[20,21]

Preoperative Prostate mpMRI and Pathologic Evaluation

In the discovery and validation cohorts, mpMRI comprising T2-weighted imaging, diffusion-weighted imaging, and dynamic contrast-enhanced imaging was obtained. All mpMRI results were re-reviewed and coregistered with whole-mount formalin-fixed paraffin-embedded RP specimens to delineate mpMRI-visible (Prostate Imaging and Reporting Data System [PI-RADS] version 2; score, 3 to 5) and -invisible foci. Additional procedural details are described in the Data Supplement. Data on mpMRI were not available for the testing cohort.[22]

Targeted DNA and RNA NGS

In the discovery cohort, DNA and RNA from each focus were co-isolated for targeted multiplex NGS as previously described[23] and detailed in the Data Supplement. Our targeted NGS assays were designed to assess relevant PCa genomic and transcriptomic alterations and derive clinically available prognostic tests.[15] The details of RNA sequencing in the validation cohort and genome-wide expression profiling in the testing cohorts have been previously described.[19,24]

Bioinformatic Analysis of the Discovery Cohort

NGS data analysis was performed using Torrent Suite (4.2.0; Thermo Fisher Scientfic, Waltham, MA) and the Coverage Analysis Plug-ins v.5.0.4. (Thermo Fisher Scientific), along with the Ion Reporter (4.2.0; Thermo Fisher Scientific). All other analyses were performed using *R* Project for Statistical Computing v.3.2.3. Details regarding targeted NGS techniques, quality control parameters, DNA copy number alterations and variant calls, fusion isoform and partner level analysis, androgen receptor (AR) and AR-splice variants detection, and prognostic scores derivation have been previously described and summarized in the Data Supplement.[15,25,26]

Differential Gene Expression Analysis of mpMRI-Visible and -Invisible Cancer Foci

To determine gene expression differences between mpMRI-visible and -invisible tumors, we analyzed RNAseq data from the discovery cohort using two approaches—differential expression (DE) analysis and random forest (RF) classifier—as described in the Data Supplement. From these two approaches, a gene expression signature comprising independent differentially expressed genes was developed to predict mpMRI tumor visibility status.

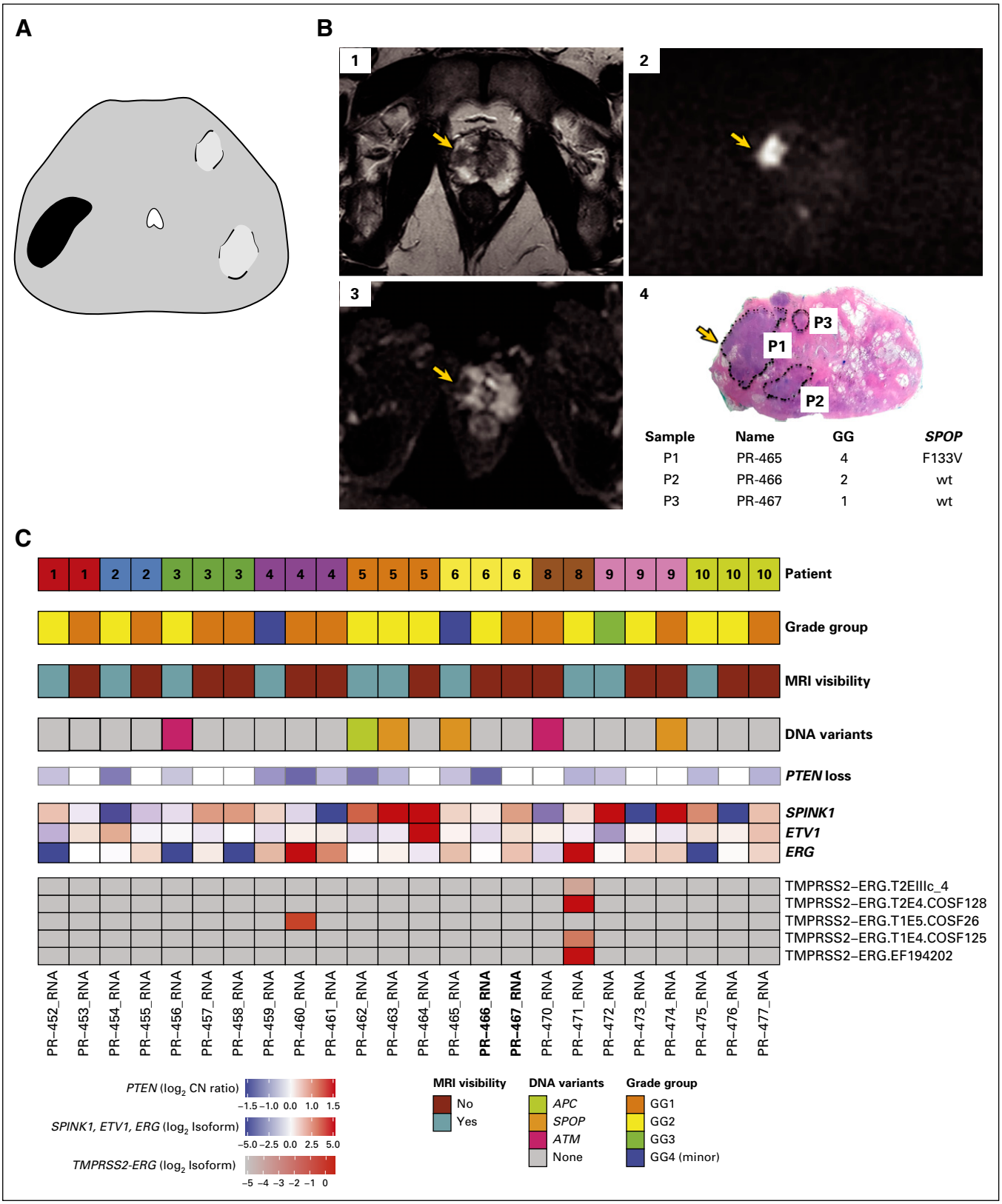


FIG 1. Radiogenomic characterization of multifocal prostate cancer. (A) Cartoon depicting multifocal prostate cancer (PCa) with both multiparametric magnetic resonance imaging (mpMRI)–visible (solid black, left) and invisible (gray with black discontinuous borders, right) lesions. (B) Coregistration of axial mpMRI images with whole-mount histopathology. (1) Axial high-resolution T2, (2) axial diffusion-weighted imaging (*b*-value = 1,600), and (3) axial apparent diffusion coefficient map shows a visible lesion corresponding to cancer focus P1 (grade group [GG] 4; arrows) on the (continued on following page)

Prognostic Significance of mpMRI-Based Gene Expression Signature

A total of 375 patients with genome-wide expression profiles were pooled from two independent case-cohort studies[20,21] to constitute the testing cohort. The mpMRI-based nine-gene expression signature was applied to the testing cohort to predict mpMRI visibility status. Kaplan-Meier curves and Cox proportional hazard regression were used to evaluate the performance of this signature in predicting oncological outcomes: biochemical recurrence-free survival (BFS), distant metastasis–free survival (DMFS), and PCa-specific mortality (PCSM). Multivariable analyses were performed to evaluate this signature as an independent predictor of oncological outcomes after adjusting for relevant clinicopathological variables, including preoperative prostate-specific antigen, pathologic grade group (GG), surgical margins, extraprostatic extension, seminal vesicle invasion, and lymph node invasion. Spearman correlation analysis was performed to measure the association of the gene signature with cellular organization pathway activity. Mean expression of genes involved in the cellular organization pathway on the Oncotype Dx genomic prostate score (GPS; Genomic Health, Redwood City, CA) assay was correlated with the mpMRI-based gene expression signature.[27] Statistical analyses were performed in *R* version 3.3.3, and all statistical tests were two-sided using a .05 significance level.

RESULTS

Study Cohorts

The discovery cohort included 10 patients from the University of Michigan PCa database with both mpMRI-visible and -invisible lesions (Fig 1). The clinicopathological characteristics of the discovery cohort are shown in the Data Supplement. Of the 26 cancer foci identified on surgical pathology specimens, 12 foci (46%) were visible on mpMRI. Among the 14 mpMRI-invisible foci (54%), five (36%) were GG2 and the remainder were GG1 (Fig 1C and Data Supplement). There were 16 patients in the validation cohort, of whom eight (50%) had mpMRI-invisible cancer lesions, and two of these (25%) were GG2 (Data Supplement). A summary of patient-level characteristics of the testing cohort (n = 375) stratified by predicted mpMRI visibility status is shown in the Data Supplement. The median age at RP was 62 years, and median follow-up time for censored patients was 8 years. During follow-up, 136 (36.3%) patients experienced biochemical recurrence, 55 (14.7%) developed metastasis, and 28 (7.5%) died as a result of PCa (Data Supplement).

Detection of Mutations and Copy Number Alterations in the Discovery Cohort

We detected high-confidence mutations in 14 of 26 (54%) tumor foci; six (43%) of the mutations were identified in mpMRI-invisible lesions. Notable somatic point mutations were in *APC*, *ARID1B*, *ATM*, *NOTCH1*, and *SPOP*. We detected *PTEN* one copy number loss in 25% (three of 12) and 14.3% (two of 14) of mpMRI-visible and -invisible foci, respectively (Fig 1C).

Discovery and Validation of a Nine-Gene Expression Signature for mpMRI Visibility

Of the 26 total tumor foci in the discovery cohort and 306 amplicons on the RNAseq panel, 24 samples and 74 amplicons, respectively, passed quality control parameters and underwent DE analysis (Data Supplement). Using DE analysis (Data Supplement) and RF classifier (Data Supplement) to identify candidate differentially expressed genes, we interrogated four separate logistic regression models for predicting mpMRI tumor visibility status using the 19 DE analysis genes, 20 RF genes, 11 shared genes between the DE analysis and RF gene sets, and 11 shared genes combined with the mutually exclusive genes (Data Supplement). A multivariable RNAseq-based logistic regression model with the best performance for predicting mpMRI visibility status, comprising a nine-gene expression signature, was developed from the intersection of the DE analysis and RF gene sets (Fig 2A; Data Supplement). This signature correctly predicted seven (70%) of the mpMRI-visible and 13 (93%) of the mpMRI-invisible foci in the discovery cohort, yielding an area under the curve of 0.89. The optimal probability cutoff for predicting mpMRI-visible tumor was greater than 0.46, with a sensitivity and specificity of 80% and 86%, respectively, in the discovery cohort (Figs 2A and 2B). We observed underexpression of seven of the nine genes in mpMRI-visible tumors, the majority of which were stromal, cellular organization, and structure genes (Fig 2A; Data Supplement).

The nine-gene expression signature was then evaluated in the independent validation cohort (Cedars-Sinai Medical Center) using the predetermined optimal probability cutoff (from the discovery cohort) to predict mpMRI visibility status. The receiver operating characteristic curve in the validation cohort is shown in Fig 2B, with an area under the curve of 0.88. The sensitivity and specificity of the signature

FIG 1. (Continued). radical prostatectomy specimen (hematoxylin and eosin, panel 4). Cancer foci P2 (GG 2) and P3 (GG 1) were both mpMRI invisible. (C) Integrative summary of the primary multifocal PCa cohort. Ten patients comprising 26 distinct PCa foci were evaluated. Two samples (patient 7) did not pass initial RNA quality thresholds and were thus omitted. Each patient had at least one MRI-visible and one MRI-invisible cancer focus. Recurrent DNA variants are shown. Log_2 copy-number ratio for *PTEN* is also shown. *PTEN* one copy number loss was observed in 25% (three of 12) and 14.3% (two of 14) of mpMRI-visible and invisible cancer foci, respectively (false discovery rate, less than 5%). Expression of *SPINK1*, *ERG*, and *ETV1*, as well as expressed isoforms of *TMPRSS2-ERG* are shown.

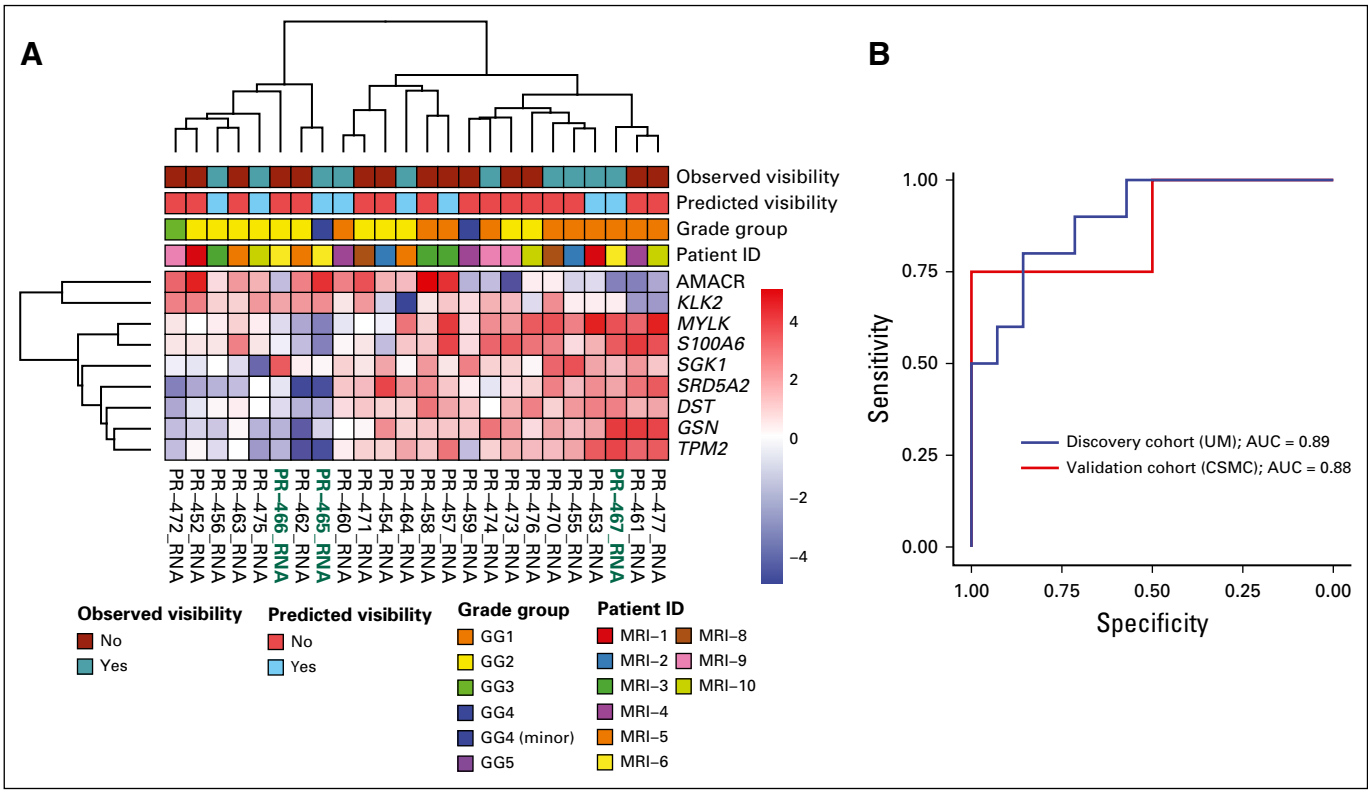


FIG 2. Development and validation of a nine-gene signature to predict multiparametric magnetic resonance imaging (mpMRI)–visible tumors. (A) Annotated heat map of differentially expressed genes in the training cohort. Differentially expressed genes were identified with the EdgeR package using a Benjamini-Hochberg procedure adjusted false discovery rate cutoff of less than 0.05. Targets and samples were clustered using hierarchical clustering on the basis of Euclidian distances. Comparisons of observed (first row) versus predicted (second row) mpMRI visibility using the nine-gene signature are shown in the annotation, as well as International Society of Urological Pathology grade group. (B) Receiver operating characteristic curves for the signature in the discovery (University of Michigan [UM]) versus the validation (Cedars-Sinai Medical Center [CSMC]) cohorts. The signature was developed with multivariable ridge logistic-regression model using cross-validation for λ hyperparameter selection. The area under the curve (AUC) for the signature was not significantly different between the discovery and the validation cohorts (0.89 *v* 0.88, Delong's unpaired *t* test, $P = .877$). The optimal probability cutoff for predicting mpMRI-visible tumor was greater than 0.46, with a sensitivity and specificity of 75% and 100% in the validation cohort, respectively.

for predicting mpMRI visibility status were 75% and 100%, respectively, in the validation cohort. Notably, the signature correctly predicted two GG2 cancers that were mpMRI invisible in the validation cohort.

Prognostic Significance of the Nine-Gene mpMRI Visibility Expression Signature

The distribution of each gene composing the nine-gene signature in the normalized microarray data (testing cohort) from the Decipher GRID mapped to The Cancer Genome Atlas Prostate Adenocarcinoma (TCGA-PRAD) RNAseq closely resemble that of the discovery cohort (Data Supplement). We applied the expression signature to the testing cohort as a proxy for mpMRI tumor visibility. Of the 375 patients in the testing cohort, 177 (47.2%) were classified as mpMRI visible (Data Supplement). Using the predicted probability as a surrogate for mpMRI, we found that the mpMRI visibility signature was not a predictor of BFS, DMFS, or PCSM (Fig 3; all log-rank $P > .05$). Similar findings were observed when the testing cohort data were not mapped to the TCGA-PRAD RNAseq cohort (Data Supplement; all log-rank $P > .05$). Adjusting for relevant clinicopathological variables on multivariable analysis, we found that genomic signature–determined mpMRI visibility status was not an independent predictor of BCR, metastasis, or PCSM (Fig 4; Data Supplement; all $P > .05$). Similar findings were observed when the testing cohort data were not mapped to the TCGA-PRAD RNAseq data (Data Supplement; all $P > .05$)

Molecular Basis of Cancer Visibility on mpMRI

Using our multiplex (mx) RNAseq data from the discovery cohort, we derived commercially available tissue-based prognostic biomarker test scores (Myriad Prolaris cell cycle progression [mxCCP] score, Oncotype DX [mxGPS], and the GenomeDX genomic classifier [mxGC]) for each cancer focus, as previously described.[15] We found no significant difference in the mxCCP, mxGPS, and mxGC scores between mpMRI-visible and -invisible foci (Fig 5A; all $P > .05$). However, as described above, we observed

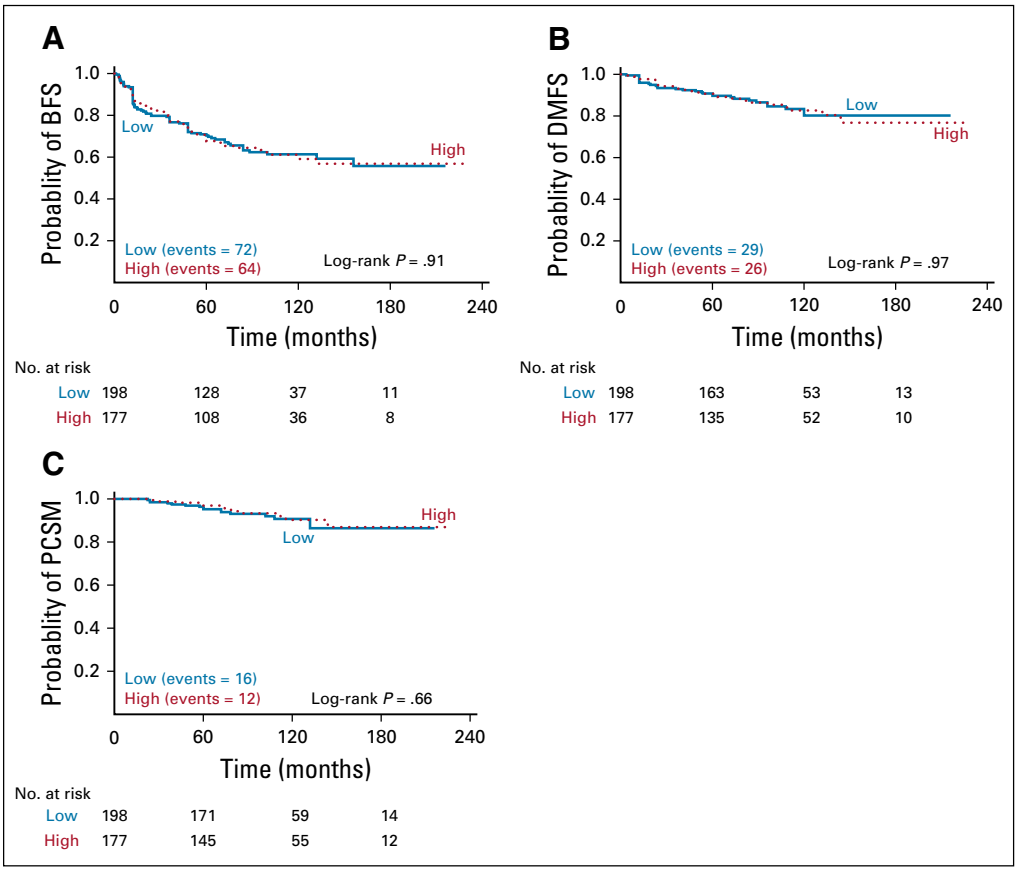


FIG 3. Prognostic significance of predicted multiparametric magnetic resonance imaging (mpMRI) visibility status. Patients (n = 375) in the testing cohort were pooled from two independent case-cohort studies (Johns Hopkins Medical Institute [n = 260] and Mayo Clinic [n = 235]) to test the capacity of predicted mpMRI visibility status to predict (A) biochemical recurrence-free survival (BFS), (B) distant metastasis-free survival (DMFS), and (C) prostate cancer–specific mortality (PCSM). The expression data in this cohort were generated using Affymetrix human exon 1.0 ST array (Santa Clara, CA). Normalization was performed to match the distribution of the genomic data from this cohort to The Cancer Genome Atlas Prostate Adenocarcinoma RNAseq data, as described in Methods, to facilitate testing of the RNASeq-based nine-gene signature to predict mpMRI-visible tumors (Data Supplement). mpMRI visibility status was computed using the signature: high score denotes mpMRI-visible and low score denotes mpMRI-invisible tumor. Kaplan-Meier survival curves were plotted and compared between predicted mpMRI-visible and -invisible tumor using log-rank test. There were no significant differences in BFS, DMFS, and PCSM between predicted mpMRI-visible and -invisible tumors (all $P > .05$). Similar results were obtained using the Affymetrix microarray data that were not matched to the distribution of the The Cancer Genome Atlas Prostate Adenocarcinoma RNAseq data (Data Supplement).

underexpression of seven of the nine genes in mpMRI-visible tumors, the majority of which were stromal, cellular organization, and structure genes (Fig 2A; Data Supplement). We then computed three subcomponents of the OncotypeDx GPS, as previously described,[15,27] and compared these between mpMRI-visible and -invisible tumors. There were no significant differences in the expression of OncotypeDx GPS androgen signaling and stromal response submodules between mpMRI-visible and -invisible tumors (Fig 5B; both $P > .05$). However, we found underexpression of the cellular organization submodule of the OncotypeDx GPS panel in mpMRI-visible tumors consistent with the results of the nine-gene signature (Fig 5B; all $P = .014$). Similarly, using data from the testing cohort, we found underexpression of the OncotypeDx GPS cellular organization module in predicted mpMRI-visible compared with -invisible foci (Data Supplement; all $P < .05$). Taken together, these findings suggest that loss of cellular organization and structure contributes to PCa visibility on mpMRI.

DISCUSSION

To better understand the molecular alterations associated with mpMRI visibility and prognostic significance of mpMRI-invisible disease, we performed a comprehensive molecular characterization of primary multifocal PCa inclusive of both mpMRI-visible and -invisible tumor foci

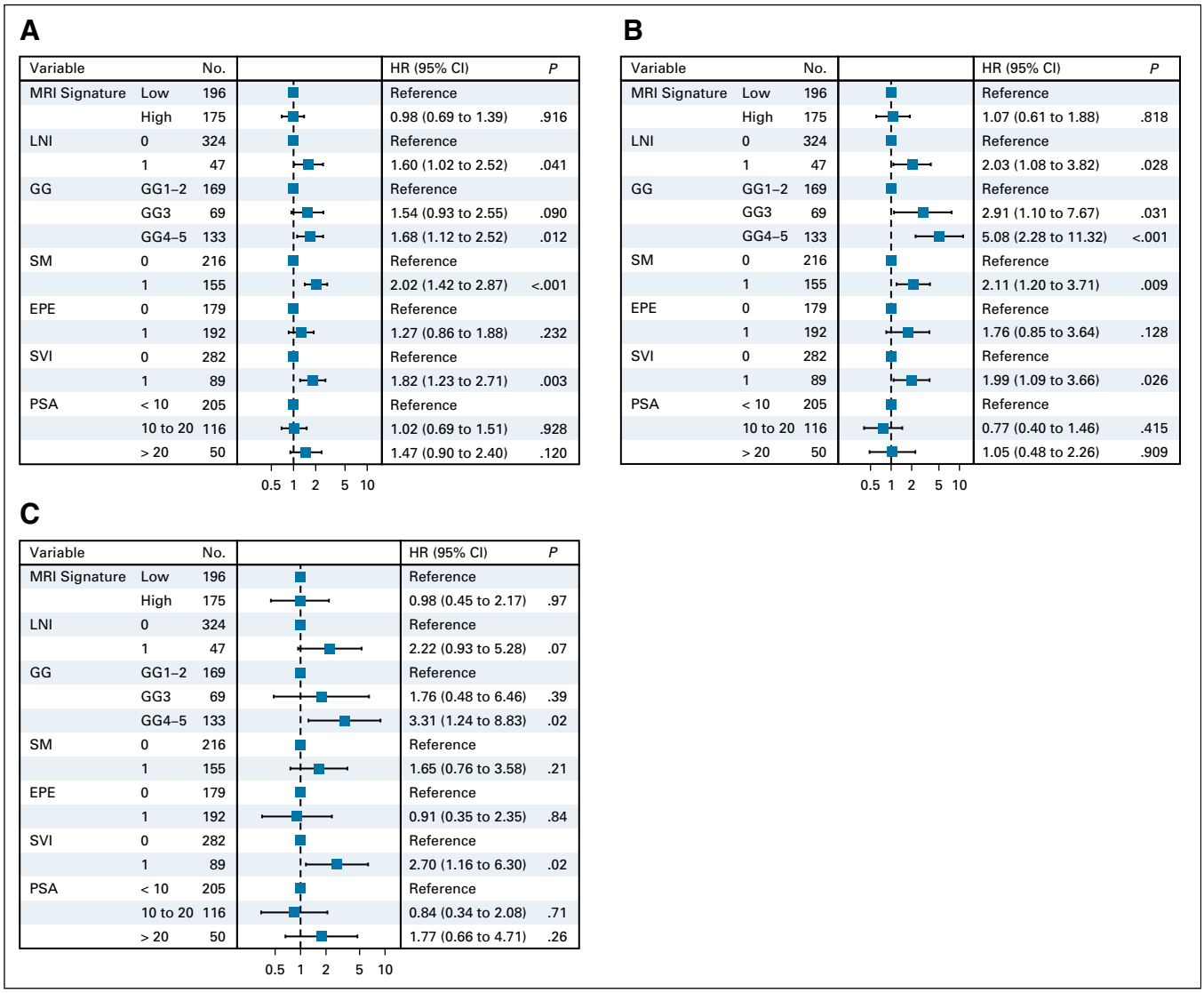


FIG 4. Multivariable analysis to assess the prognostic significance of predicted multiparametric magnetic resonance imaging (mpMRI) visibility status. Using data from the testing cohort described in Figure 3 (Affymetrix microarray data matched to the distribution of the The Cancer Genome Atlas Prostate Adenocarcinoma RNAseq data; n = 375), multivariable Cox proportional hazard models were developed to assess the capacity of predicted mpMRI visibility status to predict: (A) biochemical recurrence-free survival (BFS), (B) distant metastasis–free survival (DMFS), and (C) prostate cancer–specific mortality (PCSM), adjusting for relevant clinicopathological variables. mpMRI visibility status was not an independent predictor of BFS, DMFS, and PCSM (all adjusted $P > .05$). Similar results were obtained when the Affymetrix microarray data were not matched to the distribution of The Cancer Genome Atlas Prostate Adenocarcinoma RNAseq data (Data Supplement). EPE, extraprostatic extension; GG, grade group; HR, hazard ratio; LNI, lymph node invasion; PSA, prostate-specific antigen; SM, surgical margins; SVI, seminal vesical invasion.

using a targeted multiplex NGS approach. We observed that mpMRI-invisible cancer may possess mutations in known cancer-associated genes, with close to 15% harboring *PTEN* one copy number loss. Using robust biostatistic methods, we developed and validated a novel nine-gene signature to predict PCa mpMRI visibility status. Interrogation of this signature in a distinct cohort with long-term follow-up revealed no significant association with BFS, DMFS, or PCSM. Intriguingly, additional analyses revealed that underexpression of genes associated with cellular organization and structure may underpin the molecular basis of PCa visibility on mpMRI. Taken together, these findings indicate that mpMRI-invisible cancer foci harbor many of the same aggressive molecular features as mpMRI-visible foci and may also be clinically significant.

The molecular basis of PCa visibility on mpMRI is poorly understood. Although tumor size and grade contribute to cancer visibility on mpMRI, the architecture of the glands may play an important role.[28-31] For example, tumors harboring cribriform Gleason pattern 4 were less likely to be detected by mpMRI compared with poorly formed or fused glands, suggesting that tumor size and grade alone do not

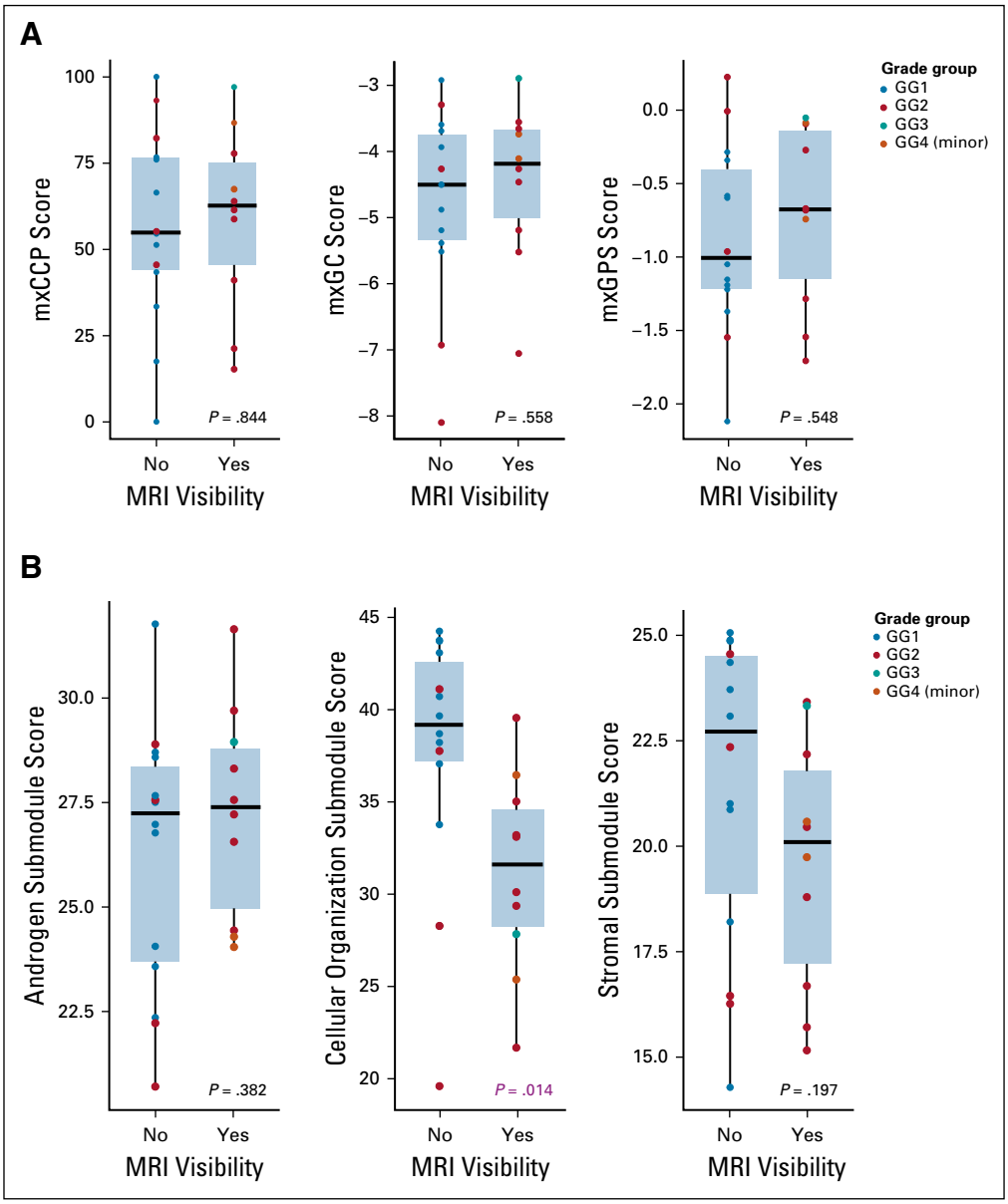


FIG 5. Derivation and comparison of expression-based prognostic scores between multiparametric magnetic resonance imaging (mpMRI)–visible and -invisible lesions. (A) Box plots of derived Prolaris cell cycle progression (mxCCP) score, Oncotype DX genomic prostate score (mxGPS), and Decipher genomic classifier (mxGC) stratified by mpMRI visibility status in the discovery cohort (n = 10 patients; 26 cancer foci). Points represent individual cancer focus colored according to International Society of Urological Pathology grade group (GG). Unpaired *t* tests were used to test for significant differences in mean score. There was no statistically significant difference between the derived prognostic scores of mpMRI-visible and -invisible lesions ($P > .05$). (B) Comparisons of derived mxGPS submodules stratified by mpMRI visibility status. Box plots of derived mxGPS androgen, cellular organization, and stromal submodules stratified by mpMRI visibility status are shown, with each point representing an individual cancer focus colored according to GG. Unpaired *t* tests were used to compare mean subcomponent scores. Only the cellular organization submodule had a significant difference in mean expression ($P = .014$), suggesting that at the RNA expression level mpMRI visibility is related to underlying cellular organization of the tumor.

explain cancer visibility on mpMRI.[28] In a recent report, Li et al[19] observed significant fold changes of differentially expressed genes on the basis of mpMRI visibility regardless of Gleason score or tumor size, including genes involved in cytoskeleton organization. Similarly, the majority of genes composing our novel mpMRI visibility signature are involved in cytoskeletal organization and structure. Other smaller-scale studies have reported the possible role of *CHD1* deletion[32] and *PTEN* loss[33,34] in PCa mpMRI visibility. In the current study, we found that 25% of

mpMRI-visible foci in the discovery cohort demonstrated *PTEN* one copy number loss compared with 14% in mpMRI-invisible foci. In aggregate, our work and that of others suggests that cellular (dis)organization contributes significantly to the underlying basis of PCa visibility on mpMRI. Additional studies are needed to further characterize the fundamental basis of PCa visibility on mpMRI.

The prognostic significance of mpMRI-invisible PCa foci is unknown. Although PCa is multifocal and mpMRI may miss up to 35% of intermediate- to high-grade PCa, the absence of visible lesions on mpMRI has been proposed as a reason to defer confirmatory biopsy when considering active surveillance.[1,3,16,17] In addition, mpMRI is increasingly being used to identify the index or dominant cancer foci for focal therapy. To be sure, although size and grade are believed to be important, how best to define the biologically dominant cancer in multifocal disease is not known.[35] To date, no study has demonstrated the clinical trajectory of mpMRI-visualized lesions. Such a study would be a challenge to perform, given the long duration of follow-up required and the multifocal nature of PCa, with frequent coexistence of mpMRI-visible and -invisible cancers within the same gland.[31]

Salmasi et al[36] reported that the PI-RADS (a grading system for mpMRI lesion visibility) was not a significant predictor of adverse pathology at the time of RP. Similarly, Parry et al[18] found that 50% of mpMRI-invisible cancers harbored one or more genetic alterations commonly observed in metastatic castrate-resistant PCa, suggesting that mpMRI-invisible tumors may be as important as visible ones. In the study by Li et al,[19] a four-gene signature comprising genes differentially expressed between mpMRI-visible and -invisible PCa was shown to predict BFS in two external data sets. However, this signature was not developed as a predictor of or a surrogate for mpMRI cancer visibility, but rather it was selected on the basis of their common association with mpMRI visibility and metastasis. By contrast, in this first study of its kind to our knowledge, using a validated novel mpMRI-based RNAseq signature as a surrogate instrument for mpMRI visibility status, we have demonstrated that predicted mpMRI visibility status was not associated with BFS, DMFS, or PCSM during long-term follow-up. Put another way, mpMRI-invisible PCa does not seem to represent purely indolent disease; mpMRI-invisible lesions may be just as clinically relevant as mpMRI-visible disease. Future studies aimed at better defining the biologically dominant nodule and prognostic significance of mpMRI are warranted.

Our findings have significant clinical implications in the management of PCa. First, in the diagnostic setting, these data corroborate findings from several institutions indicating that a negative mpMRI does not rule out the presence of clinically significant PCa[3,17,31] and should therefore not preclude a prostate biopsy without consideration of clinical risk.[37,38] Second, in the setting of active surveillance, our findings underscore the potential for mpMRI-invisible cancer foci to harbor similar biologic trajectories as mpMRI-visible disease. Although additional studies are needed to delineate the utility of mpMRI in reducing the frequency of surveillance biopsies, the current literature supports systematic in addition to targeted biopsies in men undergoing active surveillance.[3,6] Last, for men considering focal therapy, our data demonstrate that mpMRI alone is not sufficient to rule out the presence of a potentially lethal, nondominant cancer focus.

Our study has several limitations. First, we used a targeted NGS approach; thus it is conceivable that other potential alterations implicated in mpMRI cancer visibility may have been missed. Notwithstanding, our novel RNAseq signature developed from a targeted NGS approach demonstrated high fidelity for predicting mpMRI visibility in the validation cohort, where tumors underwent whole-transcriptome profiling. Second, we did not use the commercially available platforms for Oncotype Dx, Prolaris, and Decipher assays in the discovery cohort. The validity and consistency of deriving these scores from RNAseq data has been previously reported.[15] Third, there were no GG4 and 5 lesions in our cohort. However, our novel RNAseq signature demonstrated high accuracy for predicting mpMRI visibility in the validation cohort, including 19% GG5 lesions. Moreover, GG4 and 5 lesions are generally mpMRI visible, and such patients routinely undergo whole-gland therapy. Fourth, the discovery cohort was made up of a relatively small sample, with low proportion of *ERG*-positive tumors. Nonetheless, we similarly observed high test performance in the validation cohort with ERG overexpression in 31% of samples. Fifth, what constitutes mpMRI-visible or -invisible lesions is not purely objective. To facilitate reproducibility, all lesions in the current study were scored according to the validated PI-RADS v2 system. PI-RADS 1 and 2 lesions were classified as mpMRI invisible, and PI-RADS 3 to 5 were classified as mpMRI visible. Finally, the prognostic significance of mpMRI-invisible cancer was evaluated in the testing cohort using a surrogate molecular marker for mpMRI visibility status. Thus, additional studies are needed to delineate the prognostic significance of mpMRI-invisible PCa in a prospective clinical cohort.

Discerning aggressive from indolent disease remains a significant clinical challenge in the evaluation and management of men with primary PCa. Our findings indicate that mpMRI-invisible cancers were no less likely to harbor lethal biologic potential than visible tumors, highlighting the limitation of using mpMRI alone to guide patient management or delineate specific index cancer foci for ablative therapy. Our results also highlight the continued need for biopsy strategies that detect mpMRI-invisible tumors. Future PCa molecular studies are needed to further characterize the molecular basis of cancer visibility on mpMRI and determine its prognostic significance.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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