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Evaluation of a 24-Gene Signature for Prognosis of Metastatic Events and Prostate Cancer-Specific Mortality

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Abstract

OBJECTIVES—To determine the prognostic potential of Sig24 for identifying prostate cancer patients at risk of developing metastases or experiencing PCSM following radical prostatectomy.

SUBJECTS AND METHODS—Sig24 scores were calculated from previously collected gene expression microarray data from the Cleveland Clinic and Mayo Clinic (I and II). The performance of Sig24 was determined using time-dependent c-index analysis, Cox proportional hazards regression and Kaplan-Meier survival analysis.

RESULTS—Higher Sig24 scores were significantly associated with higher pathologic Gleason scores (GS) in all three cohorts. Analysis of the Mayo Clinic II cohort, which included time to event information, indicated that patients with high Sig24 scores also had an increased risk of developing metastasis (HR: 3.78, 95% CI: 1.96–7.29, $p < 0.001$) or experiencing PCSM (HR: 6.54, 95% CI: 2.16–19.83, $p < 0.001$).

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Conflicts of Interest

Qi Long and Carlos Moreno are co-inventors on a patent application related to the Sig24 gene signature. María Santiago-Jiménez, Mandeep Takhar, Nicholas Erho, Kasra Yousefi, and Elai Davicioni are employees of GenomeDx Biosciences.

CONCLUSIONS—The findings of this study demonstrate the applicability of Sig24 for the prognosis of metastasis or PCSM following radical prostatectomy. Future studies investigating the combination of Sig24 with available prognostic tests may provide new approaches to improve risk stratification for patients with prostate cancer.

Keywords

Biomarker; Prostate Cancer; Metastasis

INTRODUCTION

Radical prostatectomy (RP) is a curative intent treatment that is frequently employed for patients with clinically localized prostate cancer, significantly reducing the risk of metastasis and prostate cancer-specific mortality (PCSM) (1–3). Periodic measurement of serum PSA levels post-RP can identify disease recurrence at an early stage so that additional treatment such as radiation therapy or androgen deprivation therapy (ADT) can be initiated. However, there are many patients who would benefit from adjuvant radiation or ADT immediately following prostatectomy to prevent the recurrence of ultimately fatal disease (4–6). Pathologic analysis of the primary tumor is conducted to identify features that indicate the cancer may have metastatic potential such as invasion of the seminal vesicles or lymph nodes, and extracapsular extension, in addition to the presence of more poorly differentiated tissue architecture as described according to the Gleason grading system. While there are various methods and nomograms to determine risk group, typically over 50% of patients classified as high risk will experience PCSM within 10 years of RP (7). Although a lower proportion of patients with low and intermediate risk cancer experience PCSM, there are still many patients in these groups with aggressive cancers that could benefit from adjuvant therapies.

More recently, a number of multi-gene expression panels demonstrating prognostic potential for outcomes such as biochemical recurrence, metastasis, or PCSM have been described (8–10). Integration of clinical and genomic parameters can allow a more detailed understanding of each patient's tumor pathology and their associated risk of adverse events, offering additional information for the identification of high-risk patients before disease recurrence. We recently described the development and validation of a 24-gene signature (referred to as Sig24) that improved the stratification of patients at increased risk of biochemical recurrence following radical prostatectomy (11). In our current study, we sought to determine whether Sig24 also had prognostic value for the identification of patients at risk of metastasis or PCSM following radical prostatectomy.

SUBJECTS AND METHODS

Patient Cohort

Decipher GRIDTM (Genomic Resource Information Database) is a genomic sharing and collaborative research platform for oncology that contains tumor RNA microarray expression profiles for thousands of patients and was used to assess the expression of the Sig24 genes in primary prostate tumor specimens collected at the time of radical

prostatectomy for patients in three independent cohorts: (1) a case control set of 182 patients from Cleveland Clinic (12), (2) a case control set of 545 patients from the Mayo Clinic (10, 13), and a case cohort set of 235 patients from the Mayo Clinic (14). The Institutional Review Boards of each participating institution approved the research protocols under which the data were collected and shared as part of Decipher GRIDTM. The relevant characteristics of each cohort are summarized in Supplementary Table 1.

Calculation of Sig24

The assayed specimens in the Cleveland Clinic cohort were prepared by taking two 0.6 mm diameter tissue cores with a biopsy punch tool to sample the primary Gleason grade of the index lesion of the FFPE RP block (12). For both Mayo Clinic cohorts, the assayed specimens were prepared by macrodissecting tumor from surrounding stroma for up to four 10 µm tissue sections of the primary Gleason grade within the index lesion (the lesion with the highest pathologic Gleason score) of the RP (10, 13, 14). Gene expression analysis for all patients had been conducted previously by GenomeDx using the Affymetrix Human Exon 1.0 ST GeneChip platform according to manufacturer recommendations (10). This data was normalized using the Single Channel Array Normalization (SCAN) algorithm as described previously (15) and the Sig24 score for each patient was calculated by combining the expression of the 24 component genes with the relevant coefficient (11). These genes and coefficients were originally discovered and trained on a cohort of 100 men for the endpoint of biochemical recurrence, and include genes involved in processes such as cell cycle progression, angiogenesis, apoptosis, chromatin modification, and transcription (11).

Statistical Analyses

Descriptive statistics of variables focused on medians, interquartile ranges (IQR), 95% confidence intervals (95% CI), frequencies, and proportions as appropriate. Correlations between Sig24 score and Gleason score were computed using Spearman's rank correlation. In time to event analyses, event times were defined as the time from RP to outcome. Time-dependent c-indices were used to evaluate the performance of Sig24 in Mayo Clinic II cohort using the nearest neighbor estimator with a span parameter of 0.001 as described by Heagerty, *et al.* (16). Decision curve analysis was used to evaluate the net benefit of the combined clinical+Sig24 model across probability thresholds as compared to treat all or treat none scenarios (17). Survival curves were constructed using Kaplan-Meier methods. Univariable (UVA) and multivariable (MVA) Cox proportional hazards models were used to evaluate the impact of clinicopathologic features and Sig24 score on the outcomes. In the case of the Mayo Clinic II cohort, control patients were re-weighted by the inverse of the sampling fraction (20%) and the Prentice method for case-cohort design was used in the UVA and MVA models to inflate the variance (18). Accordingly, the re-weighted Mayo Clinic II cohort was used to determine time-dependent c-indices (19). For the combination of clinical features with Sig24, Hosmer-Lemeshow analysis was used to verify that the risk predicated by the combined model accurately reflected observed risk in the study population (Supplementary Figure 1). Optimal Sig24 thresholds for metastasis and PCSM were developed based on the Mayo Clinic I cohort using the Youden index statistic and survival curves were constructed for the Mayo Clinic II cohort using Kaplan-Meier methods. All

statistical tests were two-sided and had a significance level of 0.05. Analyses were performed in R v3.1 (R Foundation, Vienna, Austria).

RESULTS

Sig24 Score was Significantly Associated with Aggressive Tumor Pathology

To investigate the association our gene signature with tumor aggressiveness, we analyzed the correlation of Sig24 scores with pathological Gleason score (GS) in the Cleveland Clinic (CC), Mayo Clinic I (MCI), and Mayo Clinic II (MCII) cohorts. Amongst all cohorts, higher Gleason scores were significantly associated with higher Sig24 scores, with positive correlation values of 0.46 (95% CI: 0.34–0.57), 0.32 (95% CI: 0.25–0.40), and 0.32 (95% CI: 0.20–0.42) for the CC, MCI, and MCII cohorts, respectively (all $p < 0.001$). In the CC cohort, the median Sig24 score was observed to increase by at least 0.1 for each Gleason grade increase ($p < 0.001$; Figure 1A). A similar trend was observed for MCI and MCII, although in these cohorts the most apparent increase in Sig24 score occurred when comparing patients with GS 7 versus those with GS 8 ($p < 0.001$; Figures 1B and 1C). Across the three cohorts, the majority of patients with a pathological Gleason score 6 had a Sig24 score below the median (74.5%, 79 of 106 patients), while patients with a pathological Gleason score 8 tended to have Sig24 scores above the median (90.4%, 320 of 354 patients).

Patients with Higher Sig24 Scores were More Likely to Develop Metastatic Disease

To determine the prognostic ability of Sig24, we focused our analyses on the Mayo Clinic II cohort as it included information on time to event that was necessary for survival analysis. Of the 235 patients in the MCII cohort, 76 experienced metastasis (32.3%) with a median time to metastasis of 39 months (IQR: 20–62 months). Median follow-up time for patients who did not experience metastasis was 84 months (IQR: 59–109 months). Overall, the 5- and 10-year metastasis-free survival rates were 93.2% (95% CI: 91.5–94.9%) and 88.7% (95% CI: 86.1–91.3%), respectively. The median Sig24 score was higher for patients who experienced metastasis within the follow-up period (0.13, IQR: 0.01–0.23) than for those who did not (−0.03, IQR: −0.15–0.10; Figure 2A), and the time-dependent AUC of Sig24 at 5 years was 0.69 (95% CI: 0.61–0.75; Figure 2B and 2C). This was higher than the AUCs for serum PSA (0.52, 95% CI: 0.50–0.58), pathologic Gleason score (0.64, 95% CI: 0.55–0.69), and pathologic tumor stage (0.60, 95% CI: 0.55–0.71), while a clinical model combining all of these parameters also had an AUC of 0.69 (95% CI: 0.62–0.77; Figure 2B and 2C). The addition of Sig24 score to this clinical model improved the time-dependent AUC to 0.73 (95% CI: 0.66–0.78; Figure 2B and 2C). Decision curve analysis indicated that this combined model offered a net benefit across threshold probabilities of 5–20% for metastasis within 5 years (Figure 2D).

Univariable analysis demonstrated that Sig24 had a hazard ratio (HR) of 3.60 (95% CI: 2.04–6.37), which meant that Sig24 scores above the median were accompanied by a 3.6-fold greater risk of developing metastasis ($p < 0.001$; Supplementary Table 2). Other individual parameters that were significantly associated with risk of metastasis included pathological GS 8, extracapsular extension, seminal vesicle invasion, and androgen

deprivation therapy (Figure 2E). When the combined effects of these factors were considered in multivariable analysis, only Sig24 score and pathological GS 8 remained significantly associated with risk of metastasis (Figure 2F). Of these factors, Sig24 score had the highest HR (3.78, 95% CI: 1.96–7.29, $p < 0.001$; Supplementary Table 2).

The Risk of Prostate Cancer-Specific Mortality was Positively Associated with Sig24 Scores

We also investigated the association of Sig24 score with prostate cancer-specific mortality (PCSM), which 34 of the patients (14.5%) in the MCII cohort experienced. Median time to PCSM was 59 months (IQR: 37–79 months) and median follow-up among those who did not experience PCSM was 84 months (IQR: 60–107 months). Overall, the 5- and 10-year PCSM-free survival rates were 97.5% (95% CI: 96.3–98.6%) and 92.7% (95% CI: 90.0–95.6%), respectively. The median Sig24 score was 0.18 (IQR: 0.09–0.26) for patients who experienced PCSM within the follow-up period, and 0.00 (IQR: –0.14–0.12) for those who did not (Figure 3A), and the time-dependent AUC of Sig24 at 10 years was 0.72 (95% CI: 0.63–0.81; Figure 3B and 3C). This was higher than the AUCs for serum PSA (0.52, 95% CI: 0.50–0.71), pathologic Gleason score (0.67, 95% CI: 0.59–0.77), pathologic tumor stage (0.62, 95% CI: 0.47–0.71), and the overall clinical model (0.69, 95% CI: 0.67–0.87; Figure 3B and 3C). The consideration of Sig24 in combination with the clinical model improved the time-dependent AUC to 0.74 (95% CI: 0.63–0.85; Figure 3B and 3C). Decision curve analysis indicated that the combined model offered a net benefit across threshold probabilities of 5–25% for prognosis of PCSM within 10 years (Figure 3D).

Individual parameters significantly associated with higher risk of PCSM included Sig24 score, pathological GS 8, extracapsular extension, seminal vesicle invasion, and androgen deprivation therapy, with Sig24 score having the highest HR (4.71, 95% CI: 2.02–11.02, $p < 0.001$; Figure 3E and Supplementary Table 3). Sig24 score was also significantly associated with PCSM in multivariable analysis (HR: 6.54, 95% CI: 2.16–19.83, $p < 0.001$), as were pathological Gleason score 8 (HR: 7.41, 95% CI: 2.45–22.40, $p < 0.001$), extracapsular extension (HR: 2.95, 95% CI: 1.01–8.59, $p = 0.05$), and serum PSA between 10 and 20 ng/mL, which showed an inverse association (HR: 0.26, 95% CI: 0.08–0.92, $p = 0.04$; Figure 3F and Supplementary Table 3).

Sig24 has Prognostic Potential for Metastasis and PCSM

To establish the prognostic performance of Sig24, we conducted Kaplan-Meier analyses of the MCII cohort using thresholds determined by applying the Youden index statistic to the MCI cohort. These Sig24 thresholds were –0.1322 for metastasis (75% sensitivity, 42% specificity) and –0.0316 for PCSM (54% sensitivity, 64% specificity; Supplementary Figure 2). The 5-year metastasis-free survival rate for patients with high Sig24 scores was 90.9% (95% CI: 88.5–93.2%) compared to 98.7% (95% CI: 97.3–100.0%) for patients with low Sig24 scores. The 10-year metastasis-free survival rates were 85.4% (95% CI: 81.9–89.0%) and 96.2% (95% CI: 93.5–99.1%) for patients with high and low Sig24 scores, respectively ($p < 0.001$; Figure 4A). The 5-year PCSM-free survival rate for patients with high Sig24 scores was 92.6% (95% CI: 90.1–95.1%) compared to 98.6% (95% CI: 97.4–99.8%) for patients with low Sig24 scores. The 10-year PCSM-free survival rates were 89.1% (95% CI:

85.5–92.9%) and 97.8% (95% CI: 96.2–99.5%) for patients with high and low Sig24 scores, respectively ($p < 0.001$; Figure 4B).

DISCUSSION

In this study, we have evaluated the Sig24 gene panel for prognosis of metastasis and PCSM. We began by establishing that Sig24 scores were significantly associated with pathological Gleason score. As the pathological Gleason score is frequently used as a measure of prostate cancer aggressiveness, gene expression by Sig24 could be of particular use in situations where tumor pathology is ambiguous or borderline. When we investigated the direct association between Sig24 and outcome, we found significantly higher scores in patients that went on to develop metastasis or experience PCSM. This was further supported by both univariable and multivariable analysis where higher Sig24 scores were significantly associated with an increased risk of metastasis and PCSM, indicating that Sig24 offers prognostic information in addition to other clinical parameters. Kaplan-Meier analysis also demonstrated that patients stratified by Sig24 had significantly different metastasis and PCSM outcomes. It is important to note that only the Mayo Clinic II cohort included the time to event data that allowed us to study the association of Sig24 with metastasis and PCSM. Further analysis of this association in additional independent cohorts would provide an understanding of how generalizable these results are.

The work presented here offers a complementary method for determining a patient's risk of disease recurrence, which could promote more specific prognosis and initiation of additional therapies before recurrence is detected. Previous studies have shown that physicians are willing to incorporate multi-gene expression data into their clinical practice to assist in post-RP treatment decisions – both to encourage the initiation of additional treatments in patients identified as high-risk, and to identify low-risk patients that can safely proceed with observation (20–24). In a specific example of this, the use of Decipher GC was shown to improve the identification of patients most likely to benefit from adjuvant radiotherapy (25). This demonstrates a scenario in which adjuvant radiotherapy could be specifically targeted to the patients who will benefit, while avoiding unnecessary treatment for patients who can be safely managed with a salvage approach. The continued investigation of Sig24 will focus on how best to combine it with currently available gene signatures and whether it has particular relevance for other clinical decision points.

Sig24 has been included in Decipher GRID and will be calculated for all Decipher patients as part of GRID, setting the stage for future study of Sig24. In the current study, we have established that the Sig24 score is associated with a higher risk of metastasis and PCSM. Further development of this biomarker panel will focus on the optimal approach for combining Sig24 with available assays to improve the stratification of patients with high-risk prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Distribution of Sig24 scores amongst patients with different Gleason scores
 Sig24 scores were determined for the Cleveland Clinic (A), Mayo Clinic I (B), and II (C) cohorts. For each Gleason group, the median Sig24 score is represented by the horizontal black line, with the interquartile range and 1.5x the IQR represented by the box and whiskers, respectively. Outliers are shown as points beyond the boxplot whiskers.

Figure 2. Analysis of Sig24 in metastasis (Mets)

The association between Sig24 and metastasis was investigated in the Mayo Clinic II cohort. Distribution of Sig24 scores amongst patients that either did or did not develop metastasis within the follow-up period is shown in the box plots, where the horizontal black lines represent the median score and the notches indicate the 95% confidence interval (A). Time-dependent AUCs were determined for the association of metastasis at 5 years post-RP with Sig24 score, serum PSA, pathologic Gleason score, and pathologic tumor stage, individually and in combination (B and C). Decision curve analysis was conducted to determine the net benefit of the combined model versus scenarios where no prediction models are required or used (D). Univariable (E) and multivariable (F) analyses of Sig24 and other parameters relevant to metastasis were carried out by Cox proportional hazards regression ($p < 0.05$ shown with filled diamonds).

Figure 3. Analysis of Sig24 in prostate cancer-specific mortality (PCSM)

The association between Sig24 and PCSM was investigated in the Mayo Clinic II (E–H) cohort. Distribution of Sig24 scores amongst patients that either did or did not experience PCSM within the follow-up period is shown in the box plots, where the horizontal black lines represent the median score and the notches indicate the 95% confidence interval (A). Time-dependent AUCs were determined for the relationship of PCSM at 10 years post-RP with Sig24 score, serum PSA, pathologic Gleason score, and pathologic tumor stage, individually and in combination (B and C). Decision curve analysis was conducted to determine the net benefit of the combined model versus scenarios where no prediction models are required or used (D). Univariable (E) and multivariable (F) analyses of Sig24 and other parameters relevant to PCSM were carried out via Cox proportional hazards regression ($p < 0.05$ shown with filled diamonds).

Figure 4. Kaplan-Meier analysis of metastasis (Mets) and prostate cancer-specific mortality (PCSM) in the Mayo Clinic II cohort

Patients in the Mayo Clinic II cohort were grouped as low or high based on cut-points determined by Youden Index analysis of the Mayo Clinic I cohort (Supplementary Figure 1), and Kaplan-Meier analysis was conducted for metastasis (A) and PCSM (B).