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Transcriptomic Heterogeneity of Gleason Grade Group 5 Prostate Cancer

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eururo.2020.05.009.

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Abstract

Gleason grade group (GG) 5 prostate cancer has been associated with an aggressive natural history, and retrospective data support a role for treatment intensification. However, clinical outcomes remain heterogeneous in this cohort, and intensified treatments carry an increased risk of adverse events. We sought to explore the transcriptomic heterogeneity of GG 5 tumors by querying transcriptomic data from the tumors of 2138 patients with GG 5 disease who underwent prostatectomy. Four distinct consensus clusters were identified with respect to differential transcriptional activation of hallmark pathways, with distinct molecular subtyping profiles and

different average genomic risks (AGRs). One cluster, accounting for 325 tumors (15.2% of the population), was enriched for genes related to the cell cycle/proliferation, metabolic pathways, androgen response pathways, and DNA repair, and had a higher AGR than the other clusters ($p < 0.001$). This clustering, with an identification of a high genomic risk cluster, was subsequently validated in a separate cohort of 1921 patients as well as a third cohort of 201 patients. The latter cohort had outcomes available, and it was found that patients in the high genomic risk cluster had significantly worse distant metastasis-free survival than the other clusters. Tumors in this high genomic risk cluster of GG 5 disease may be particularly likely to benefit from treatment intensification.

Patient summary: In this report, we examined differences in gene expression in tumors from men with Gleason grade group 5 prostate cancer. We identified significant diversity, with one specific subgroup of tumors associated with expression profiles that suggest a worse prognosis.

Keywords

Gleason grade group 5; Gleason score 9; Gleason score 10; Biomarkers; Transcriptomics

Gleason grade group (GG) 5 prostate cancer (PCa) is now recognized as a distinct histopathological entity [1] with significantly worse PCa-specific mortality outcomes following definitive radiotherapy [2] or radical prostatectomy [3] than all classes of lower-grade tumors. Patients with GG 5 disease may stand to benefit from local treatment intensification, as suggested by recent retrospective data evaluating patients treated with definitive radiotherapy and upfront radical prostatectomy [4,5]. The impact of systemic therapy intensification, for example, with earlier incorporation of second-generation antiandrogen therapeutics or cytotoxic chemotherapy, is unknown, but is supported by the observation of lower benefit to long-term conventional androgen deprivation therapy with definitive radiotherapy in patients with GG 5 cancers when compared with patients with GG 4 disease [2].

However, clinical outcomes remain quite heterogeneous. In a large multi-institutional cohort of 1809 patients with GG 5 disease, patients treated with external beam radiotherapy and androgen deprivation therapy or radical prostatectomy had 10-yr distant metastases rates of ~43%, suggesting that many men with GG 5 will not develop rapid metastases [4]. As neither local nor systemic treatment intensification can be delivered sans an increased risk of adverse events, a better understanding of this interpatient heterogeneity is required. We sought to explore the transcriptomic heterogeneity of GG 5 tumors, with the hypothesis that this histopathological entity could be clustered in an unbiased manner into subgroups based on distinct expression profiles that may harbor different prognoses with respect to long-term outcomes.

To probe the transcriptomic heterogeneity of GG 5 disease, we queried the Decipher Genomic Resource Information-Database, which contains prospectively obtained transcriptomic data from radical prostatectomy specimens for which the Decipher test was run between February 2014 and August 2017 (NCT02609269). For all specimens, tumor RNA was extracted from formalin-fixed, paraffin-embedded blocks, with microdissection of the Gleason pattern 5 component guided by a genitourinary pathologist. At least 0.5

mm^2 of tumor and ≥60% cellularity was required. Whole transcriptomes of 2138 patients with GG 5 disease were obtained. The Molecular Signatures Database was queried for 56 hallmark gene sets, with gene set scores computed by averaging the abundance of each gene in the set [6]. Patient pathway expression profiles were partitioning around medoids clustered based on Spearman's correlation distances. Consensus clustering [7] bootstrapped over 1000 iterations with 75% sampling of both patients and pathways was used to arrive at a robust clustering solution. Quantitative variables were summarized using quartiles (median and interquartile range), and differences among the clusters were compared using Kruskal-Wallis test. Qualitative variables were summarized using frequencies and percentages, and compared using chi-square tests. Dunn's test and pairwise chi-square test for variables with significant global p values ($p < 0.05$) across clusters were performed in a series of post hoc analyses in which p values were adjusted for multiple testing using the Benjamini and Hochberg method. As outcomes data are not available for patients in this prospective cohort, we also chose to evaluate the association between consensus clusters and two prognostic biomarkers. The first, the Decipher score, has been validated extensively and consists of a 22-gene signature with locked cut points of low (<0.45), intermediate (0.45–0.60), and high (≥0.6) risks [8]. The second, the average genomic risk (AGR), is a previously reported metascore derived from combining scores across 18 prognostic signatures that had been found to achieve univariate significance for the endpoint of distant metastasis specifically [9].

We identified four distinct clusters, representing putative pathway-based subtypes of GG 5 PCa. Clusters 1 (the high genomic risk cluster [gray], $n = 325$, 15%) and 2 (the low genomic risk cluster [brown], $n = 383$ patients, 18%) had almost reciprocal patterns of hallmark pathways. The third and fourth clusters (named the blue [$n = 624$, 29.2%] and the purple [$n = 806$, 37.7%] cluster, respectively) were much more similar to one another. The low genomic risk cluster showed transcriptional activation of the immune response, angiogenesis, transforming growth factor beta signaling, *KRAS* signaling, and certain developmental pathways. The high genomic risk cluster is enriched for genes related to the cell cycle/proliferation, metabolic pathways, androgen response pathways, and DNA repair. Blue and purple clusters have intermediate RNA activation of these pathways, with slightly higher representation of proliferative and metabolic pathways in the purple cluster and immune response pathways in the blue cluster (Fig. 1). Boxplots for DNA repair, immune response, and androgen response pathways by cluster are presented in Fig. 2.

We compared Decipher and AGR scores across clusters (Fig. 2 and Table 1). The median AGR for patients in the high genomic risk cluster was 0.69, which was significantly higher than the median AGR of 0.40 in the low genomic risk cluster ($p < 0.001$). We also compared clinical and pathological features between clusters. Global p values, indicative of a difference across clusters but not specifically identifying which pairwise comparisons may manifest significant differences, are presented in Table 1. Age, lymph node involvement, and margin status did not significantly differ across clusters, while pretreatment prostate-specific antigen, extraprostatic extension, and seminal vesicle invasion did (Table 1). Significantly more patients in the high genomic risk cluster would be classified to have luminal B by the PAM50 classifier [10] and were more likely to demonstrate Phosphatase and tensin homolog (PTEN) loss than the other clusters ($p < 0.001$). Significant differences in *ERG*-fusion status

were noted as well. The androgen receptor activity score was higher in the high genomic risk cluster than in the low genomic risk cluster [11]. PTEN loss was significantly more frequent in the high genomic risk cluster than in all other clusters ($p < 0.001$). Post hoc comparisons between clusters for variables found to be significantly different across clusters are shown in Supplementary Table 1.

We then validated this clustering in an independent dataset of 1921 patients. Cluster characteristics are presented in Supplementary Table 2, and heatmap and boxplots are shown in Supplementary Figs. 1 and 2. Once again, we identified four clusters, including a high (median AGR 0.65) and low (median AGR 0.37) genomic risk cluster. The high genomic risk cluster was again characterized by upregulation of pathways related to androgen receptor signaling, DNA repair, and proliferation, while the low genomic risk cluster had upregulation of pathways related to the immune response.

In order to explore whether clinical outcomes might differ between clusters, we performed a second validation in a cohort of 201 patients with known outcomes. Cluster characteristics are presented in Supplementary Table 3, and heatmap and boxplots are shown in Supplementary Figures 3 and 4. Crude incidences of distant metastasis and PCa-specific mortality are shown in Supplementary Table 4. Time to metastasis and PCa-specific mortality were modeled in a Cox proportional hazard model with clusters as independent covariate and summarized using hazard ratios with 95% confidence intervals; the results are shown in Table 2 and the corresponding Kaplan-Meier curves are presented in Supplementary Figure 5. Patients in the high genomic risk cluster had significantly shorter time to developing metastases than patients in any of the other clusters ($p < 0.03$).

Overall, these results suggest that GG 5 PCa exhibits significant transcriptomic heterogeneity. A subset of GG 5 tumors, accounting for ~15% of all GG 5 tumors, has significantly higher AGR scores than other GG 5 subsets and displays transcriptomic activation of proliferation, metabolic activity, androgen response, and DNA repair. This subset of GG 5 tumors is also associated with a shorter time to metastasis.

There are several limitations of our study. First, the transcriptomic profiles are derived from microdissected Gleason grade 5 tissue but may include contributions from adjacent nonmalignant cells. Second, the Decipher and AGR scores are surrogates for clinical outcomes but may not fully capture the determinants of prognosis for any given tumor. Next, the proportion of patients with a component of intraductal tumor is unknown, and prior studies have demonstrated that intraductal disease is associated with unique transcriptomic features [12]. Race and ethnicity were not available; should one attempt to develop a genomic risk score calculator, clinical variables such as these two must be integrated. Additionally, the transcriptomic data analyzed herein are clearly restricted to genes included in the microarray panel, and the experimental approach does not take into account single cell transcriptomics. Since all microarray data are based on the dissected Gleason pattern 5 component, our study cannot account for other areas of tumor that were not sampled and yet could be driving a clinically aggressive (or nonaggressive) course.

Nonetheless, these findings suggest that this identified subgroup of GG 5 tumors (the high genomic risk cluster) may be the most likely to derive benefit from treatment intensification, and rational approaches may include the use of interventions active against the pathways that are dysregulated. Further investigation into the heterogeneity of GG 5 disease, and particularly into this aggressive subgroup, is clearly warranted. Additionally, future studies of GG 5 disease identified on biopsy are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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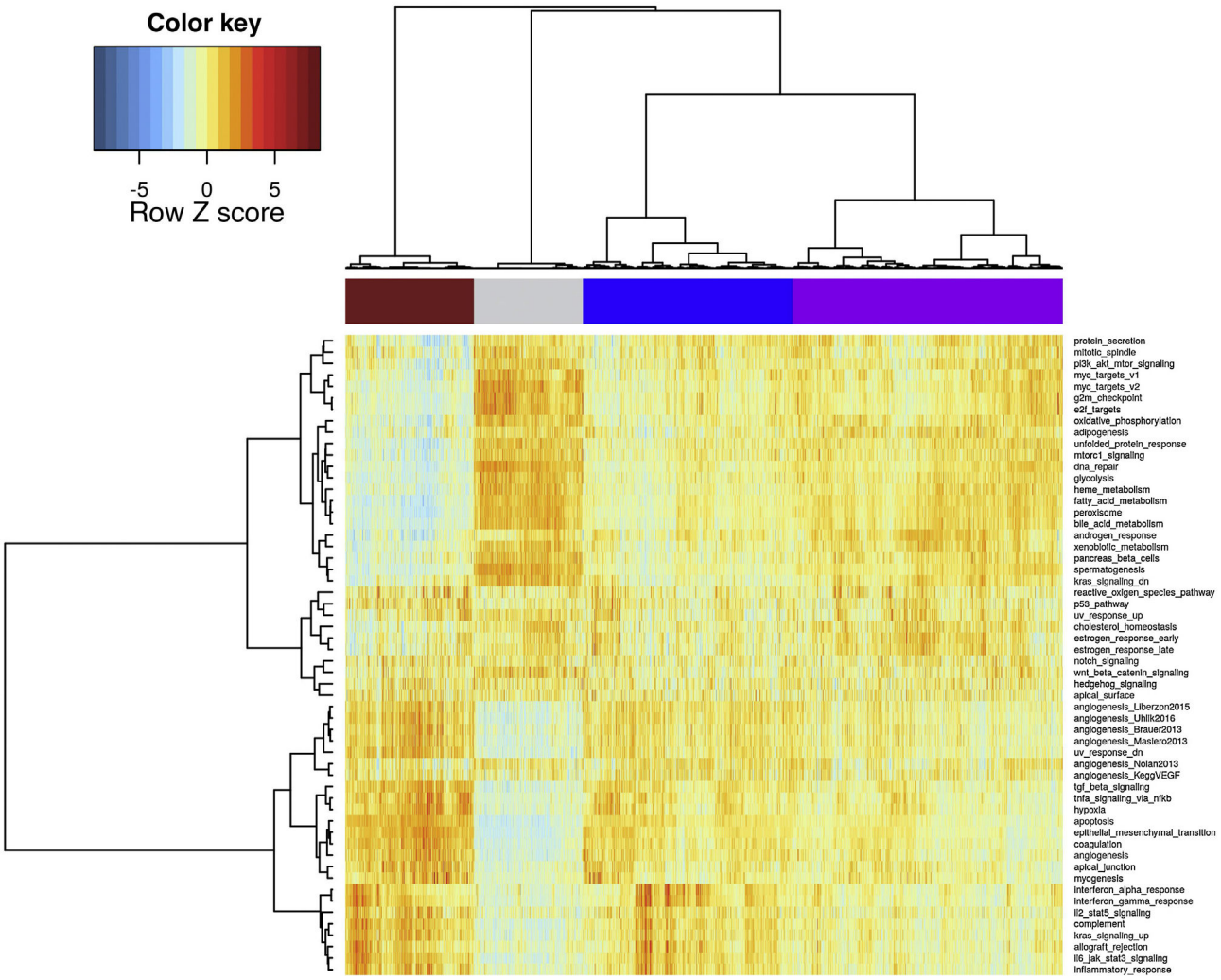

Fig. 1 –.

Heatmap of 2138 patients with Gleason grade group 5 disease after consensus clustering based on transcriptomic activation of 56 hallmark gene sets. Four clusters were identified: brown, low genomic risk cluster; gray, high genomic risk cluster; blue, blue cluster; and purple, purple cluster.

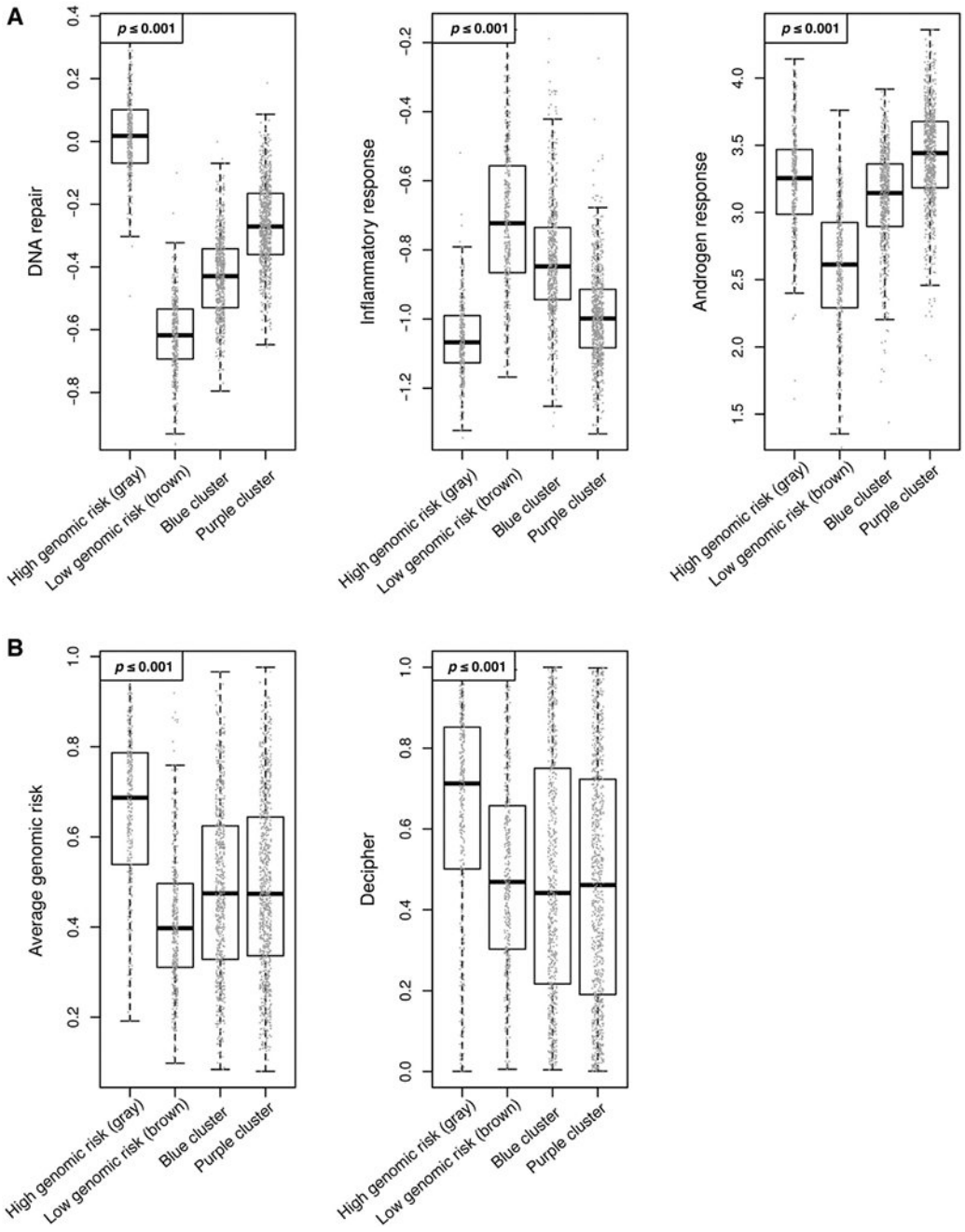

Fig. 2 –.

Box plots showing (A) transcriptional activation of hallmark pathways related to DNA repair, immune response, and androgen response, segregated by cluster; and(B) average genomic risk scores and Decipher scores.

Table 1 –

Characteristics of clusters derived from hallmark gene set transcriptomic activation profiles in the initial dataset (*n* = 2138).

	High genomic risk (gray) *n* = 325	**Low genomic risk (brown) *n* = 383**	**Blue cluster *n* = 624**	**Purple cluster *n* = 806**	***p* value**
Age, median (IQR)	66 (61–70)	66 (61–67)	67 (61–71)	67 (61–71)	0.9
Preoperative PSA, median (IQR)	8.0 (5.2–13.9)	6.8 (4.8–11.3)	7.2 (5.3–11.6)	7.8 (5.5–12.1)	0.041
Gleason score, *n* (%)					
4 + 5	263 (81)	308 (80)	493 (79)	675 (84)	0.08
5 + 4	52 (16)	62 (16)	119 (19)	120 (15)	
5 + 5	10 (3.1)	13 (3.4)	12 (1.9)	11 (1.4)	
Seminal vesicle invasion, *n* (%)	121 (42)	178 (48)	288 (47)	309 (39)	0.001
Extraprostatic extension, *n* (%)	229 (71)	282 (75)	488 (79)	575 (73)	0.02
Lymph node involvement, *n* (%)	49 (16)	53 (15)	97 (16)	111 (14)	0.8
Positive surgical margins, *n* (%)	181 (56)	189 (50)	334 (54)	399 (50)	0.2
Average genomic risk score, median (IQR)	0.69 (0.54–0.79)	0.40 (0.31–0.50)	0.47 (0.33–0.62)	0.47 (0.34–0.64)	<0.001
Decipher score, median (IQR)	0.72 (0.50–0.85)	0.47 (0.30–0.66)	0.44 (0.22–0.75)	0.46 (0.19–0.72)	<0.001
PAM50 classifier, *n* (%)					
Basal	23 (7.1)	271 (71)	224 (36)	195 (24)	<0.001
Luminal A	20 (6.1)	93 (24)	207 (33)	215 (27)	
Luminal B	282 (87)	19 (4.9)	193 (31)	396 (49)	
ERG-fusion positive, *n* (%)	102 (31)	147 (38)	260 (42)	231 (29)	<0.001
PTEN status, *n* (%)					
Normal	101 (31)	323 (84)	395 (63)	461 (57)	<0.001
Loss	139 (43)	15 (3.9)	112 (18)	171 (21)	
Indeterminate	85 (26)	45 (12)	117 (19)	174 (22)	
Androgen receptor activity, *n* (%)					
Average	302 (93)	182 (48)	530 (85)	748 (93)	<0.001
Low	23 (7.1)	201 (52)	94 (15)	58 (7.2)	

IQR = interquartile range; PSA = prostate-specific antigen.

Table 2 –

Cox proportional hazards in the second validation cohort (n = 201).

	HR (95% CI)	*p* value
Distant metastasis		
Low genomic risk vs high genomic risk	0.54 (0.32–0.91)	0.02
Cluster blue vs high genomic risk	0.46 (0.22–0.94)	0.03
Cluster purple vs high genomic risk	0.50 (0.32–0.76)	0.001
Prostate cancer–specific mortality		
Low genomic risk vs high genomic risk	0.84 (0.40–1.78)	0.7
Cluster blue vs high genomic risk	0.82 (0.31–2.22)	0.7
Cluster purple vs high genomic risk	0.52 (0.27–0.98)	0.045

CI = confidence interval; HR = hazard ratio.