Identification of a high-risk subgroup in primary prostate cancers presenting with targetable immune biology

Reilly E[1], McCavigan A[1], Walker S[1,2], McCabe N[1,2], Parkes E[2], Harkin D[1,2], Kennedy R[1,2], Knight L[1,2]

[1] Almac Diagnostics, 19 Seagoe Industrial Estate, Craigavon, UK;
[2] Centre for Cancer Research and Cell Biology, Queens University Belfast, Northern Ireland, UK;
Email: emma.reilly@almacgroup.com

Background and Methods

Background

Recent studies have demonstrated limited success of immune checkpoint therapies in unselected prostate cancer. We therefore assessed an immune based DNA Damage Repair Deficiency (DDRD) assay (Mulligan et al. 2014), that we have previously reported represents activation of the cGAS STING pathway (Parkes et al. 2017), in the TCGA prostate cancer dataset to investigate the presence of targetable immune biology in prostate cancer. In addition we applied a second assay (the prostate cancer metastatic signature-PCM) that predicts the risk of metastatic recurrence for early prostate cancer (Walker et al. 2017), in order to assess if immune therapy could have a role in treating high risk disease.

Methods

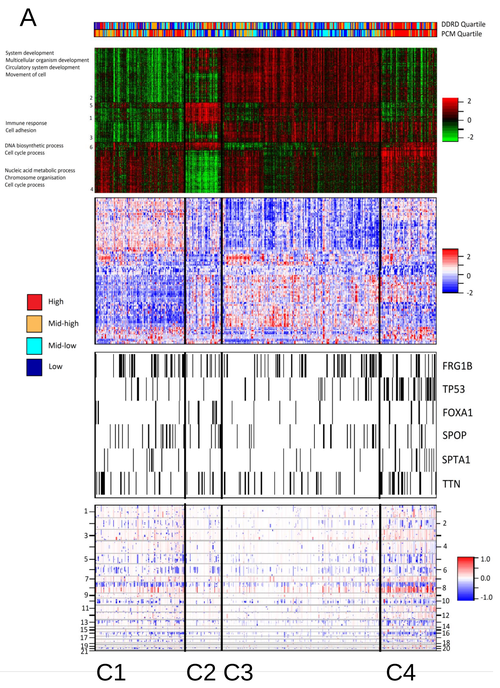
498 samples with RNA sequencing data were scored with the PCM and DDRD assays. Integrative analysis was performed on 488 samples with RNA sequencing, promoter site methylation, somatic mutation and somatic copy number variation. The clustering was performed with iClusterPlus (Qianxing Mo and Ronglai Shen (2016)) using 5380 genes that were differentially expressed between low and high PCM quartiles, 480 promoter sites with methylation data, 158 frequently mutated genes and 1868 copy number regions. Gene expression of n=6 immune checkpoint targets was investigated with the subgroups identified using T-tests. The prevalence of immune infiltration in each subgroup was tested by applying a cut off to the leukocyte fraction. The viability of reproducing the subgroups was tested in the TCGA dataset and an independent validation dataset of 321 resected primary prostate cancers. Cox proportional hazards regression analysis was performed for biochemical recurrence and metastatic events in both datasets.

Integrative Clustering

Figure A shows the results of the integrative clustering with the four data types from top to bottom: RNA, promoter site methylation, somatic mutation and copy number alteration. Four patient subgroups were identified, labelled C1:C4. These clusters were defined by differences in 1345 RNA features, 113 methylated promoter sites, 6 mutated genes and 467 somatic copy number regions. The assay quartiles are overlaid showing that:

- C1 & C4 represent patients with increased risk of developing metastatic disease - this corresponds to 42% of patients in the cohort.
- C4 differentiates from C1 in that it also represents a subgroup of DDRD positive patients.

Results

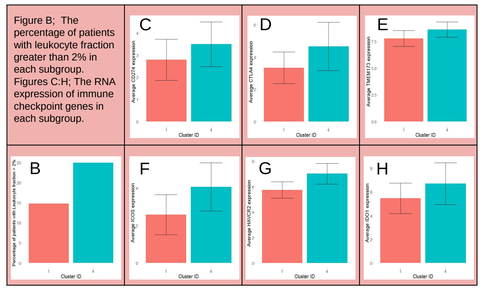


Genomic Instability

The patients with metastatic-like biology are characterised by increased copy number alteration & frequency of somatic mutation :

- C1 & C4 both show greater loss of 8p compared to C2 & C3.
- The DDRD positive cluster, C4, also shows the gain of 8q.
- In general, patients in C4 show higher CNV than those in C1.
- Patients in the Metastatic-like DDRD subgroup also exhibit a much higher prevalence of TP53 somatic mutations at 38% compared to 7% in C1.

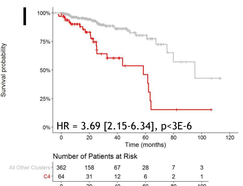
Immune Biology



The differences in RNA expression between C1 & C4 is most prominent in gene cluster 3. In this gene cluster the RNA expression in C4 is characterised by the switch on of immune signalling. Assessing n=6 immune checkpoint genes we also found that patients in C4 have elevated expression of immune checkpoint genes and also increased leukocyte infiltration compared to those in C1.

Survival

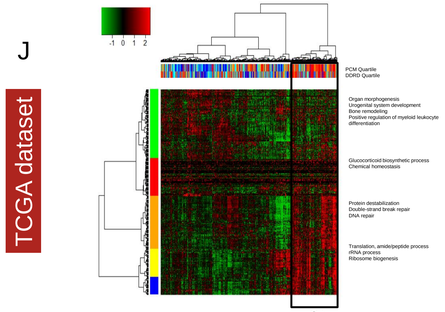
	HR	95% CI	P-value
All other clusters			
C4	2.586	1.392 - 4.803	0.003 **
Gleason 3+4			
Gleason 4+3	3.002	0.782 - 11.527	0.109
Gleason ≤6	2.128	0.354 - 12.795	0.410
Gleason ≥8	3.171	0.898 - 11.200	0.073
PSA	1.034	1.003 - 1.066	0.030 *
Age	0.979	0.938 - 1.021	0.327
T Stage 1-2			
T Stage 3	2.494	0.913 - 6.807	0.075
T Stage 4	1.976	0.351 - 11.139	0.440



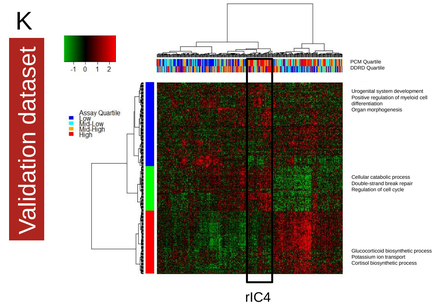
Cox proportional hazards regression analysis was performed in n=426 patients with available Biochemical recurrence follow-up information. The analysis revealed that subgroup C4 has a significantly poorer survival outcome compared to all other clusters (C1, C2 & C3 combined) in both univariate analysis and after adjustment for clinically relevant covariates.

Independent Validation

The viability of reproducing the Metastatic-like DDRD subgroup (C4) with RNA sequencing alone was tested in the 498 samples of the TCGA dataset. Given the prevalence of 8q gains in C4, genes present on the long arm of chromosome 8 were used in hierarchical clustering of the RNA Sequencing data.

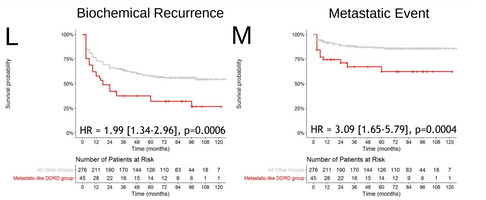


Four sample clusters were identified. The rC4 cluster, characterised by overexpression of double-strand break repair genes, captured the C4 subgroup with high sensitivity and specificity (80.2 [70.3-87.5] % & 85 [81.2-88.2] % respectively).



Hierarchical clustering using the 8q genes was also performed in an independent validation set of n=321 resected primary prostate cancers with gene expression profiles on microarray. The data were also scored for the DDRD and PCM assays. 8 sample clusters were identified, rIC4 consists of two such clusters representing 14% of the cohort which similarly to rC4 display overexpression of double-strand break repair genes and score highly for the DDRD and PCM assays.

Survival in Validation Set



Cox proportional hazards regression analysis was performed for biochemical recurrence and metastatic events in n=321 patients in the validation set. Similarly to the TCGA dataset, the Metastatic-like DDRD subgroup (rIC4) showed significantly poorer prognosis in both univariate and multivariate analysis. Multivariate biochemical recurrence analysis: HR = 1.78 [1.19-2.66], p=0.00514, Multivariate metastatic events analysis: HR = 2.47 [1.31-4.69], p =0.00549.

Conclusions

- Integrative clustering of multi-omics data revealed 2 subgroups of patients with metastatic-like biology that have distinct biology.
- 17% of the patients show greater genomic instability and present with targetable immune biology – that can be identified with the combination of the DDRD and PCM assays.
- This subgroup can be reproduced with the expression of 8q genes alone
- The group is also identified in an independent dataset and represents 14% of the n=321 cohort
- The Metastatic-like DDRD group is shown to be at increased risk of biochemical recurrence & metastatic disease
- It may represent a viable target population for immune checkpoint and DNA damaging treatment

References

- Jude M. Mulligan, et al. Identification and Validation of an Anthracycline/Cyclophosphamide–Based Chemotherapy Response Assay in Breast Cancer, *JNCI: Journal of the National Cancer Institute*, Volume 106, Issue 1, 1 January 2014
- Eileen E. Parkes, et al. Activation of STING-Dependent Innate Immune Signaling By S-Phase-Specific DNA Damage in Breast Cancer, *JNCI: Journal of the National Cancer Institute*, Volume 109, Issue 1, 1 January 2017
- Walker SM, et al. Molecular Subgroup of Primary Prostate Cancer Presenting with Metastatic Biology. Eur Urol (2017)
- Qianxing Mo and Ronglai Shen (2016). iClusterPlus: Integrative clustering of multi-type genomic data. R package version 1.10.0.