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EDITORIAL

Molecular Diagnostics

Precision medicine for prostate cancer—improved outcome prediction for low-intermediate risk disease using a six-gene copy number alteration classifier

A multiplex 6-gene copy number classifier was used to distinguish between low- or intermediate-risk prostate cancer patients. The study analysed a cohort of 448 patients and previously published datasets from radical prostatectomies. The classifier performs better than conventional stratification methods, is low cost, and can be performed easily in clinical laboratories.

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Prostate cancer (PCa) is a heterogeneous disease with varying clinical outcomes. PCa risk classification is based on prostate-specific antigen, T stage, Gleason score, impact treatment planning, clinical trial design, and outcome reporting, although individual clinical outcomes may not be predicted by the available stratification tools [1]. The lack of precision for patients with low and intermediate (favourable and unfavourable) risk tumours continues to lead to the overtreatment of clinically insignificant diseases and the undertreatment of potentially aggressive cancers [2]. In contrast to other tumours, precision medicine has developed more slowly for PCa. The addition of new molecular prognostic factors may improve risk stratification, impacting treatment strategy and clinical trials.

Copy number alterations (CNAs) are frequent events in PCa, which can lead to the loss or gain of genomic regions containing key oncogenes or tumour suppressor genes that contribute to tumour development and progression. To create a copy number abnormality classifier for outcome prediction, the genes or chromosomal regions frequently altered are first selected from the PCa literature. Next, large-scale genomic datasets, such as those generated by genome-wide sequencing or microarray analysis, are used to identify the copy number status of the genes or recurrent regions in PCa patient cohorts. Machine learning algorithms, such as logistic regression or random forest, are then trained using the copy number data and associated clinical information to generate predictive models that can be applied to specific clinical cohorts. The model's accuracy is then validated using several independent cohorts of patients. Several recent studies in PCa have used these approaches to highlight the potential of various CNA classifiers of outcome and treatment response [3, 4].

The intrinsic genomic heterogeneity of PCa tumours makes accurate and reproducible evaluation of a new CNA classifier particularly challenging. The design of the classifier must consider intratumour genotypic tumour heterogeneity, in which there may be distinct subpopulations of tumour cells with different CNA profiles and potentially different responses to treatments. In addition, the CNA interpretation must address variations in sample quality, the possibility of normal tissue contamination, and technical variables related to the analytical platform.

Multiplex ligation-dependent probe amplification (MLPA) copy number analysis is particularly beneficial in cancer settings since it is unaffected by formalin fixation and can provide a comprehensive analysis of multiple genes that may have a collaborative impact on the outcome (reviewed in [5]). Compared to other CNA techniques, such as array comparative genomic hybridization, next-generation sequencing, and fluorescence in situ hybridization, MLPA is a relatively simple and inexpensive method that requires less DNA consumption (~50 ng per assay) and has a low cost (~$6 USD per reaction). The MLPA CNA assay can also be easily run using standard molecular genetic laboratory equipment [3].

In this issue, Ebrahimizadeh et al. present a promising new six-gene CNA classifier that has the potential to improve clinical management for PCa patients with low- or intermediate-risk disease [6]. The authors employed Random Survival Forest analysis of their MLPA targeting 14 genes to identify the best predictive CNAs [3]. They identified 6 features, two new deletions at 1p21.3 (*RWDD3*) and 8p12 (*WRN*) in addition to two established deletions at 10q23 (*PTEN*) and 17p13 (*TP53*) and known gains at 8q24 (*MYC*) and 16p13.3 (*PDPK1*). They then examined the predictive power for biochemical recurrence of this six-gene classifier using 448 radical prostatectomy samples by testing the association with biochemical recurrence and comparing their results to the known prognostic index CAPRA-S score. A unique aspect of their experimental design was to compare intratumour classifier variation using DNA from two separate needle core biopsies taken from tumour regions with the highest and lowest Gleason scores. This double-sampling strategy also ensured that a CNA profile was still obtained in instances of inadequate DNA quality/quantity or failed MLPA reaction of one of the samples. In addition, the authors rigorously validated the predictive agreement of their six-gene classifiers using three previously published radical prostatectomy datasets [7–9] and one radiation dataset [10] blinded to clinical endpoints (see Fig. 1).

The study included an investigation of the predictive value of well-known genes such as *RB1*, *CHD1*, and *NKX3.1* in low-intermediate risk diseases. Surprisingly, these genes did not contribute to outcome prediction in the studied cohort, despite their established role in

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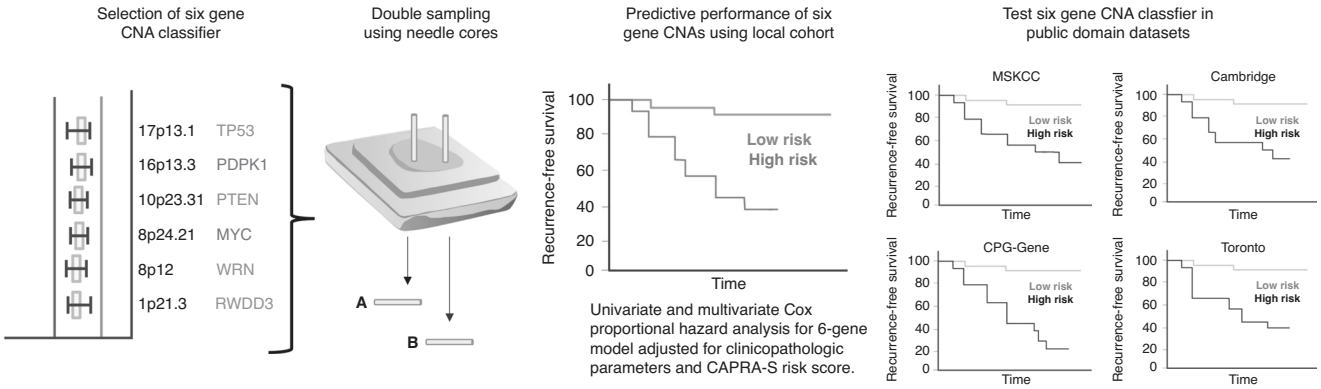

Fig. 1 Development of prostate cancer six-gene classifier for accurate prediction of recurrence in patients with low-intermediate disease. Schematic depiction of gene copy number selection for classifier using Random Forest survival and Cox proportional hazard modelling. Genes are shown in blue = loss and red = gain. The cohort analysis involved double sampling using needle cores from differing Gleason score regions (A and B) that can address variation in tumour sampling due to technical issues or genetic heterogeneity. Analysis of CNAs for all six genes from both needle core samples was used to determine the risk of recurrence. The classifier's performance was compared to the CAPRA-S risk score and four public domain outcome cohorts.

tumour progression and recurrent CNAs in PCa. Instead, the researcher's six-gene classifier includes two genes, *RWDD3* and *WRN*, which have not previously been reported in cohort studies of outcome. Loss of *RWDD3* has been found to increase the expression of RSUME, a protein that stabilises and enhances the function of PTEN. As Ebrahimizadeh et al. discuss, loss of *WRN* has previously been linked to biochemical recurrence and genomic instability in PCa. Further investigations are needed to better understand the role of *RWDD3* and *WRN* in the biology of PCa tumour progression.

FUTURE PROSPECTIVE

Precision medicine for PCa management requires new genetic biomarker classifications of patient subgroups to benefit from specific therapies. The CNA approach presented by Ebrahimizadeh et al. provides a valuable foundation for utilising diagnostic needle core biopsies to reduce unnecessary treatment for patients with PCa. Active surveillance, which involves closely monitoring the disease and intervening when necessary, may now be a more feasible option for low-risk PCa patients [11]. Hopefully, clinicians will soon be able to use specific CNA classifiers designed to accurately distinguish low-risk tumours that can be monitored without immediate treatment from aggressive tumours that require early intervention to prevent disease progression. If this approach is validated on diagnostic needle biopsies of PCa, it could lead to a more personalised approach to patient management that reduces unnecessary treatments and improves therapeutic outcomes.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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