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Platinum Priority – Brief Correspondence

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A Multigene Assay Identifying Distinct Prognostic Subtypes of Clear Cell Renal Cell Carcinoma with Differential Response to Tyrosine Kinase Inhibition

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Abstract

Patients with clear cell renal cell carcinoma (ccRCC) have divergent survival outcomes and therapeutic responses, which may be determined by underlying molecular diversity. We aimed to develop a practical molecular assay that can identify subtypes with differential prognosis and response to targeted therapy. Whole-genome expression analysis of formalin-fixed paraffin-embedded (FFPE) material from 55 ccRCC patients was performed and two molecular subtypes with differential clinical outcomes were identified by hierarchical clustering. An eight-gene quantitative polymerase chain reaction assay for classification into two subtypes was developed for FFPE material. The primary objective was to assess assay performance by correlating ccRCC prognostic subtypes to cancer-specific survival (CSS) and, for patients receiving targeted therapy, radiologic response. In three validation cohorts, patients could be distinguished into prognostic subtypes with differential CSS (Singapore General Hospital FFPE cohort: $n = 224$; $p = 1.48 \times 10^{-8}$; the Cancer Genome Atlas RNA-Sequencing cohort: $n = 419$; $p = 3.06 \times 10^{-7}$; Van Andel Research Institute microarray cohort: $n = 174$; $p = 0.00743$). For 48 patients receiving tyrosine kinase inhibitor (TKI) treatment, the prognostic classification was associated with radiologic response to treatment ($p = 5.96 \times 10^{-4}$) and prolonged survival on TKI treatment ($p = 0.019$). The multigene assay can classify ccRCCs into clinical prognostic subtypes, which may be predictive of response in patients receiving TKI therapy.

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About 30% of localized clear cell renal cell carcinomas (ccRCC) relapse after curative surgery [1]. While tumor stage at presentation remains the most reliable predictor of clinical course of disease after surgery, survival outcomes are heterogeneous within each staging group [2]. For advanced ccRCC, survival and treatment response are similarly variable, even in the era of targeted therapy [3,4]. Extensive molecular characterization of ccRCC suggests that subtypes exist with distinct survival advantages [5,6]. In such a heterogeneous disease setting, discovering reliable biomarkers that can improve prognostic determination and identify patients likely to benefit from treatment is of high

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priority. Consideration of molecular features of ccRCCs in existing risk-stratification models for predicting survival after treatment may enhance clinical decision making [7,8]. In this study, we developed a practical expression-based assay with utility in formalin-fixed paraffin-embedded (FFPE) material that assigns biologic subtypes of ccRCC, characterized by differential prognosis and treatment response.

The study was conducted retrospectively with a cohort of 279 ccRCC patients who underwent surgery at Singapore General Hospital (SGH) between 1999 and 2012. Patient characteristics are described in Supplemental Table 1 and the clinical data collection process in the Supplement. The overall analysis pipeline is described in Supplemental Figure 1. Initially, to identify relevant biologic subtypes of ccRCC, RNA was extracted from a set of 55 FFPE samples (SGH-55) and processed for whole-genome expression analysis by Whole Genome (WG)-DASL (Illumina, San Diego, CA, USA) (Supplement 1).

Hierarchical clustering based on expression of 3740 transcripts measured by WG-DASL partitioned samples from SGH-55 into two main groups (Fig. 1A; Supplemental Table 2). Kaplan-Meier analysis showed that the two biologically determined subtypes of ccRCC differed in cancer-specific survival (CSS) (hazard ratio [HR]: 8.70; 95% confidence interval [CI], 1.69–44.89; $p = 0.00185$) (Fig. 1B). The subtypes were also associated with relevant clinicopathologic features: tumor grade, stage, and size (Supplemental Fig. 2). Potential prognostic genes were selected from gene features that were significantly different between the two subtypes (Supplement; Supplemental Table 3).

Quantitative polymerase chain reaction (qPCR) assays were designed for measuring expression in FFPE tissue; expression data for potential prognostic and normalization genes for SGH-55 were collected. Processing of qPCR expression data, selection of prognostic genes, and development of the prediction model are described in the Supplement. A model assigning prognostic subtype was developed based on the combination of qPCR expression values of eight genes: chemokine (C-X-C motif) ligand 5 (*CXCL5*), ephrin A5 (*EFNA5*), endomucin (*EMCN*), laminin beta3 (*LAMB3*), plasminogen (*PLG*), preferentially expressed antigen in melanoma (*PRAME*), retinoic acid receptor responder (tazarotene induced) 1 (*RARRES1*), and solute carrier family 6 (neutral amino acid transporter), member 19 (*SLC6A19*).

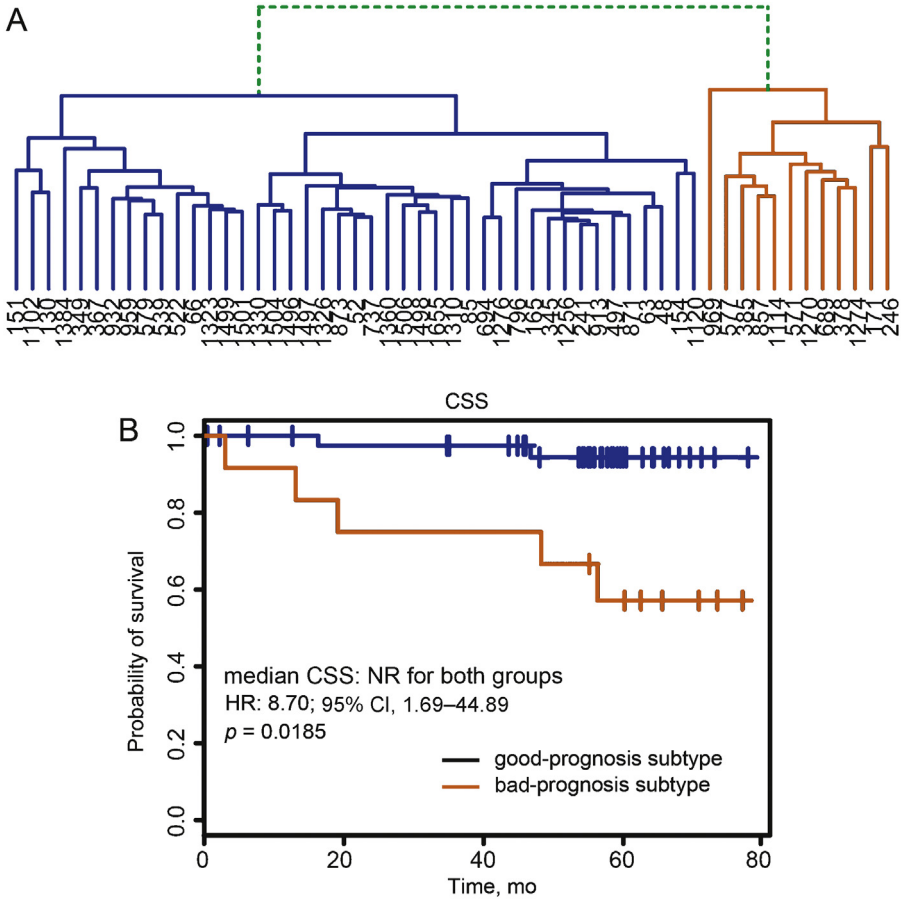

Fig. 1 – Hierarchical clustering analysis of 55 clear cell renal cell carcinomas (ccRCC) based on DASL expression data identifies two prognostic subtypes. (A) Cluster dendrogram of 55 ccRCC samples grouped by expression of 3740 genes measured by DASL analysis. Two main groups are formed ($n_1 = 43$ and $n_2 = 12$), denoted by blue branches and orange branches in the dendrogram. (B) Kaplan-Meier curves of cancer-specific survival (CSS) for two prognostic subtypes generated by hierarchical clustering. Survival in the good-prognosis subtype is significantly better than in the poor-prognosis group (log-rank test $p = 0.0185$).
CI = confidence interval; HR = hazard ratio; NR = not reached.