Molecular Dissection of Clear Cell Renal Cell Carcinoma Reveals Prognostic Significance of Epithelial-Mesenchymal Transition Gene Expression Signature

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Abstract

Background: There is an ongoing need to develop prognostic biomarkers to improve the management of clear cell carcinoma (ccRCC).

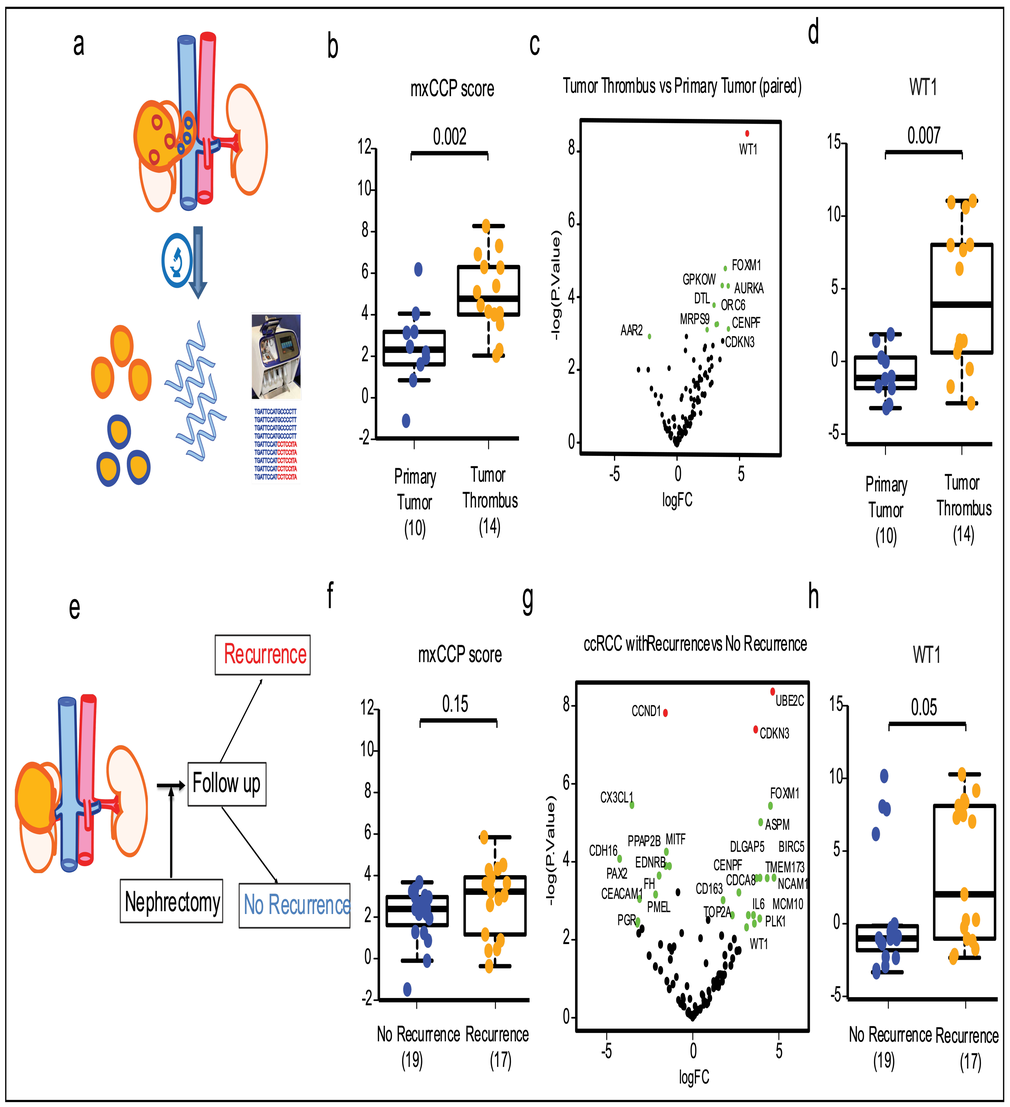
Methods: We retrospectively identified two complementary discovery cohorts of patients with ccRCC who underwent: 1) radical nephrectomy (RNx) with inferior vena cava (IVC) tumor thrombectomy (Patients=5, Samples=24); and 2) RNx for localized disease and developed recurrence vs. no recurrence (n=36). Using TCGA ccRCC cohort for validation (n=386), Kaplan-Meier (KM) survival analysis and multivariable cox-proportional hazard testing were utilized to investigate the prognostic impact of cell cycle proliferation (CCP) and a novel 22-gene epithelial mesenchymal transition (EMT) score on progression free survival (PFS) and disease specific survival (DSS).

Results: In the discovery cohorts, we observed over-expression of WT1 and CCP genes in the tumor thrombus vs. the primary tumor, as well as in patients with recurrence vs. those without recurrence. Hallmark pathway analysis demonstrated enrichment of EMT and CCP related pathways in patients with high WT1 expression in the TCGA (validation) ccRCC cohort. CCP and EMT scores were derived in the validation cohort and stratified into four risk groups using Youden-Index cut points:

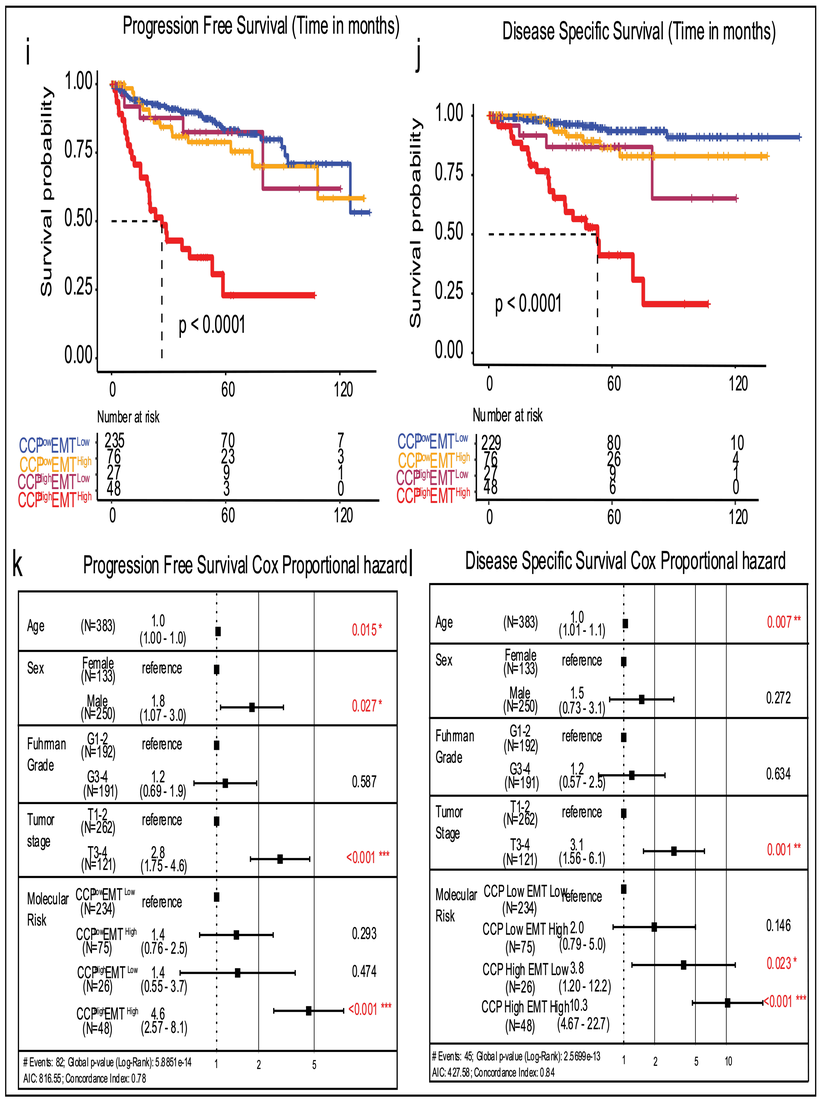
CCP^{low}/EMT^{low} **CCP^{low}/EMT^{high}**
CCP^{high}/EMT^{low} **CCP^{high}/EMT^{high}**

CCP^{high}/EMT^{high} risk group was associated with the worst PFS and DSS (both p<0.001). In a multivariable analysis, CCP^{high}/EMT^{high} was independently associated with poor PFS and DSS (HR=4.6 and 10.3, respectively; p<0.001).

Conclusions: We demonstrate the synergistic prognostic impact of EMT in tumors with high CCP score. Our novel EMT score has the potential to improve risk stratification and provide potential novel therapeutic targets.



a) Molecular dissection of primary kidney cancers and synchronous inferior vena cava (IVC) tumor thrombi in the *discovery* cohort. Patients with ccRCC and synchronous IVC tumor thrombus (n=5) who had undergone radical nephrectomy with IVC tumor thrombectomy were retrospectively identified (# of samples = 30). **b**) Boxplot displaying derived cell cycle proliferation (mxCCP) scores. Tumor thrombi demonstrated higher CCP scores compared with matched primary ccRCC (unpaired two-sided t-test, p <0.01). **c**) Differential gene expression analyses. Paired differential expression analysis revealed over-expression of *WT1* and proliferation genes in tumor thrombi compared to primary ccRCC tumors. Genes with Log-likelihood ratio test FDR < 5% were deemed to be significantly differentially expressed. **d**) Boxplots displaying *WT1* log2 expression. Tumor thrombi demonstrated higher *WT1* expression compared with matched primary ccRCC (unpaired two-sided t-test, p <0.01). **e)** Molecular profiling of primary ccRCC with and without recurrence following nephrectomy (n=36). We matched patients who developed recurrence to patients without recurrence during follow up in ratio 1:1 based on tumor stage, grade and duration of follow up. **f**) Boxplot displaying derived mxCCP scores. There was no significant difference between the CCP scores of patients with and without recurrent disease (unpaired two-sided t-test, p = 0.15). **g**) Differential expression analysis revealed significant over-expression of *WT1* and CCP genes in patients with disease recurrence (FDR < 5%). **h**) Boxplot displaying *WT1* log2 expression. Patients who developed disease recurrence demonstrated higher *WT1* expression compared with those without recurrence (unpaired two-sided t-test, p = 0.05; The p < 0.05 when the *four* patients without recurrence but with outlier *WT1* expression were excluded.



i-j) Novel EMT score and derived CCP score and survival analyses. Youden index cut-point values were determined and used to stratify tumors into low and high CCP (cut-off = 0.7) as well as low and high EMT (cut-off = 1.22). Next, all tumors were stratified into *four* risk groups: CCP^{low}/EMT^{low}; CCP^{low}/EMT^{high}; CCP^{high}/EMT^{low}; and CCP^{high}/EMT^{high}. Kaplan-Meier survival analysis was then performed demonstrating the worst progression free survival (PFS) and poorest disease specific survival (DSS) in CCP^{high}/EMT^{high} risk group. **k-l**) Multivariable Cox proportional hazard analyses. Adjusting for clinicopathologic variables, high CCP and EMT scores were significantly associated with PFS and DSS, with the CCP^{high}/EMT^{high} risk group having the worst outcome.

Results

- WT1 over-expression was observed in paired tumor thrombus vs. the primary tumor, as well as in patients with recurrence vs. those without recurrence. Differential expression and pathway analysis of TCGA high WT1 vs low WT1 tumors revealed EMT pathway as top enriched pathway in high WT1 tumors.
- Derived CCP and EMT scores in TCGA cohort stratified primary tumors into four groups.
- Tumors with high CCP and high EMT scores were significantly associated with poor PFS and DSS.

Conclusions

- The synergistic impact of cell cycle proliferation and epithelial-mesenchymal transition pathway scores can significantly identify patients that are going to develop recurrence and eventually die from ccRCC.
- Our novel molecular stratification performs better than the clinical variables like Fuhrman grade and pathologic tumor stage.
- Our results open new potentials for the development of prognostic biomarkers & identification of novel therapeutic targets.

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