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Molecular characterization of renal cell carcinoma with venous tumor thrombus: molecular signatures related to renal vein wall invasion

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Predicine 慧渡医疗

INTRODUCTION

Background:

Renal cell carcinoma (RCC) patients with tumor thrombus (TT) have been observed with distinct phenotypes of TT: deeply invading into the wall of the renal vein/inferior vena cava (IVC) (DITT) or minimal/non-invasion (NITT), with different surgical strategy and post-thrombectomy outcome. However, the molecular characteristics of DITT/NITT have not been investigated, due to operational challenges and limited patient access.

Patients & Sample collection:

In this study, primary tumor and TT samples from 68 patients having IVC thrombectomy were prospectively collected for DNA mutation profiling and RNA expression profiling, using Predicine panel-based next generation sequencing and whole transcriptome RNA-SEQ, respectively. Next-generation sequencing and bioinformatics data analysis were conducted in the Colleges of American Pathologist (CAP)-accredited laboratory in Shanghai, China (Huidu Shanghai Medical Sciences Ltd.). Kaplan–Meier survival analysis was performed to analyze the correlation between DITT/NITT and clinical outcome, and p-values were calculated using the log-rank test.

Results:

When metastasis was not present, patients with DITT exhibited significantly shorter overall survival compared to patients with NITT (P<0.05, log-rank test). However, this difference was not observed in the metastasis group. Successful sequencing was performed in 100% (68/68) patient samples. NITT tumor samples harbored significantly higher frequencies of mutations in PTEN, TP53 and epigenetic regulators such as SETD2, PBRM1 and KDM5C and had higher expression of cell cycle related pathway genes. In contrast, DITT had lower expression of genes encoding cell adhesion and extracellular matrix molecules such as ADAM33, NCAM1 and FLRT2, which could contribute to higher invasiveness.

NGS METHODS

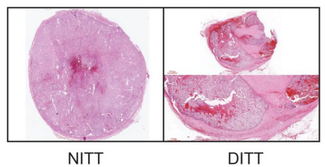
NGS assays:

The 600-gene PredicineATLAS™ NGS assay was used to profile somatic alterations and RNA-SEQ assay was used to measure gene expression in tumor samples from 68 patients.

Workflow for PredicineATLAS™, a targeted NGS assay for tissue, urine and blood

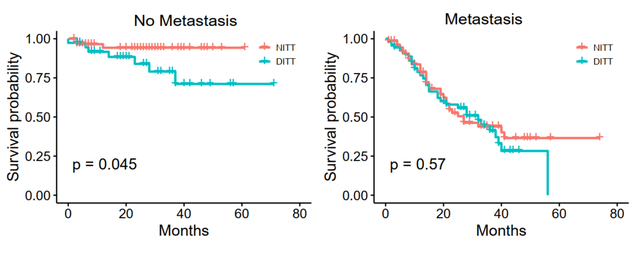
RESULTS

Figure 1. HE staining of thrombus tissues in NITT and DITT patients



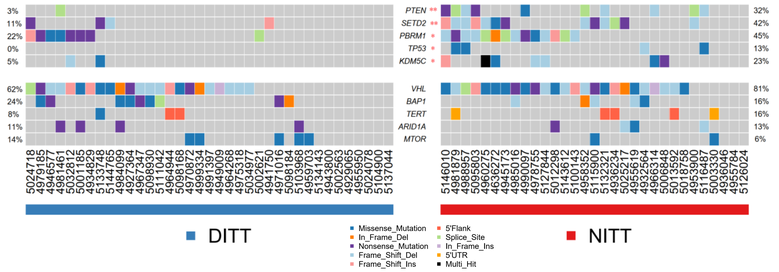
For DITT patients, IVC/ renal vein invasion was diagnosed at the time of surgery; for NITT patients, IVC wall invasion was not detected during surgery, and the final histopathologic reports confirmed the absence of vessel wall invasion.

Figure 2. Patients with invasive TT had shorter survival in the non-metastasis group



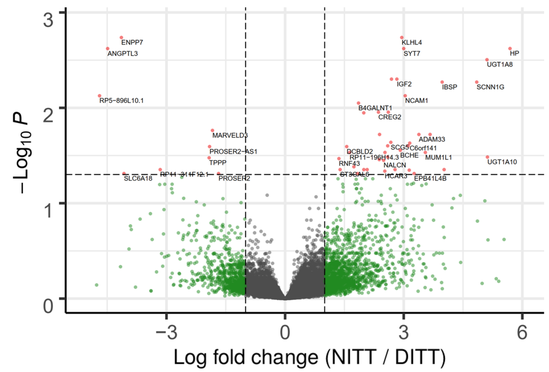
We retrospectively analyzed the outcome of 226 patients who underwent caval thrombectomy between 2015 and 2019. 128 patients had tumor metastasis in other body sites and 98 did not have metastasis at time of surgery. Among the metastasis group, patients with DITT and NITT did not show significant difference in overall survival, whereas in non-metastasis group, patients with DITT had significantly shorter survival comparing to patients with NITT.

Figure 3. Epigenetic regulator genes were more frequently mutated in NITT group



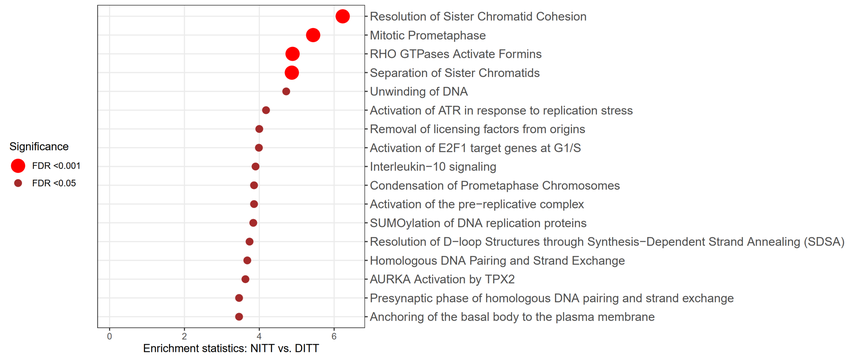
Gene-wise analysis revealed that 5 genes were more frequently mutated in NITT compared to DITT patients (PTEN, SETD2, PBRM1, TP53 and KDM5C). SETD2, PBRM1 and KDM5C are epigenetic regulators previously shown to be associated with RCC development. VHL, the most frequently mutated gene across all samples, exhibited similar mutation frequencies in DITT and NITT patients. * * P< 0.01, * P< 0.05

Figure 4. Cell-adhesion genes had lower expression in invasive RCC primary tumors (DITT)



RNA-SEQ data was analyzed with DESeq2. In primary tumors, 47 genes had higher expression in NITT tumors and 9 genes had higher expression in DITT tumors. Notably, cell adhesion genes such as IBSP, ADAM33, NCAM1 and FLRT2 had lower expression in invasive tumors (DITT).

Figure 5. Cell cycle related pathways had higher expression in NITT group



Differential expression of Reactome pathways in NITT or DITT groups was analyzed using R package GAGE. A positive enrichment statistic for NITT vs. DITT indicates higher expression of the pathway in the NITT group. Most pathways with higher expression in the NITT group are cell-cycle related.

CONCLUSIONS

To our knowledge, this is the first large study to systematically interrogate the genomic landscape and molecular features of invasive and non-invasive tumor thrombus phenotypes in RCC patients. The differential genomic mutation and gene expression landscapes across the two groups may reflect biologic differences and clinical implications such as tumor invasiveness and aggressiveness.