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Analiza wartości prognostycznej markerów molekularnych raka nerkowokomórkowego z czopem w układzie żylnym

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

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Streszczenie w języku angielskim

Analysis of the prognostic value of molecular markers in renal cell carcinoma with venous tumor thrombus.

Introduction

A venous tumor thrombus (VTT) associated with renal cell carcinoma (RCC), represents a distinct compartment within the cancer, located in the frontline of contact with the bloodstream that remains with the continuous interaction with host blood cells. Various immune cells of the host blood may potentially interplay with VTT influencing its biology. While many authors have reviewed the current state-of-art of the management of VTT, its biology and microenvironment have not been comprehensively reviewed to date. In this thesis, I have described current concepts regarding the formation of thrombus, its histopathology, immune microenvironment, genetic and molecular features with potential impact on prognostication and tailored therapy. Although it is the sophisticated and challenging surgery that remains the primary modality in the management of RCC with VTT, recent advances in the research on cancer biology and microenvironment shed some light on the numerous future perspectives. The formation of tumor thrombus is a complex process, understanding of which may trigger onset of novel therapies leading to the improvement of not only the oncological results but also patient's safety in these life-threatening conditions. In my dissertation, I focused also on the assessment of the expression of novel immune marker, P-selectin glycoprotein ligand-1 (PSGL-1) and cytoplasmic stimulator of interferon genes (STING) in the cohort of patients with primary RCC with VTT, in conjunction with the evaluation of tumor infiltrating leukocytes (TILs) in two compartments, i.e. the primary tumor and the venous tumor thrombus. In parallel, the co-expressions of other immune-related markers, including V-domain Ig suppressor of T-cell activation (VISTA) and programmed death-ligand 1 (PD-L1), were also analyzed.

Materials and Methods

Study Cohort

The study cohort consisted of 82 patients with primary clear cell renal carcinoma (ccRCC) and venous tumor thrombus (clinical stage cT3a or higher), who underwent surgical treatment between 2012 and 2019 at two tertiary urological centers. Surgical management comprised radical nephrectomy with thrombectomy, and cavotomy, when indicated. No neoadjuvant therapy was administered prior to surgery. All procedures were performed using open

approach via laparotomy or lumbotomy. The study group also included patients with clinically positive regional lymph nodes (cN1), in whom lymphadenectomy was performed. Patients with distant metastases were treated primarily with surgery, either with the intention of complete tumor removal or by means of cytoreductive nephrectomy. The following data were collected: age, sex, tumor stage based on computed tomography or magnetic resonance imaging of the chest, abdomen, and pelvis according to the 2017 Tumor-Node-Metastasis (TNM) classification system (AJCC version) [1], preoperative hematological parameters (counts of neutrophils, platelets, lymphocytes, and monocytes, as well as derived ratios: neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR)) obtained from certified local laboratories (FACS, Sysmex XM200, Sysmex Poland, Poland), pathological findings, follow-up and survival data retrieved from institutional database. In case of missing data, telemedicine visits were arranged to complete the records.

The study was approved by the Bioethics Committee of Medical University of Warsaw (approval no AKBE/72/2021). Written informed consent was obtained from all participants.

Tissue Microarrays and Immunohistochemistry

Hematoxylin and eosin-stained ccRCC specimens obtained from the primary tumor and venous thrombus were reviewed by two pathologists (R.P. and M.K). Sarcomatiod/rhabdoid differentiation and tumor necrosis were assessed using binary scoring systems (present vs absent). All cases were evaluated for the presence of tumor associated immune cells, including mainly leucocytes (tumor infiltrating leucocytes, TILs). Representative areas containing tumor cells and TILs were selected. Tissue micorarrays (TMAs) comprising representative samples of the primary tumor mass and venous tumor thrombus from 82 ccRCC cases were constructed using a manual Tissue Arrayer MTA-1 (Beecher Instruments, Inc., Sun Prairie, WI, USA) with 1,5mm diameter needles. Three cores were obtained from each compartment (two peripheral and one central core from the thrombus and three cores from the primary tumor) [2]. TMA blocks were cut into 5 µm thick sections for immunohistochemical analysis [2, 3]. Subsequently, the constructed TMAs were stained with antibody against PSGL-1 (clone KPL-1, dilution 1:200, Sigma Aldrich, St. Loius, MO, USA), anti-VISTA antibody (clone D5L5T, dilution 1:300, Cell Signaling Technology, Danvers, MA, USA), and/or PD-L1 antibody (clone 22C3, dilution 1:50, DAKO, Agilent, CA, USA). PSGL-1 expression was evaluated separately in tumor cells (TC) and TILs in both tumor compartments. Subsequently, the prepared tissue microarrays were stained with anti-STING antibody (OTI4E12, product no. MA5-26032, dilution 1:100, Thermo Fisher Scientific, Waltham, MA, USA). PD-L1 and VISTA expressions were evaluated as previously described [3, 4]. Tonsils and placenta tissues served as positive controls, whereas liver samples were used as negative controls [5]. Cytoplasmic and membranous STING expression in tumor cells was quantified using H-score method (range: 0-300), calculated by multiplying the percentage of positive cells (0-100%) by staining intensity (0, 1+, 2+, 3+), as previously proposed [6]. Cytoplasmic staining of tumor cells was quantified,

with immune and endothelial cells serving as internal positive controls. A cutoff value of H-score >100 was applied to define high STING expression, corresponding to the 75th percentile of STING expression in venous tumor thrombus. TILs were assessed dichotomously as present (≥1% stromal leukocytes) or absent (≤1%), based on practical considerations to ensure reproducibility. The percentage of positive cells was independently evaluated by two pathologists experienced in uropathology (R.P. and M.K). Discrepancies were resolved by consensus after joint review.

Statistical Analysis

Statistical analyses were performed using Statistica version 13.3 (TIBCO, Palo Alto, CA, USA; license granted to the Medical University of Gdansk) and the R statistical environment [7]. Association between categorical variables and PSGL-1 expression were assessed using the $\chi 2$ test or Fisher's exact test, as appropriate. Continuous variables were analyzed using the Wilcoxon test, Kruskal-Wallis test or Spearman's rank correlation, depending on data distribution. Kaplan-Meier survival curves were generated using the "survminer" package and compared with the log-rank test [8]. Hazard ratios were calculated using Cox proportional hazards regression models. Overall survival (OS) was defined as the interval between diagnosis and death from any cause. Univariate survival analysis for STING expression was performed using Kaplan-Meier curves and the log-rank test. Multivariate survival analysis was conducted using Cox proportional hazards regression with variance inflation factor (VIF) analysis to assess collinearity. Data visualization was performed using the ggplot2 and survminer packages in R [8]. All statistical tests were considered significant at $p \leq 0.05$.

Results

PSGL-1 expression varied between tumor compartments, with higher prevalence in tumor cells (TC) located within VTT, and TILs stained in primary tumor. PSGL-1 positive TC correlated with high-grade histology, while PSGL-1 positive TILs were associated with tumor necrosis. Univariate analysis identified PSGL-1 positivity in TC within thrombus as indicator of poorer overall survival.

The frequency of STING expression in both analyzed compartments was similar (p=0.18). Its presence correlated with no clinicopathological features but for necrosis in VTT only (p=0.0023). PD-L1 expression in the primary tumor was associated with STING in TC in the same compartment (p=0.02). On the contrary, VISTA expression was correlated with the presence of STING in VTT. TILs presence was associated with positive PD-L1 (p=0.008) and STING (p<0.05) expression in the primary tumor. Strong STING expression in VTT was associated with inferior overall survival (OS) (p=0.0061). TILs presence emerged as a robust prognostic factor for OS in both primary tumor (p=0.021) and VTT (p=0.034).

Conclusions

The increased expression of PSGL-1 in venous thrombus suggests its potential role in facilitating TC interactions with platelets and endothelium, potentially contributing to metastatic spread and worse outcomes. Additionally, this study demonstrates for the first time the prognostic values of STING in contemporary cohort of RCC patients with VTT. STING expression in VTT showed prognostic potential, while TILs assessment proved to be a particularly valuable prognostic tool that can be readily implemented in routine pathological evaluation. The use of biomarkers analyzed in this work may help develop new therapeutic strategies and personalize treatment methods for patients with RCC with concomitant VTT.