МЕДИЦИНСКИ УНИВЕРСИТЕТ - ВАРНА
„Проф. д-р Параскев Стоянов“
Ул.“Марин Дринов“ 55, Варна 9002, България
Тел. : 052/ 65 00 57, Факс: 052/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

MEDICAL UNIVERSITY - VARNA
“Prof. Dr. Paraskev Stoyanov”
55, Marin Drinov Str., 9002 Varna, Bulgaria
Tel.: +359 52/ 65 00 57, Fax: + 359 52/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

Fund “Nauka” Project № 21002 Resume – Competition-Based Session 2021:

“Predictive and prognostic role of immunohistochemical expression of apoptosis – inducing factor and RIPK3, marker for necroptosis in renal cell carcinoma”

Project leader: Prof. Maria Angelova Tzaneva, MD, PhD

According to GLOBOCAN data for 2020, renal cell carcinoma is about 2% of malignant tumors in the world and about 2% as a cause of death among malignant neoplasms.

Apoptosis is a process of programmed cell death that is activated by an external apoptotic pathway through receptors for death on the cell membrane and an internal pathway that is triggered at the level of mitochondria as a result of internal damage. It is initiated by activating several caspases. It begins with the activation of caspases 8 and 9, which generate a signaling pathway that potentiates cell death. There is another signaling pathway for caspase-independent activation of apoptosis that is mediated by apoptosis-inducing factor (AIF). In tumors, defects in the mechanism of programmed cell death of neoplastic cells are often observed and this leads to an increase in their resistance to various conventional antitumor therapies. Targeting alternative pathways to cell death is an attractive strategy for improving antitumor therapy. Necroptosis is a relatively new form of cell death that is stimulated by classical cell death receptors identical to those of the external apoptosis pathway. It combines the morphological characteristics of necrosis and part of the molecular regulatory mechanisms of apoptosis. It is mediated by the receptor-interacting protein kinase family (RIPK): RIPK1 and RIPK3.

Analysis of both markers, apoptosis and necroptosis, in relation to histological characteristics, differentiation and tumor stage, would contribute to predicting the course of the disease, and on the other hand allows the discovery of new signaling pathways for therapeutic response.

The aim of the present study is to investigate the immunohistochemical expression of the apoptosis marker, apoptosis-inducing factor (AIF) and the necroptosis marker, Receptor-interacting protein kinase 3 (RIPK3), in patients with renal cell carcinoma and to determine their prognostic and predictive value.

The following tasks are formulated to achieve the set goal:

1. Study of the clinical and morphological characteristics and survival of patients with renal cell carcinoma;

2. Assessment of semi-quantitatively the immunohistochemical expression of AIF and RIPK3 in renal cell carcinoma tumor tissue and compare with neighboring non-tumor tissue;
3. Study of the immunohistochemical expression of AIF and RIPK3 in connection to the clinical and pathological characteristics of patients with renal cell carcinoma: tumor stage, histological type, degree of differentiation, tumor necrosis;
4. Conduct a complex analysis of apoptosis and necroptosis in connection with the clinical and morphological parameters and survival of patients and to determine their prognostic and predictive role in renal cell carcinoma.

To perform the set tasks, suitable paraffin blocks will be selected from patients with kidney cancer. The histological variant, TNM classification stage and patient survival will be determined. Immunohistochemically, AIF and RIPK3 expression will be determined by H-score. Different statistical methods will be used for comparative analysis of the indicators and the obtained results will be published.

Renal cell carcinoma (RCC) is resistant to chemotherapy, and the 5-year survival rate for metastatic disease is between 5 and 15%. Many chemotherapeutic agents act by induction of apoptosis, and apoptotic deficiency can cause drug resistance. Necroptosis is another form of regulated cell death in cells where apoptosis is suppressed.

The study was performed in 80 patients with RCC.

The following clinical-morphological parameters were analyzed: gender, tumor location, histological type, TNM stage, degree of differentiation, area of necrosis, tumor-infiltrating lymphocytes (TILs), vascular invasion, and patient survival. Immunohistochemical expression of RIPK3 and AIF was evaluated semiquantitatively using H-score (histo-score) on tissue samples. The following statistical methods were used: descriptive analysis, variance analysis, testing for normality of data distribution, Student's t-test, t-test, dispersion analysis, Pearson correlation analysis, Kaplan-Meier method, $\chi 2$ – test.

Achieved results:

- In RCC tumor tissue, the mean cytoplasmic expression of AIF was 168.3 (SD=36.88), with a minimum value of 95 and a maximum of 250, while nuclear expression was 2.1 (SD=10.57), with a minimum value of is 0 and a maximum of 85.
- There was no difference in the cytoplasmic expression of AIF in tumor and non-tumor tissue of RCC and between the primary tumor and metastases.
- The intensity of AIF expression in the cytoplasm of RCC tumor cells did not correlate with sex, histological type, degree of differentiation, TILs, area of tumor necrosis, and patient survival.
- The cytoplasmic expression of AIF was higher in the absence of vascular invasion than in patients with tumor emboli.

- There was a statistically significant difference in the cytoplasmic expression of AIF only between T1 and T3 stages.
- The nuclear expression of AIF in RCC did not correlate with any clinical-morphological parameters studied.
- The mean cytoplasmic expression of RIPK3 in RCC tumor tissue was 137.1 (SD=43.96) with a minimum value of 0 and a maximum of 285, while nuclear was 27.9 (SD=46.89) with a minimum value of 0 and a maximum of 195. Cytoplasmic expression of RIPK3 in tumor tissue was lower than in adjacent non-tumor tissue. There was no correlation between the intensity of cytoplasmic expression of RIPK3 and clinical-morphological parameters: sex, histological type, T stage, TILs, area of necrosis, and patient survival. There was no difference between the intensity of RIPK3 cytoplasmic expression in the primary tumor and metastases. Higher cytoplasmic expression of RIPK3 in tumor tissue correlates with better renal carcinoma differentiation and the absence of vascular invasion.
- The nuclear expression of RIPK3 in RCC tumor tissue did not correlate with any of the clinical-morphological parameters studied.
- Our results show that in RCC tumor tissue, necroptosis is suppressed while the apoptotic signaling pathway of cell death is preserved.