UPSTATE MEDICAL UNIVERSITY

UPSTATE CANCER CENTER

Review of the doctoral dissertation by Dr. Grzegorz Stawarz, entitled "Molecular Changes in Clear-Cell Renal Cell Carcinoma."

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Summary of the review. Despite quite-well understood etiology and identified risk factors, clear-cell renal cell carcinoma (ccRCC) is a malignancy that continues to pose a clinical challenge—especially at metastatic stages. Data from the last two decades indicate an increase in the incidence of ccRCC—on average by about 2% annually, both in Europe and worldwide. Consequently, searching for new therapeutic pathways for this type of cancer is critical. While localized, organ-confined kidney tumors, generally respond quite well to treatment, distant metastases remain a major challenge for modern medicine. Resistance to existing therapeutic regimens can appear even many years after initial therapy seems to arise from the continuous evolution of the tumor and appears to originate from significant heterogeneity of cancer cells. This reality makes it even more necessary to explore the molecular basis of pathogenesis to discover potential new treatment methods for ccRCC.

Current cancer research focuses not only on genetic mutations that serve as initiators of tumorigenesis but also on the relationship between the tumor and the patient's immune system. Given the numerous failures of classical chemotherapy and radiotherapy, along with their serious side effects, immunotherapy appears to be a source of hope—both for healthcare systems and, most importantly, for oncology patients who are counting on transforming cancer at least into a chronic disease. Although immunotherapy regimens for ccRCC are already in use, their effectiveness remains unsatisfactory, particularly in advanced stages of the disease. Therefore, such in-depth study of the molecular alterations in ccRCC continues, may bring us closer to the development of effective therapeutic strategies for advanced cases.

I consider the topic undertaken by the doctoral candidate as extremely important and timely. The candidate analyzed the presence of molecular changes in ccRCC in correlation with lymphocytic infiltration within in the tumor microenvironment. The main aim of this study was to determine whether the identified molecular changes in ccRCC could serve as a potential target for the development of immunotherapy-based treatments.

Based on the conducted analyses, the following conclusions can be drawn regarding the role of OSMR, IDO-1, and TMPRSS2 proteins in ccRCC. First, the presence of OSMR is characteristic of both tumor cells and T lymphocytes infiltrating the tumor microenvironment, suggesting its important role in the interactions between the tumor and the immune system. Second, a high level of T lymphocytes with positive OSMR expression correlates with more advanced disease, reaching maximum values in the metastatic stage. Additionally, an elevated level of OSMR in cancer cells is also associated with advanced ccRCC. Regarding the other proteins, ccRCC is characterized by dysregulated expression of IDO-1 and TMPRSS2. Although IDO-1 expression

in ccRCC is relatively low, its level shows a positive correlation with the TNM stage of disease. Furthermore, TMPRSS2 is found in both T lymphocytes infiltrating the tumor and cancer cells themselves, highlighting its potential role in ccRCC pathogenesis.

The conclusions presented in the thesis confirm the importance of the undertaken research topic. The findings may provide a basis for broader investigations into the molecular alterations in ccRCC and contribute to the development of effective immunotherapy for metastatic disease.

Evaluation of the Dissertation

Research Rationale and Aim.
Oncology research has begun emphasizing not only the genetic mutations that drive tumorigenesis but also the interplay between the tumor and the patient's immune system. Given the limited success of classical chemotherapy and radiotherapy in advanced disease, immunotherapy holds promise for transforming lethal cancers into more manageable chronic conditions. However, effectiveness of immunotherapy in advanced ccRCC remains insufficient, underscoring the need for continued exploration of the molecular dysfunctions that fuel this cancer.

Dr. Stawarz's study addresses this pressing need by examining ccRCC's molecular changes, with a particular focus on the interactions of tumor-infiltrating lymphocytes. The specific goal was to determine whether identified molecular abnormalities in ccRCC could serve as potential targets for immunotherapy.

Study Design and Methodology.
The research is a single-center retrospective study conducted at the National Institute of Oncology – National Research Institute in Warsaw. A total of 161 paraffin-embedded samples were analyzed from patients treated and observed between 2014 and 2020, each diagnosed with ccRCC. These 161 patients were divided into three groups:

1. **No relapse**: 60 patients with organ-confined disease and no progression for at least five years.
2. **Late progression**: 49 patients initially free of metastases, who later progressed within at least five years of follow-up.
3. **Primary metastatic disease**: 52 patients with metastatic disease at diagnosis.

Samples underwent immunohistochemical and transcriptomic analyses to measure the expression of specific proteins in both tumor cells and infiltrating lymphocytes. Results were correlated with clinical and pathological data (sex, age, histological grade, and TNM classification). Comprehensive statistical methods were employed, including tests such as Shapiro–Wilk, Kruskal–Wallis, ANOVA, and Cox regression, implemented through R software and complemented by graphical tools like GraphPad Prism and BioRender.

Key findings of the study

1. OSMR (Oncostatin M Receptor)

- **Expression in Tumor and T Cells**: OSMR was found in both ccRCC cells and T lymphocytes infiltrating the tumor microenvironment.
- **Correlation with Advanced Disease**: A higher level of OSMR-positive T cells aligned with more advanced stages, peaking in metastatic cases. Similarly, elevated OSMR in tumor cells was associated with higher disease progression. These findings point to OSMR's potential as both a disease marker and therapeutic target.

2. IDO-1 (Indoleamine 2,3-Dioxygenase 1)

- **Relatively Low but Meaningful Expression**: Although IDO-1 levels in ccRCC samples were generally low, the protein's expression positively correlated with disease progression (TNM stage).
- **Immune Suppression Pathways**: IDO-1's role in immune modulation suggests that its rising expression may facilitate immune evasion and tumor growth, making it a potential immunotherapeutic target.

3. TMPRSS2 (Transmembrane Protease, Serine 2)

- **Presence in Tumor and Immune Cells**: TMPRSS2 was observed in ccRCC cells and tumor-infiltrating T lymphocytes.
- **Implications for Tumor Biology**: Its presence in both cell populations highlights a possible role in cancer progression and immune escape. Further research into TMPRSS2 could reveal new angles for targeted therapy.

These molecular insights not only enhance our understanding of ccRCC's pathology but also illuminate promising avenues for immunotherapy, providing potential biomarkers that might guide more personalized treatment strategies.

Significance of the Study for Basic Science and Clinical Practice.

By integrating molecular data with clinical outcomes, Dr. Stawarz's study offers a more nuanced perspective on ccRCC progression and immune interactions. The findings suggest that OSMR, IDO-1, and TMPRSS2 could be harnessed as diagnostic or prognostic biomarkers, paving the way for novel immunotherapeutic approaches. Moreover, the planned survival analyses, incorporating data from the National Cancer Registry, will help elucidate how these molecular factors influence overall survival—crucial for optimizing treatment strategies in advanced ccRCC. The rigorous use of immunohistochemical methods, performed in a specialized department of experimental immunotherapy, and the robust statistical approach lend strong credibility to the study's conclusions. These results contribute meaningful data to the broader field of oncological research, particularly in understanding how tumor immunology can be exploited for improved clinical outcomes.

Methodological Assessment

A. Choice of literature and the ability to use sources. The dissertation is based on 193 bibliographic references, of which 192 (99.5%) are publications in English and one (0.5%) is in Polish. The majority of sources come from reputable scientific journals worldwide and were published after 2010, attesting to the author's skill in selecting current scientific literature. The sources used were carefully curated and varied, providing the latest perspectives and research findings in the field.

B. Accuracy in formulating problems and hypotheses (research assumptions). The main objective of the study was to evaluate molecular changes in ccRCC, including quantitative analyses of OSMR, IDO-1, and TMPRSS2 proteins in both tumor-infiltrating lymphocytes and ccRCC tumor cells in three patient groups: those without relapse, those with relapse, and those who initially presented with metastatic disease. The study additionally examined the relationship between the expression levels of these proteins and clinical-pathological patient data from the observation period. The questions posed in the study were satisfactorily addressed in the results and conclusions, and both the objectives and the conclusions were appropriately formulated.

C. Relevance of the chosen methods and research tools and ability to apply them. The choice of methods and research tools in the presented study is highly appropriate and demonstrates the doctoral candidate's precision in their use. The sample-preparation process—including slicing the paraffin blocks, deparaffinization, and further immunohistochemical analysis—was conducted according to rigorous laboratory standards. The material obtained during surgery underwent a comprehensive process of fixation in formalin, dehydration, and embedding in paraffin at the Department of Pathology, National Institute of Oncology – National Research Institute. These carefully selected steps yielded high-quality samples enabling reliable analysis of OSMR, IDO-1, and TMPRSS2 proteins in different patient groups. This was followed by a complete deparaffinization procedure using xylene and ethanol of gradually decreasing concentrations, allowing for the exposure of antigenic sites in the samples.
The candidate also used appropriate statistical techniques, such as analysis using the R software, which enabled an objective evaluation of the results. Relevant statistical tests were applied, including the Shapiro–Wilk test, Kruskal–Wallis test, ANOVA, and Kendall's correlation coefficient, permitting accurate between-group comparisons and the evaluation of relationships between variables. Graphical methods, such as plots generated in GraphPad Prism and illustrations created with BioRender, enhanced the clarity of data presentation. Overall, the dissertation confirms the candidate's professional approach to using modern research tools and advanced knowledge of histological and statistical techniques.

D. The paper's organization and content structure. The dissertation under review follows a typical structure. It has 125 pages and comprises a theoretical section (the introduction), divided into six chapters. In these chapters, the author discusses the anatomy of the kidney, describes ccRCC in detail, and delves into immunotherapy-related topics. The aim of the study is then presented, followed by a chapter on research methodology. In the subsequent sections, the candidate presents results, discussion, and conclusions. The paper also includes a list of tables, figures, abbreviations, and summaries in Polish and English, as well as a bibliography. An

appropriate portion of the dissertation is devoted to describing the results, statistical analyses, and discussion. Both the structure and format of the dissertation merit the highest praise.

E. Formal, linguistic, and stylistic correctness. Dr. Grzegorz Stawarz's monograph is carefully published and does not raise major editorial concerns. Including the table of contents and bibliography, it spans 125 pages. It is characterized by clear, logical, and orderly presentation. The tables and figures it contains are legible and intuitively understandable. The paper only needs minor stylistic and grammar corrections.

Summary and Recommendation

Dr. Stawarz's dissertation advances current knowledge of ccRCC by mapping critical molecular players involved in tumor progression and immune system interplay. The carefully designed study and its statistically grounded findings underscore the value of exploring OSMR, IDO-1, and TMPRSS2 as potential therapeutic targets. Such insights have considerable relevance not only for guiding future research but also for informing clinical decision-making in advanced ccRCC treatment.

Based on the presented evidence, the dissertation meets the formal criteria for a doctoral degree. The candidate's work exhibits originality, scientific rigor, and direct clinical applicability. Therefore, I recommend admitting Dr. Grzegorz Stawarz to the next stages of the doctoral process and propose that this outstanding work be considered for distinction.

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