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Review of the Doctoral Dissertation:

Characterization of the function and heterogeneity of infiltrating T cells, especially Th17/Treg cells in colorectal cancer and inflammation

Performed by M. Sc. Dominika Miroszewska
in the Laboratory of Experimental and Translational Immunology
Intercollegiate Faculty of Biotechnology
University of Gdańsk & Medical University of Gdańsk
Supervised by Dr. Zhi Chen University Prof.

The dissertation focuses on the role of T-helper 17 (Th17) and regulatory T cells (Tregs) in colorectal cancer (CRC) and inflammatory bowel diseases (IBD). The research is highly relevant because CRC and IBD represent significant global health challenges. The study investigates immune system interactions in the tumor microenvironment (TME) and

inflammatory processes, which are crucial for developing future therapeutic strategies. It comprises experimental studies using mouse models, proteomic and transcriptomic analysis, and patient serum studies to identify biomarkers and mechanisms in immune response.

Formal evaluation:

The doctoral dissertation of Ms. Dominika Miroszewska submitted for evaluation consists of seven scientific publications (published and unpublished) presented as complete manuscripts. The dissertation is written in English. The work begins with abstracts in English and Polish, lists of abbreviations, a list of communications, a list of figures, a table of contents, an introduction, the aims of the thesis, and seven publication sections. This is followed by general conclusions, literature, supplemental materials, and co-authorship statements. The Introduction includes relevant subsections: colorectal cancer, immune system, T cell subsets, inflammation in cancer, and IBD – the role of Th17/Treg and tumor microenvironment with logically formulated subtitles. The chapter serves as a well-considered introduction to the research issues of the dissertation and is very clear and enjoyable to read. In Chapter Two - Aims of thesis (p. 17), the doctoral candidate concisely and precisely presents five general aims of the research and the specific objectives.

Significance:

The work is structured around key immunological and molecular mechanisms involved in disease progression, particularly in Intestinal microbiota and its impact on colitis, T-cell differentiation, immunosuppressive functions, Proteomic and transcriptomic profiling of tumor environments, and Potential biomarkers for CRC and inflammatory conditions. This demonstrates that the dissertation addresses a significant research problem in biotechnology and immunology.

Results:

Results include seven publications; in two, the candidate is the first or co-first author, and in five, she is a co-author. In the first publication, authors aimed to investigate the influence of microbiota on the development and progression of colitis through T-cell-mediated mechanisms in mice with subsequent changes in the epithelium impacted by aberrant immune response. Publication II focused on the role of USP28 on Tregs and Th17 functions in the inflammatory

strategy, LC-MS/MS proteomics. Publication VII is a review article describing antibody-based and MS-based proteomics approaches applied in research on TME and cancer immune responses with an emphasis on CD4 + T-cells.

As mentioned, the central part of the reviewed dissertation consists of six original and one review scientific articles authored or co-authored by the PhD student. The reviewers who reviewed their acceptance for publication had already made a detailed assessment of them regarding the selection of methodology, the quality of the obtained results, their interpretation, and discussion. My opinion in this respect is, therefore, by definition, secondary. Nevertheless, I highly value the research contained in the publications presented to me for assessment. They used modern and correctly selected methods and obtained reliable results that were correctly interpreted and discussed against the background of the available scientific literature.

Novelty:

The dissertation contributes new insights into the following:

- The role of gut microbiota in modulating immune responses related to colitis and CRC.
- How USP28 deficiency influences T-cell differentiation and IL-22/STAT5 signaling.
- Spatial interactions of immune cells within CRC tumor microenvironment.
- Identification of new molecular markers for CRC progression and immune evasion.

The findings are novel and clinically relevant, especially in identifying potential biomarkers for cancer immunotherapy and inflammatory disease treatment.

The dissertation provides compelling insights, but several aspects could benefit from further exploration:

1. How does this type of study control for potential microbiota variations in human CRC patients?
    - The mouse models highlight the influence of gut microbiota, but human CRC patients have highly diverse microbiomes.
    - Are clinical validation studies confirming the correlation between microbiota composition and T-cell infiltration in human CRC tissues?
2. Can Treg depletion serve as a therapeutic strategy for CRC?
    - Since Tregs suppress inflammation yet promote tumor growth, targeting them could be a double-edged sword.
    - Could a therapy that modulates Tregs (rather than depleting them) be more effective?

3. How reliable are the proposed biomarkers for clinical application?

- The biomarker study uses patient serum proteomics, but how do these biomarkers compare to existing diagnostic tools (e.g., CEA tests, colonoscopy)?
- Does the dissertation discuss biomarker validation in independent cohorts?

4. The Role of USP28 in Colitis and CRC

- The study assumes that USP28 knockout primarily affects Tregs and IL-22/STAT5 signaling.
- However, USP28 may influence other immune cells, which the study does not explore in depth.

5. Spatial Transcriptomics as a Reliable Indicator of CRC Progression

- The dissertation assumes that spatial transcriptomic gradients directly indicate immune interactions.
- However, gene expression does not always translate to protein activity, and additional functional assays could strengthen this claim.

In conclusion:

The dissertation includes multiple experimental studies, employing a variety of modern methodologies like animal models of colitis to study immune responses in CRC and IBD, spatial transcriptomics and proteomics to map gene expression in tumors, mass spectrometry and immunohistochemistry for biomarker discovery, in vitro and in vivo models of immune cell differentiation. The candidate critically interprets findings, linking experimental results to broader biological mechanisms and developing and applying advanced techniques (e.g., proteomics, single-cell transcriptomics). These aspects highlight the candidate's strong ability to conduct independent and interdisciplinary research.

Final Evaluation:

To sum up, I state that the work meets all the statutory requirements for doctoral dissertations, constitutes an original solution to a scientific problem, presents the required theoretical knowledge of the candidate in the scientific discipline, and confirms the ability to conduct scientific work independently. I state that the results obtained by Dominia Miroszewska are substantively valuable, contain a significant element of innovation, and are evidence of proficiency in complex research tools in the field of biotechnology and molecular biology - both in the context of knowledge of the method itself and proficiency in using available tools for analysis and interpretation of results. The reviewed doctoral dissertation meets all the

conditions specified by Article 191, paragraph 1 of the Act of 20 July 2018, the Law on Higher Education and Science.

Recommendation:

I want to express my recognition for the submitted thesis and the tremendous amount of work behind it, including a variety of experimental, novel, time-consuming, and not easy-to-perform methods. I recommend that the doctoral candidate be granted permission to proceed to the next stages of the doctoral process. I also recommend the award of a PhD degree.