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Review of the doctoral dissertation of M.Sc., Víctor Urbiola-Salvador entitled " Proteomics characterization of immune responses in inflammation and cancer". in the proceeding of the awarding of the PhD degree in the field of science in the discipline of biotechnology. The following review was prepared based on the decision of the Scientific Council of the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk which appointed me as a reviewer of the above-mentioned dissertation.

Formal evaluation

The doctoral dissertation of M.Sc., Víctor Urbiola-Salvador entitled " Proteomics characterization of immune responses in inflammation and cancer" has been made on Intercollegiate Faculty of Biotechnology UG&MUG under supervision of Prof. Dr Zhi Chen. The dissertation was prepared in English I have got it as a pdf file. The dissertation has 105 pages and consists of an Abstract in English and Polish, Abbreviations, List of communications. The first chapter is an overview of the immune system in homeostasis and disease. Chapter 2 includes the aims of the thesis Next chapter presents the publications included in the thesis.

Chapter 3. Plasma Proteomics Elucidated a Protein Signature in COVID-19 Patients with Comorbidities and Early-Diagnosis Biomarkers

Chapter 4. Plasma proteomics unveil novel immune signatures and biomarkers upon SARS-CoV-2 infection

Chapter 5. Mass spectrometry proteomics characterization of plasma biomarkers for colorectal cancer associated with inflammation

Chapter 6. Plasma protein changes reflect colorectal cancer development and associated inflammation

Chapter 7. Deep proteomics characterization of colorectal cancer tumor microenvironment enriched in CD4+ T cells

Chapter 8 Conclusions and future directions presents conclusion of the entire dissertation. The next chapters are Acknowledgements, References, and Appendices.

This arrangement of dissertations is typical for this type of work and I have no objections and I rate it as very good. This Dissertation is based on a publication and according to our law should be a series of thematically related scientific articles published in scientific journals. In my opinion, there are two main topics here concerning the immune system in infections and cancers but one would be enough.

Substantive assessment

Inflammation is a very important process in the reaction of organisms to the challenges related to cancer or infectious diseases. The PhD student during his research of inflammation in cancer had the opportunity to study an exacerbated immune response and acute inflammation with aggravating effects in the course of COVID-19. The role of the immune system and its regulatory processes in the course of inflammation is still little understood. The imbalance between regulatory and inflammatory CD4⁺ T-cell populations and other immune cells and in protein abundance and mRNA expression is observed. A deeper understanding of these processes and relations allows a better understanding of the course of inflammation, which will contribute to the development of knowledge and increase the possibilities of applying this knowledge in therapy. This dissertation focuses on the application of proteomic approaches to characterize immune responses in the context of inflammation and cancer, intending to identify novel immune regulators and discover potential biomarkers. From a scientific point of view, the research is fully justified and contributes to increasing knowledge in the field.

The PhD thesis is based on the publication

1. Urbiola-Salvador V, Miroszewska D, Jabłńska A, Qureshi T, Chen Z. Proteomics approaches to characterize the immune responses in cancer. *Biochim Biophys Acta Mol Cell Res.* 8, 119266 (2022)
2. Urbiola-Salvador V, Lima de Souza S, Macur K, Czaplewska P, Chen Z. Plasma Proteomics Elucidated a Protein Signature in COVID-19 Patients with Comorbidities and Early-Diagnosis Biomarkers. *Biomedicines.* 12,840 (2024)
3. Urbiola-Salvador, V.; Lima de Souza, S.; Grešner, P.; Qureshi, T.; Chen, Z. Plasma Proteomics Unveil Novel Immune Signatures and Biomarkers upon SARS-CoV-2 Infection. *Int. J. Mol. Sci.* 24, 6276 (2023)
4. Urbiola-Salvador V, Jabłńska A, Miroszewska D, Kamysz W, Duzowska K, Drężek-Chyła K, Baber R, Thieme R, Gockel I, Zdrenka M, Śrutek E, Szyłberg Ł, Jankowski M, Bała D, Zegarski W, Nowikiewicz T, Makarewicz W, Adamczyk A, Ambicka A, Przewoźnik M, Harazin-Lechowicz A, Rys J, Macur K, Czaplewska P, Filipowicz N, Piotrowski A, Dumanski JP, Chen Z. Mass spectrometry proteomics characterization of plasma biomarkers for colorectal cancer associated with inflammation. *Biomark. Insights.* 19, 11772719241257739 (2024)
5. Urbiola-Salvador V, Jabłńska A, Miroszewska D, Huang Q, Duzowska K, Drężek-Chyła K, Zdrenka M, Śrutek E, Szyłberg Ł, Jankowski M, Bała D, Zegarski W, Nowikiewicz T, Makarewicz W, Adamczyk A, Ambicka A, Przewoźnik M, Harazin-Lechowicz A, Rys J, Filipowicz N, Piotrowski A, Dumanski JP, Li B, Chen Z. Plasma protein changes reflect colorectal cancer development and associated inflammation. *Front. Oncol.* 13:,1158261 (2023)
6. Urbiola-Salvador V, Miroszewska D, Jabłńska A, Duzowska K, Drężek-Chyła K, Zdrenka M, Śrutek E, Szyłberg Ł, Jankowski M, Bała D, Zegarski W, Nowikiewicz T, Makarewicz W, Adamczyk A, Ambicka A, Przewoźnik M, Harazin-Lechowicz A, Rys J, Filipowicz N, Piotrowski A, Dumanski JP, Chen Z. Deep proteomics characterization

of colorectal cancer tumor microenvironment enriched in CD4+ T cells. Unpublished manuscript.

Five of them were published in reputable journals confirming the high scientific value of these studies.

The aim of the studies was to identify and characterize protein changes associated with chronic inflammation and cancer contexts by proteomics approaches, especially focused on immune-related proteins related to CD4+ T cell subsets and other immune cells. In my opinion, the aim of the work was formulated clearly and precisely. Methods used in studies were described in each publication separately according to tasks planned to be performed and the methods were correctly chosen to perform the planned research. In the studies 97 CRC and 58 COVID-19 patients were studied. Considering the very advanced research, the number of samples tested was sufficient to achieve significant results.

In studies of SARS-CoV-2 using the combination of orthogonal proteomics LC-MS/MS and proximity extension assay technologies the alteration of several immune proteins was observed. Elevated level of markers of tissue remodeling and damage such as 22E, MATN2, COL6A3, and ECM was denoted. Authors postulated that reduction of RNF41 may be a potential biomarker of COVID-19

In studies of CRC altered protein levels caused by CRC development were observed. Mainly, pro-inflammatory proteins LBP and SAA4 and cytokines such as MDK, IL6, CSF3, and CCL20, and SERPIN family members were increased in CRC. They show that among related proteins, Treg/Tfol ARG1 production may be a novel immunosuppressive mechanism in CRC and findings suggest that the novel chemotactic MCEMP1 may be involved in the migration and adhesion of CD4+ T cells in tumor tissue, especially Tregs.

The author concludes that the study of processes inside the tumor focused on proteomic studies or RNA expression, must be taking into account the architecture of the tumor. For a proper understanding of the processes in the tumor, the tissue structure and location information have to be kept. PhD student promotes spatial transcriptomics where resolution below the size of a single cell has already been achieved in the case of HD spatial transcriptomics

Summary

The work is very extensive and multi-threaded, in my opinion, it was enough to present for a dissertation either research related to COVID-19 or CRC, I rate the scientific level of the research very high, which is confirmed by the prestige of the journals where the results were published. I rate the conclusions drawn as correct and resulting from the research performed and the original works presented.

In my estimation the doctoral dissertation of M.Sc., Víctor Urbiola-Salvador entitled "Proteomics characterization of immune responses in inflammation and cancer". meets the requirements specified in Article 187 of the Act of 20 July 2018 - "Law on Higher Education" and I ask the Scientific Council of the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk to admit the doctoral dissertation of M.Sc.,

Víctor Urbiola-Salvador to the further stages of the doctoral procedure. I highly value the level of Víctor Urbiola-Salvador's dissertation, I would like to kindly ask for this dissertation to be distinguished by the High Scientific Council of the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk.


Andrzej Pławski