



RNDr. Daniel Smrž, Ph.D.

Department of Immunology

Charles University, Second Faculty of Medicine
and University Hospital Motol



V Úvalu 83

150 06 Praha 5

Email: daniel.smrz@lfmotol.cuni.cz

Tel. 244-43-5968

Referee report on a Doctoral Thesis of **RNDr. Fabian Čaja**

"Inflammation and cancer in germ-free vs. conventionally reared animals"

The Doctoral Thesis was elaborated in the research laboratory of Dr. Luca Ernesto Vanucci (supervisor) at the Institute of Microbiology, Czech Academy of Sciences of the Czech Republic, v. v. i. in Prague. Dr. Vannucci's laboratory (The laboratory of Immunotherapy) has been dedicated to the research of tumor development in experimental animal models for many years and has authored many publications in this field.

The Doctoral Thesis of RNDr. Čaja attempts to understand the basic mechanisms of tumor development and the contribution of the immune system to these mechanisms. The work hypothesizes that chronic inflammation, even at barely detectable levels long before, precedes tumor development and that this inflammation is affected by commensal microbiota. The first part of the work investigates the role of the microbiome in shaping the colon mucosa. To study this topic, conventional vs. germ-free Wistar-AVN rats were used as the animal models. The study's findings showed that commensal microbiota is responsible for enhanced activation of the local immune microenvironment, and this enhancement was associated with changes in the collagen scaffold organization (architecture) of the colon mucosa. The research results were summarized in the primary-authored publication in the Journal of Immunotoxicology (IF 3.00). The findings of the study were further elaborated in the second part of the work, where changes in collagen architecture in the colon mucosa were investigated in the conventional Wistar-AVN rats after chemically-induced colitis or chemically-induced carcinogenesis. The work results indeed showed that chemically-induced pathological inflammation of the colon mucosa also induced local changes in collagen architecture and that these changes occurred under conditions of "smoldering" inflammation. These findings indicated that changes in collagen architecture

could represent a novel pre-neoplastic marker. The findings of the study were summarized in the primary-authored publication in *Cancers* (IF 6.64). In the third part of the work, the changes in collagen architecture were investigated in human samples, namely the colon tumors, peritumoral (the tumor close) tissue, and the adjacent healthy tissue of CRC patients. The study confirmed that the disease-elicited inflammatory burden had impacted the collagen architecture, the extent of which was progressively increasing towards the tumor. These novel findings have not been yet published but, indeed, warrant a speedy publication because these findings have great diagnostic potential in clinics.

The Doctoral Thesis is mostly well-written and structured according to the established style for this type of work. The introduction first presents the reader with the immune system and its relation to tumor development. Next, the introduction focuses on colon tumors, tumor microenvironment, the tumor microenvironment, and collagen architecture. The final part of the introduction describes the study models and methods, namely two-photon confocal microscopy, which is central for evaluating collagen architecture. The Thesis aims are well defined and adequately addressed in the following experimental/result part. The results of the work were summarized in two primary-authored original research articles in impacted scientific journals with a cumulative IF 9.64. Many of the results presented in the Thesis have not been published yet, but presumably await a soon publication. The work also includes one non-primary-authored original research article, three first-authored reviews, and two non-primary-authored reviews.

Critical points and questions:

- 1/ The English writing is, in general, very good. And it needs to be appreciated that the work is written in English. However, the quality of the writing is a bit erratic throughout the work, where some parts are written in excellent English, and some parts are littered with "very long sentences".
- 2/ The collagen and collagen architecture is central to the work. The introductory part on this topic is not satisfactorily described, particularly when the collagen structure is only text-described and no figure/illustration with basic collagen structural motifs is shown.
- 3/ There is often a short description of the graphs of the non-published results. There is often missing information about the sample size (n), a statistical method used, the error pads meanings (SD or SEM), or whether the mean or median is shown.
- 4/ The findings of the changes in collagen architecture induced by a smoldering and often undetectable inflammation are impressive, particularly after these findings were confirmed on human samples of CRC patients. Is it possible to quantitatively evaluate these changes? If so, is it possible to consider that these evaluations could find a way, for instance, to non-invasive screening diagnostics of pre-neoplastic/pre-carcinogenic lesions? Would it be technically feasible to design an instrument that could be used for such a purpose?

5/ Have you looked or do you plan to look at whether the changes in collagen architecture are associated with changes in the expression of checkpoint molecules in the tumor tissue or the immune cells?

In summary, the presented Doctoral Thesis of RNDr. Fabian Čaja shows the author's scientific qualification to theoretically and experimentally conduct independent research, evaluate and process the results, and disseminate them to the scientific community. Therefore, it has to be stated that **the Doctoral Thesis "Inflammation and cancer in germ-free vs. conventionally reared animals" meets all the qualification criteria required by the Charles University on Doctoral Theses. Based on that, I am delighted to recommend this Doctoral Thesis for acceptance for the Thesis Defense.**

Prague, Jul 14th 2021

RNDr. Daniel Smrž, Ph.D.