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Review of the doctoral dissertation by Beatriz Hernández Suárez, MSc

entitled

THE ROLE of TUMOR ASSOCIATED PROTEINS IN DNA DAMAGE and UNFOLDED PROTEIN RESPONSES and THEIR USE AS TARGET FOR THE DEVELOPMENT of NOVEL CANINE CANCER THERAPY

Supervisor: Aleksandra Pawlak, DVM, PhD, associate professor

Second supervisor: David A. Gillespie PhD, FRSE

Formal and legal basis for the review

The review was prepared on the basis of Resolution of the Scientific Council of Discipline of Veterinary Science of Wrocław University of Environmental and Life Sciences of September 19 2023, according to which I was designated as the reviewer of the doctoral dissertation of Beatriz Hernández Suárez, MSc, entitled „*The role of tumor associated proteins in DNA damage and unfolded protein responses and their use as target for the development of novel canine cancer therapy*”. The legal basis of this review is the Act of 20 July 2018 - The Law on Higher Education and Science (Journal of Laws of 2023, item 742).

General description of the doctoral dissertation and the doctoral candidate's contribution to the research and articles

The doctoral dissertation submitted for review constitutes a series of three peer-reviewed thematically related scientific articles under the common title “*The role of tumor associated proteins in DNA damage and unfolded protein responses and their use as target for the development of novel canine cancer therapy*”. The series includes one review and two original articles:

Publication no 1 - review article

Hernández-Suárez B, Gillespie DA, Pawlak A. DNA damage response proteins in canine cancer as potential research targets in comparative oncology. *Vet Comp Oncol.* 2022 Jun;20(2):347-361. doi: 10.1111/vco.12795. IF: 2.1 MEiN: 200

Publication no 2 - original research

Hernández-Suárez B, Gillespie D.A, Dejnaka E, Kupczyk P, Obmińska-Mrukowicz B, Pawlak A. Studying the DNA Damage Response pathway in hematopoietic canine cancer cell lines – a

necessary step for finding targets to generate new therapies to treat cancer in dogs. *Front. Vet. Sci.* 2023 Aug; vol. 10 doi: 10.3389/fvets.2023.1227683. IF: 3.2 MEiN: 70

Publication no 3 - original research

Hernández-Suárez B, Gillespie D.A, Obmińska-Mrukowicz B, Pawlak A. An initial characterization of the Unfolded Protein Response pathway in hematopoietic canine cancer cell lines -a necessary step for the introduction of new therapies in dogs with cancer. *J. Vet. Res.*, 67, 2023. doi:10.2478/jvetres-2023-0042. IF: 1.8, MEiN: 200

After having made the analysis of the above articles, especially original ones, I can state that the title of the dissertation reflects very well the content of these publications. All papers included in the doctoral dissertation were published in recognized scientific journals indexed in JCR, i.e. *Veterinary and Comparative Oncology*, *Frontiers in Veterinary Science* and *Journal of Veterinary Research*. The papers were published between 2022 and 2023. The total Impact Factor of articles of the doctoral dissertation is 7.1, and the sum of points of these papers, according to the scoring of the list of scientific journals announced by the Polish Ministry of Education and Science in 2023, is 470. As a reviewer I have to point out that the Doctoral Candidate in her doctoral dissertation provided outdated values of IF, i.e. IF for 2021 instead of 2022. Moreover, the value of points for publication no 3 according to above mentioned list is 200 instead of 140 – thus, in this case the Doctoral Candidate made a mistake to her disadvantage.

All papers were the outcomes of collective work of 3, 6 and 4 authors, but the Doctoral Candidate is the first and corresponding author in each of them. Considering this, as well as author's contribution statement in the articles and enclosed statements in this respect, it should be stated that the Doctoral Candidate played a leading role in the research and preparation of the manuscripts. As regards both original articles, the contribution of the Doctoral Candidate was to conduct research, analyze and visualize the results, prepare the figures, write the first draft and final version, submit the manuscript to the journal, and apply all the revisions requested by reviewers under the revision process prior to paper acceptance. As for the review paper, the contribution of the Doctoral Candidate was to establish the concept of the manuscript, to prepare the figures, write first draft and final version, submit the manuscript to the journal, and apply all the revisions requested by reviewers under the revision process prior to paper acceptance.

In conclusion, although the Doctoral Candidate did not develop concept of research, her role as a whole in the implementation of the project and preparation of the articles constituting the doctoral dissertation was the leading one.

Article 187 of the Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2023, item 742) states that, as in the present case, the doctoral dissertation may constitute a series of published and thematically related scientific articles. Thus, only these papers constitute essential doctoral dissertation and hence they are proper subject to evaluation in terms of meeting the requirements for the doctoral dissertation and the Doctoral Candidate described in the above-cited Article. But nevertheless for the purpose of a review of a doctoral dissertation, the principle adopted was that this form of the doctoral dissertation

should be accompanied by elaboration which describes and discusses the studies and findings described in the articles constituting the doctoral dissertation. However, it should be pointed out both the legislator and the Council of Scientific Excellence did not require such elaboration, so there are no rigid rules on the composition of such elaboration, and therefore there are no legal-based criteria to assess it. Therefore, an assessment of such elaboration is an arbitrary one and cannot have a decisive importance for the final assessment of the present work.

The above mentioned elaboration consists of the following chapters: list of publications constituting the doctoral dissertation, abstract in polish and english (1 page per abstract), abbreviation list, introduction (about 5 pages), aim and hypothesis of the research (half page), methodology (9 pages), forewords to all publications (about 5 pages), summary (1 page), conclusions (half page) and literature (about 7 pages). In general, this layout of the elaboration should be considered as logical and in majority is typical for such work. In my opinion the elaboration is well written in clear and concise manner. The language is comprehensive and coherent. However, it is a pity that the elaboration does not include discussion section. Such section would create an opportunity for combined discussion of the results described in both original articles.

The importance and urgency of the undertaken scientific problem and its formulation

Taking into consideration the aims of the research formulated in the publication 1 and 2 and forewords to them as well as in the summary and conclusions of the elaboration accompanying these papers, it can be stated that the research described in the assessed dissertation was methodological one with the essential aim of validating the different techniques and reagents needed in order to further explore the molecular mechanisms of the DNA Damage Response (DDR) pathway, and the Unfolded Protein Response (UPR) pathways in canine cancer. With respect to human medicine, it is currently known that defects in these pathways can lead to an increase in genomic instability, which is one of carcinogenesis mechanisms in various cancers. Before the articles no 1 and 2 were published by the Doctoral candidate, little was know about those two pathways in dogs, despite their potential importance and expected similarity to humans. If we are able to identify particular event in the pathomechanism of diseases, we receive, at least theoretical, potential target for the development of a novel therapeutic strategy for the disease via appropriate pharmacological manipulation of this target. As the DDR and UPR pathways are involved in pathways are involved in the pathogenesis of cancers, they can represent a promising targets for the development of a novel therapeutic strategy humans and companion animals. Taking into consideration the above, it can be objectively stated that the scientific problem undertaken in the evaluated dissertation is original and of great importance, both cognitively and in terms of potential practical application. The research hypotheses of the project are logical and based on reliable scientific findings data. In both original publications the aim of the studies is formulated in clear and concise manner and reflects well the content of the dissertation.

However, as reviewer, I must also direct some criticism towards the formulation of the research aims in the elaboration accompanying the articles constituting the doctoral dissertation and in these papers. There are several the inconsistency between the aim of the

research formulated in the publication 2 and 3 and forewords to them and in the abstract, summary, aim and hypothesis and conclusion chapters of the elaboration:

- the inconsistency between the aim of the research formulated in the publication 2 and 3, forewords to them as well as in the summary and conclusions of the elaboration accompanying these papers – which in my opinion is correct and is fully reflected by the studies described in them – and that formulated in the chapter “Aim and hypothesis” of the elaboration: in this chapter the Doctoral Candidate claims that, and I quote, *“The aim of the presented research was to find new molecular targets whose study could facilitate the development of a new anticancer therapy in canine cancers...”* Such formulation of the aim as *“to find new molecular targets”* is not reflected in the studies described in both original papers constituting the doctoral dissertation. Definitely, the Doctoral Candidate went too far in this statement. I found this mistake as just confusion between the aim of the research and the potential implications of the obtained results/the potential usefulness of the obtained results.
- the inconsistency between the aim of the research formulated in the publication 2 and 3 and forewords to them:
 - in the foreword to publication 2 the Doctoral Candidate wrote that *“The aim of this research was to facilitate the molecular research of DDR in canine cancer by validating different techniques and reagents”*, whereas in this the introduction section of this paper the aim of study is formulated in the following manner: *“To facilitate research into the significance of DDR pathway disturbances in cancer, as well as to inform studies on the development of new therapies targeting the DDR in dogs, we conducted a series of experiments on canine lymphoma/leukemia cell lines to assess...”*.
 - in the foreword to publication 3 the Doctoral Candidate wrote that *“The aim of this research was to facilitate the molecular research of UPR in canine cancer by validating different techniques and reagents necessary to conduct such research”*, whereas in this the introduction section of this paper the aim of study is formulated in the following manner: *“The aims of the study were to determine whether there were variations in the UPR activity between canine cancer cell lines and to validate the methods used to study this pathway...”*.

By reading the articles constituting the doctoral dissertation and the elaboration accompanying them, you may think that the Doctoral Candidate changes her mind all the time with respect to the main aim of the studies. I consider this as the only significant weakness of the assessed dissertation. I want to state clearly that the criticism mentioned above do not at all influence my very positive opinion of this dissertation and its high scientific value.

The methodological correctness of the research

The assessed dissertation represents a well-planned research project that uses modern research methods and tools including molecular biology methods and flow cytometry. This made it possible to gather many original, useful and reliable results. The choice of methods and tools by the Doctoral student was fully relevant in order to succeed in achieving the

established goals of the project. Moreover, the methodology is described in detail in both original articles as well as and the elaboration accompanying these papers. Modern research methods and the relevance of their use are strengths of assessed dissertation.

The level of knowledge on the topic of the work and its presentation & The skills in analysis and interpretation of research findings, drawing conclusions and presentation of results

The first publication in the series of articles constituting the doctoral dissertation is a review article. This article is an excellent comprehensive review of the current state of knowledge regarding summarizing what is known about the DDR pathway in dogs. However, the article is not a dry overview of facts on this topic but it's the creative work as it includes original and very professionally prepared figures. What is more, the last chapter of the paper is devoted to reflections/considerations on the clinical aspects of the DDR pathway in cancer in dogs. In my opinion these reflections/considerations prove a deep understanding of the subject matter, the ability of critical thinking through analyzing existing research and the ability to create a valid scientific concept. Also the introduction chapters in the publication no 2 and 3 are prepared in very professional manner, i.e. they set the context for research work, explain the research problem, justify the need to conduct the research and indicate the purpose behind the study. The results obtained in the PhD project were published in the publication no 2 and 3. The results were presented clearly with appropriate tables and figures, analyzed, discussed and interpreted in a thorough manner; their interpretation is at high scientific level. It is worth emphasizing that in both original publications, the Doctoral Candidate coped very well with the discussion of the results obtained, which was not an easy task due to the numerous, complicated and highly specialized analyses. The results presented in the dissertation allowed the Doctoral Candidate to formulate 5 conclusions, which are correctly formulated, fully supported by the results and related to the aims of the project. Taking into account the above and that the Doctoral Candidate is the first and corresponding author of all articles constituting the Doctoral dissertation and - according to author contributions - wrote original draft of these manuscripts, it can be stated that all the above described achievements and skills should be, to a predominant extent, attributed to the Doctoral candidate. It is therefore reasonable to conclude that the overall achievements and skills described above prove the scientific maturity of that the Doctoral Candidate and that she is able to conduct research independently and prepare articles for publication in scientific journals.

Originality of the results and their contribution to knowledge enhancement

The presented results for the first time provide information about the tools and techniques that can be used to study the DDR and UPR pathways in canine cells and initially characterize these pathways in dogs. This alone is an original contribution to knowledge and meets the requirements set out in Article 187 of the Act of 20 July 2018 - The Law on Higher Education and Science, that the subject matter of the doctoral dissertation is an original solution to a scientific problem. Moreover, it must be observed that the results and the conclusions coming from them presented in the dissertation, in addition to expanding the theoretical knowledge regarding the DDR and UPR pathways in canine cells,

have the potential practical implications. I mean that the development of verified research tools the Doctoral Candidate will could facilitate/provide a basis for the development of a new anticancer therapy in canine cancers.

Final conclusion

Summing up the entire review, it can be concluded that: (a) the doctoral dissertation constitutes a series of published and thematically related scientific articles; (b) the doctoral dissertation demonstrates the candidate's general theoretical knowledge in a discipline of veterinary science and the ability to conduct research independently; (c) the subject matter of the doctoral dissertation is an original solution to a scientific problem. Taking into consideration the above, I can state that the doctoral dissertation by Ms. Beatriz Hernández Suárez, MSc, entitled "*The role of tumor associated proteins in DNA damage and unfolded protein responses and their use as target for the development of novel canine cancer therapy*" meets all the requirements set out in Article 187 of the Act of 20 July 2018 - The Law on Higher Education and Science (Journal of Laws of 2023, item 742). Therefore, I hereby apply to the Scientific Council of Discipline of Veterinary Science of Wrocław University of Environmental and Life Sciences to admit Ms. Beatriz Hernández Suárez, MSc, to further stages of the doctoral procedure. Moreover, in recognition of the high scientific quality and originality of the evaluated dissertation, I recommend to the Scientific Council to award this work with an appropriate prize.


Professor Tomasz Maślanka, DVM, PhD