REPORT OF THE PhD DISSERTATION EXAMINER

1. QUALITY AND ORIGINALITY OF THE RESEARCH AND ITS CONTRIBUTION TO KNOWLEDGE

This doctoral dissertation constitutes a significant and innovative contribution to the fields of cellular and molecular parasitology and extracellular vesicles, specifically focusing on infections caused by cestode parasites. The primary objective of this research is to isolate and characterize the extracellular vesicles (EVs) released by helminthic parasites to gain further insights into the biology, pathogenesis, and life cycle of tapeworm parasites that cause a substantial disease burden worldwide.

Conducting research in parasitology presents notable challenges due to the lack of precise and robust diagnostic and molecular tools. Parasites have complex life cycles that involve one or several hosts, resulting in limited opportunities for intervention, including treatment and prevention. Additionally, the development of vaccines against parasite infections is notoriously challenging due to the complex host-parasite relationship, and current available options are inadequate in terms of protection. EVs could be critical in developing future therapies, including both prophylactic and therapeutic approaches, and also have the potential to elucidate the complex biology of these parasites.

Despite these challenges, this PhD dissertation provides valuable insights into the biology of tapeworm parasites and their EVs by establishing new laboratory models and state-of-the-art protocols for working with these nanosized particles. Moreover, this thesis highlights the biological significance and potential applications of these vesicles. Furthermore, the research has identified a novel mechanism behind the formation of beaded protrusions and release of EVs, indicating a specific form of EVs biogenesis when tapeworms interact with the host. As such, this work holds tremendous potential for enhancing our understanding and management of complex parasite-driven infectious diseases. We unequivocally support the thesis and recommend it for its defense.

2. RESEARCH METHODOLOGY

As previously indicated, the techniques currently employed in the field of parasitology lag those used in other disciplines, such as bacteriology or cancer research. Moreover, investigations involving cestode parasites pose additional complexities, including ensuring the representativeness of model sampling and acquiring high-quality biological data that can capture the intricate events of the life cycle of these organisms. Despite these obstacles, the student in question has effectively surmounted these challenges by utilizing experimental approaches and research methodologies that not only align with the objectives of the PhD dissertation but also establish new benchmarks for the study of these life-threatening parasites.

The student has presented a clear and concise description of their methodology, showcasing their exceptional abilities in reflection, observation, and experimentation. In summary, the student's research approach is well-founded and efficient. They have demonstrated a high level of rigor in their handling of sources and data and consistently presented pertinent analyses of the data and results.

3. ACCEPTABILITY OF THE DISSERTATION FORMAT AND QUALITY OF COMMUNICATION

The student has demonstrated a comprehensive understanding of the fundamental concepts, theories, and issues pertaining to infectious diseases, parasitology, and extracellular vesicles. In particular, the student has exhibited a remarkable proficiency in these areas.

In the introductory section and literature review, the candidate has presented a well-crafted argument regarding the complex biogenesis and dynamics of EVs and their roles in the biology of parasites. The student emphasizes the need for improved models and methods to gain robust and specific knowledge about the origin and biological significance of these particles during the various developmental stages of these parasites. The literature review is supported by relevant and up-to-date references and effectively employs illustrations and tables. Overall, this section is meticulously constructed and captivating.

Throughout the entire document, it is evident that the student has applied their knowledge in their research field with critical analysis and thoughtful consideration. This is a crucial aspect of the work and indicates that the student has demonstrated a thorough understanding of the relevant concepts and theories. The document is well-organized, easy to follow, and skillfully articulated. Supporting materials are pertinent and contribute to a comprehensive understanding of the principal findings.

4. QUESTIONS

I found the articles included in this thesis dissertation to be highly enjoyable. Enclosed is a series of questions that the student could potentially consider addressing during their thesis defense. Most of these queries lack a definitive answer and are intended to stimulate dialogue, as well as encourage the candidate to engage in deeper levels of reflection.

Questions from article 1

1. According to your findings, could you talk a little bit (and try to classify) the different subtypes of EVs secreted by adult tapeworms?
2. What is the composition of the cargo in the vesicles observed in this study? How do you use this cargo content to subclassify EVs?
3. What are the mechanisms involved in the budding of surface membrane-derived vesicles in adult tapeworms?
4. What is the potential role/contribution of phospholipid scramblase, calpain, myosin, and ADP ribosylation factor 1 (ARF1) in the biogenesis of sub-tegmantic vesicles (SMVs)?
5. How do the abundance and ratio of SMVs compare to other types of EVs in the host's gut?
6. Could you elaborate al little bit on the moonlighting activity of the enzymatic proteins of *H. diminuta* observed in purified EVs, and how do they could be playing a role in host-parasite interactions?
7. Do you have any hypothesis on the role of e.g., F1LPJ1, A0A564Y2H9, A0A0R3SJL9, A0A564Z7E5, and A0A0R3SPB2 in Th1 and Th2 cell differentiation and T cell receptor signaling pathways?
8. Could you discuss about the role of immunoglobulin-related proteins Ig kappa chain and Ig heavy constant mu in the interaction of *H. diminuta*-derived EVs with the host's immune system?
9. A very general question. What further research is needed to study differences in the lipid and nucleic acid compositions of distinct EV populations, and to elucidate the roles of EVs in the host-parasite relationship?

Questions form article 2

1. What are the unique (or differential) methodological difficulties associated with studying EVs in parasitic helminths? Could you elaborate on these gaps and propose some potential solutions?
2. Are the biogenesis pathways of EVs similar in non-mammalian species, such as tapeworms, to those of mammals? Could we identify specific points for therapeutic intervention?
3. How can EVs-cargo dynamics change in extended parasite cultures, and what are the implications of these changes?
4. What is the structure and size of the isolated EVs obtained from the different fractions, and how do they compare to those observed on the surface of the tapeworm?
5. How are the observed forms of secreted vesicles shed, and what is the implication of this shedding mechanism?
6. How does the ESCRT machinery contribute to the biogenesis of EVs, and what are the implications of its presence or absence in the different fractions?
7. Could you expand a little bit on what is the role of ULK3 in the formation of beaded protrusions and delayed scission of individual bEVs?
8. Could you elaborate on how do various phospholipid-transporting ATPases, calpains, and ADP-ribosylation factors (ARFs) influence membrane reorganization and MV formation?
9. How does the phosphorylation of proteins associated with EVs biogenesis differ at different time points, and what is its potential implication on EVs shedding mechanisms?

5. GENERAL COMMENTS

After carefully considering all my general and specific comments, I have determined that this document meets the necessary requirements for a PhD Dissertation in terms of its methodology, scientific content, textual structure, and presentation. We find the Dissertation to be acceptable and we recommend proceeding to oral examination.

This PhD dissertation demonstrates a strong working competence and a high level of reflective capacity. In my opinion, the student has the potential to pursue a scientific career and develop specialized skills and expertise through postdoctoral training, which would enable them to build a unique research profile. I offer my congratulations and best wishes for their future endeavors.

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