REVIEW

Of the member of the dissertation council for the dissertation of Borisenko Ilya Evgenievich on the topic: "Regeneration of sponges Halisarca dujardinii (class Demospongiae) и Oscarella lobularis (class Homoscleromorpha): cellular mechanisms and participation of Wnt signaling pathway", submitted for the degree of Candidate of Biological Sciences in a scientific speciality 1.5.23. Developmental biology, embryology.

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Dissertation of Borisenko Ilya Evgenievich on the topic: "Regeneration of sponges Halisarca dujardinii (class Demospongiae) и Oscarella lobularis (class Homoscleromorpha): cellular mechanisms and participation of Wnt signaling pathway" meets the basic requirements established by Order No.11181/1 dd. 19.11.2021 "On the procedure for awarding academic degrees at St. Petersburg State University". The applicant Borisenko Ilya Evgenievich deserves to be awarded the academic degree of Candidate of Biological Sciences in a scientific speciality 1.5.23. Developmental biology, embryology. Paragraphs 9 and 11 of the specified Order have not been violated.

Member of the Dissertation Council

Professor, PhD Bernard Michael Degnan

21 January 2022

Bernard M. Degnan's assessment of Borisenko Ilya Evgenievich's thesis for the degree of a Candidate of Sciences in 1.5.23. Developmental biology, embryology. Dissertation title: Regeneration of sponges *Halisarca dujardinii* (class Demospongiae) *и Oscarella lobularis* (class Homoscleromorpha): cellular mechanisms and participation of Wnt signaling pathway.

Overall assessment

Mr Borisenko's thesis presents a novel, interesting and rigorous study on the cellular and molecular basis of regeneration in a species of demosponge (*Halisarca dujardinii*) and homoscleromorph (*Oscarella lobularis*). The molecular work focusses on the Wnt signalling pathway in the demosponge *H. dujardinii.* This is the first study on the expression of the Wnt pathway in an adult demosponge and primorphs, and in the process of regeneration

This thesis substantially contributes to our understanding of regeneration in these two species and poriferan classes. Comparative analyses between these species and previous studies shed light on the mechanisms of regeneration in sponges. Mr Borisenko has published a substantial portion of this thesis in high-standing international journals with rigorous peer review, lending further support to the quality and novelty of the contents of this thesis. He has presented this work at a multitude of international conferences and workshops.

The English version of this thesis is in general well written and presented, and scholarly. The results, figures and figure legends are well presented, and includes high quality scanning and transmission electron micrographs, confocal micrographs, whole mount in situ hybridization using specific *Wnt* probes, and informative schematic diagrams.

Below I list specific comments that may be worth considering. These are largely minor issues and do not detract from the overall positive impression obtained from reading his thesis. They nonetheless may be worth consideration.

Specific comments

Introduction

Well written and clearly outlines the rationale for the study, the gaps in knowledge, the scope of the thesis, the broad outcomes, the specific work undertaken by the author, and how this study has been published and communicated to the wider scientific community.

Minor points to note

"Transdifferentiation, or metaplasia, is an irreversible transformation of differentiated cells of one type into another." (p. 93).
Are all transdifferentiation transformations irreversible?

"Taking into account the currently dominant opinion that sponges (Porifera) are the mostancient taxon of multicellular animals … we can present sponges as the first multicellular animals possessing this mechanism of intercellular communication [23-25]." (p. 94).

Sponges are an early diverging phyletic lineage. This does not mean the first multicellular animals were like modern or fossilised sponges. A more accurate statement follows: “Their unique phylogenetic position coupled with a… in the last common ancestor of multicellular animals (last common ancestor)” (p. 94).

Chapter 1 – Literature review

This chapter first provides details and scholarly background to the cellular and molecular basis of tissue regeneration and wound healing in the animal kingdom with a focus on cell proliferation, migration and transdifferentiation, and the regulatory and signalling genes underlying these processes. Well-known models and taxa (e.g. cnidarian, platyhelminth and ascidian species) are emphasised. This chapter reveals that there are disparate regenerative mechanisms across the animal kingdom. The author then explores the role of Wnt signalling in reparative morphogenesis. In contrast to the cellular mechanisms, Wnt appears to play a conserved and widespread role in tissue repair.

As to be expected, the next section which covers regeneration and wound repair in sponges is in far greater detail both in the species explored (four primary experimental species) but also in the timing and location of the repair process in diverse body architectures. Transcriptional analyses of these processes reveal a range of responses including activation of TGF-beta and Wnt signalling. The author rightly acknowledges that these poriferan molecular studies to not generate a full view of the mechanism of regeneration and wound healing in sponges. This provides a good rationale for the work undertaken and presented in this thesis.

In summary, this chapter provides the necessary background to contextualise the research presented in the thesis. It is scholarly and thorough.

Minor points to note

None

Chapter 2 – Cellular mechanisms

This chapter describes the cell biology of wound and regenerative healing in *H. dujardinii* and *O. lobularis* largely based on a 72 hour time course of SEMs and TEMs. It provides an accurate description of cell type and organisation in the vicinity of the wounded area and makes inferences of cellular processes based on differences in the cell populations over the time course.

These SEM and TEM analyses are augmented by the inclusion of the use EdU incorporation to document cell proliferation. In *H. dujardinii*, this analysis provides insight into when and where cell proliferation is occurring (i.e. in deep-set choanocyte chambers but not in migratory “choanocytes”).

In *O. lobularis*, Indian ink labelling provides strong evidence for transdifferentiation of choanocytes into exopinacocytes.

In summary, together the results presented in this chapter expand our understanding of cellular processes in demosponge and homoscleromorph sponges particularly in relation to wound healing.

Of course, this study is not exhaustive and there is more to be done but the results provide a strong foundation for molecular studies. There is no doubt that this is a substantive study and research chapter. There are no major issues from my perspective.

Minor points to note

Figure 2 legend (p. 120).
What are zones 1 and 2?

Paragraph beginning "Immediately after surgery, the wound surface shrinks…" (p.120)
This paragraph provides a description of wound repair. It is unclear if this description is based on previous published work or is new results generate by Mr Borisenko. There is no citation of literature or data figures. This section could be reworked to make the source of the statements clearer.

Figure 3 (p.121)
It is unclear what the "ECM" is demarcating in panels A and B.

"This temporary structure contains undifferentiated cells such as choanocytes, endo- and exopinacocytes, and multipotent cells - archaeocytes." (p. 131).
How can these defined cell types be called undifferentiated? Following on to the next sentence, how can exopinacocytes differentiate into exopinacocytes?

Chapter 3 – Wnt signaling in axis specification and regeneration

This chapter investigates the potential role of Wnts, their receptors and signalling components in *H. dujardinii,* particularly in the adult and larva, during wound healing, and in primorphs, using whole mount in situ hybridization and quantitative gene expression profiling. Previously published expression patterns of some Wnt genes are included to allow for more comprehensive comparisons.

Recognising the challenges of producing consistent and interpretable WMISH patterns in sponges, particularly adults, Mr Borisenko should be commended for this study. It provides the most detailed account of the "Wnt code" in a demosponge. It would have been good to see high resolution micrographs of sectioned material but this would be added bonus and it not necessary for the conferment of the degree in my opinion.

Minor points to note

"Thus, the identified Wnt expression domains completely cover the apico-basal axis of adult *Halisarca*, and some genes are expressed exclusively in the osculum." (p. 153).
This implies the Wnt expression has apico-basal expression pattern. Is this correct or is it cell type-specific expression with cells dispersed along this axis?

Conclusions

The conclusions drawn from this study recognise the state-of-play of understanding regeneration in sponges compared to other metazoans, and the utility of the comparison of distantly-related sponges used in this study. As acknowledged, there are a diversity of regenerative mechanisms in

sponges, although there may be conserved core elements such as choanocytes being a reservoir of proliferating cells that can contribute to regeneration in some (many) contexts. The role of "stemness' in sponges is briefly explored. There are no additional points raised that have not been addressed in earlier chapters

Minor points to note

None

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21 January 2022
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