CHU de Québec
Centre de recherche

AXE ONCOLOGIE

Québec City, July 23th, 2021

OBJECT: Report on the dissertation of Shubhangini Kataruka

Scientific Value

The scientific work presented in this thesis is clearly innovative and well performed. The introduction section covers all scientific aspects required to understand the purpose of the Ph.D. work properly. This interesting study's overarching objective and the three specific aims pursued are timely, relevant, and clearly defined. The data from the current literature are discussed appropriately in all sections of the thesis, with a few exceptions (see the following modifications list). Overall, it is clear that the student has acquired an excellent knowledge of her field of research, which was essential to conduct this study with success.

The results obtained by the student are presented in two parts. The first part, about a detailed and thorough analysis of the microRNA activity in the mammalian oocytes, includes data found in a peer-reviewed article recently published in the excellent scientific journal Nucleic Acids Research, for which the student acts as the first author. The data presented in this section nicely demonstrate that the stoichiometry between microRNAs concentration and their mRNA targets does matter and the low abundance of microRNAs in growing oocytes is the main reason why microRNA-mediated gene silencing is not efficient in this biological context. This discovery is significant for the field as previous findings, which were not as systematic and thorough as this student's work, were proposing that the microRNA pathway was not functional in oocytes. Thus, the student's study clearly shows that the microRNA pathway can be functional in this biological setting, but the microRNA abundance related to their targets is not optimal for robust and detectable gene repression.

In the second part, the student focuses on identifying and initial characterization of isoforms of Ago2 in oocytes. The unpublished data presented here demonstrate that the truncated isoform of AGO2 previously identified by her group in mouse oocytes is also expressed in other mammalian oocytes. The student also generated by CRISPR-Cas9 a transgenic mouse carrying a deletion of a MT element cluster, representing an active retrotransposon site, and its phenotypic analysis suggests that the truncated Ago2 isoform does not have any function in the mouse oocytes. The molecular analysis of this transgenic mouse shows that the MT cluster acts as an enhancer for the Ago2 locus and is responsible for the presence of the truncated

Ago2 isoform. Interestingly, the student also observed an alternative exon in the 5' end of Ago2 in mouse and other mammalian oocytes, which lacks previously identified two phosphoresidues. The initial characterization of this new Ago2 isoform in CRISPR-Cas9 edited cell lines suggests that this isoform functions for the microRNA-mediated gene silencing, but the AGO2 capacity for cleaving fully complementary mRNA targets are altered. This part of the thesis highlights the importance and necessity of carefully identifying and characterizing in specific biological contexts all isoforms of key components of the microRNA pathway to understand their role in this essential regulatory pathway.

In general, all the data presented were performed properly using appropriate methodologies and were analyzed carefully. Overall, the work presented in this thesis is clearly original and undoubtedly contributes to improving our general understanding of microRNA-mediated gene regulation in animals.

This thesis ends with an excellent Discussion section in which the student carefully analyzed the results she obtained during her Ph.D. and discussed the significance of her work in relation to the current understanding of the field of research. For this section, it will be relevant to add a model that integrates most of the student observations as well as a new part in which future directions will be discussed to highlight the logical next steps that should be pursued after all the work made by the student.

Quality of the dissertation presentation

This is a well-written thesis; I noticed very few typographic and grammatical errors (some are listed below) that the student would easily find after a thorough reading of the thesis. I congratulate the student for her effort.

The different figures and tables presented in the Introduction and Discussion sections are useful to facilitate the comprehension of the different topics discussed in this thesis. I noted some modifications mentioned below that could be made for some of them to improve their comprehension.

General Comments

This is the 30th Ph.D. thesis I am evaluating, and this one is among the top 10%. Overall, the student accomplished an excellent work during her graduate studies. The thesis is well written and provides important insights contributing to improving our understanding of the microRNA-mediated gene regulation in animals. I, therefore, recommend the acceptance of this thesis after the applicant make the few changes requested. I wish all the best for the next steps of her scientific career.

List of modifications and changes

-Page 8: Target RNA-Directed microRNA Degradation
-Page 9: Oligonucleotide not oligonucelotide
-Figure 1: Please provide a more detailed Figure legend
-Page 21: Remove the extra space after references (...Watanabe et al., 2008)
-Page 21: RNaseIII-type Dicer not RNase II Dicer
-Figure 2: Please provide a more detailed Figure legend
-Page 23: Remove the extra space after "During this exchange ,"
-Figure 4: Please provide a more detailed Figure legend. What the black oval is referring to?
*-Page 29: Missing a **e** at Th**e** (after Kwak and Tomari, 2012)*
-Page 30: Please update the text about AGO3. The AGO3 slicing activity has been demonstrated by the Nakanishi's group (NAR 2017 and PNAS 2020)
-Page31: Please add the Golden et al, 2017 reference when you discuss about of the phosphorylation cluster in AGO PIWI domain
-Page 32, Aim 2: "the" is missing in front of miRNA pathway (please verify in all the text. The is missing in some other places.)
-Page 34, Northern blot: Precise "with 5'P"
*-Page 34, Northern blot: The RNA was then **transferred*** not blotted onto a membrane
-Figure 25: In panel A, indicate where the cleavage product is on the gel. Also, add a representative image of the target and let-7 base-pairing along with the cleavage site.
-It will be interesting to add a foreword to the NAR paper describing the detailed contribution of the student for this published work

Questions

- Can you speculate about the role of other Argonautes that are expressed in mammalian oocytes? How can their presence and function affect the interpretation of your data?

- Your mathematical modelling is quite interesting, but it seems not to take into consideration the turnover of microRNA. What do you know about molecular cues that drive microRNA stability and degradation? Can you elaborate on how this can be modulated differently in oocytes?

- You mentioned on page 50 that you validated that mimic and reporter molecules do not interact during microinjection. Why do you think this is not happening?

- Do you have any idea about the alternate N-terminal AGO2 isoform abundance relative to full and truncated isoforms? Also, how do you think the stoichiometry among these different AGO2 isoforms can affect gene regulation in oocytes?

- Based on what we know about the structural features of AGO2, can you postulate a model to explain the effect of amino acids changes/loss of phosphorylation on its slicing activity? For example, why alternate N-terminal AGO2 is not completely enzymatically inactive? Do you think that this slight alteration and AGO2 slicing capacity is sufficient to have a functional effect in oocytes?

- Along this line, other than its slicing activity, what amino acids changes in the N-terminal part of AGO2 could also be affecting?

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