TEMPLATE FOR PROJECT OUTLINE – Due Oct 20
(please type!)

Student's name:

Buffy Chen

Topic chosen:

H19, miRNA, kidney disease, epigenetics

SPECIFIC QUESTION (SORRY! Couldn't decide as H19 is too interesting!)

1. A research study has shown that the lncRNA of H19 harbors binding sites for the microRNA family "let-7". The significance of this is that the RNA molecule acts as a molecular sponge, and regulates let-7 by inhibiting it thereby preventing muscle differentiation. The H19 lncRNA also contains other binding site sequences for other microRNAs, that is associated with kidney disease. Does the H19 lncRNA also bind to these microRNAs to regulate kidney development? If they do, is it necessary or sufficient to bind all of the microRNAs to ensure proper kidney development?

2. Recent research has provided multiple lines of evidence that supports the significance of microRNA in the regulation of kidney development. The most common mutation in Wilms' tumor occurs in the microRNA processing pathway. Genes associated with the processing include Drosha and Dicer 1. H19 is a microRNA precursor and produces miR-675-5p and miR-675-3p. Does one of these transcript regulate Drosha and/or Dicer 1?

3. Timing is key in development. Does miR-675-5p and/or miR-675-3p regulate Let-7 in a developmental-stage manner?

4. The more I read of H19, the more I begin to think that there isn't a universal function for the lncRNA. I begin to think of it as flexible entity as it becomes clear that the lncRNA is involved in many different, intricate processes during development. The generated miR-675-3p and miR-675-5p promote skeletal muscle differentiation and regeneration by inhibition repressors of myogenesis. However, another study reported that H19 sponges let-7 in a kidney cell line and can inhibit myoblast differentiation. It seems that H19 functions differently depending on the type of tissue and the timing. How does the lncRNA, or its microRNA products, know what to do/target? Is their function "activated" by a signalling cascade?

HOW IS THIS QUESTION NOVEL AND ORIGINAL?

There has been plenty of research to document that importance of long non-coding RNA, H19, as an important mediator in cellular fate and function during throughout development (from birth to death), however the physiological role H19 plays for reproductive process is still ambiguous. After extensive research, a common theme that occurs is that the exact mechanism of H19 is still misunderstood, especially during kidney development.

The question is novel because it is building upon found-results to establish the underlying role of H19. It is original because someone has yet to ask this question.

POTENTIAL IMPACT OF THE PROPOSED QUESTION (WERE IT TO BE ANSWERED BY YOUR PROPOSED EXPERIMENT):

The study of lncRNAs in the kidney is still a nascent field, that is to be elucidated by researchers. Challenges that hinder the progress of our understanding include 1) lncRNAs are generally poor in conservation between species 2) lncRNAs have several transcripts which subsequently creates ambiguity to the understanding of its exact mechanism and 3) studies of lncRNA in kidney disease shows an association with the disease instead of the underlying role. H19 has been linked to several genetic diseases, including Wilm's tumorigenesis. Wilm's tumorgenesis is a type of cancer that originates in the kidney. It is also the most common type of kidney cancer found in children. Wilms tumor results as a result of epigenetic silencing of H19 (as well as IGF2). However, when not epigenetically silenced, how does H19 regulate the development of kidney?

By looking intimately at the role of H19 in kidney development during embryogenesis, it will provide researchers with a foundation to build future research upon.

HYPOTHESIS:

1. The H19 lncRNA transcript is necessary to regulate some of the miRNA associated with kidney cancer. However, for other miRNA transcripts, it will only be sufficient.

2. miR-675 regulates Drosha and Dicer 1

3. miR-675 regulates Let-7 in a developmental-stage manner

4. When the miRNA products derived from the lncRNA H19 transcript in a kidney cell is transplanted to a skeletal muscle muscle cell, we will see inhibition of skeletal muscle differentiation.

EVIDENCE ON WHICH THE HYPOTHESIS IS BASED (INCLUDE REFERENCES):

Dey, B. K., Pfeifer, K., & Dutta, A. (2014). The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. *Genes & Development, 28*(5), 491-501.

Hohenstein, P., Pritchard-Jones, K., & Charlton, J. (2015). The yin and yang of kidney development and Wilms' tumors. *Genes & Development, 29*(5), 467-482.

Kallen, A. N., Zhou, X., Xu, J., Qiao, C., Ma, J., Yan, L., et al. (2013). The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Molecular Cell, 52*(1), 101-112.

PREDICTION(S):

N/A until question is decided.

EXPERIMENTAL APPROACH TO TEST PREDICTION (INCLUDE ANY DETAILS THAT YOU HAVE WORKED OUT SO FAR):

I'd imagine that a knock-out experiment might have to be performed for some of the proposed questions?

LIST OF RELEVANT PRIMARY AND REVIEW ARTICLES READ, AND SUMMARY OF RELEVANT INFORMATION FROM EACH (this is the start of the annotated bibliography that you will need to include in your portfolio):

References

Dey, B. K., Pfeifer, K., & Dutta, A. (2014). The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. *Genes & Development, 28*(5), 491-501.

The role of the H19 RNA was examined in the context of skeletal muscle differentiation and regeneration. For this process, H19 lncRNA has a trans-regulatory function that is mediated by its own microRNA products. The microRNA products encoded by H19, miR-675-3p and miR-675-5p directly targets and down-regulates Smad transcription factors involved in the bone morphogenetic pathway and the DNA replication initiation factor Cdc6.

Hohenstein, P., Pritchard-Jones, K., & Charlton, J. (2015). The yin and yang of kidney development and Wilms' tumors. *Genes & Development, 29*(5), 467-482.

Kallen, A. N., Zhou, X., Xu, J., Qiao, C., Ma, J., Yan, L., et al. (2013). The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Molecular Cell, 52*(1), 101-112.

Keniry, A., Oxley, D., Monnier, P., Kyba, M., Dandolo, L., Smits, G., et al. (2012). The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nature Cell Biology, 14*(7), 659.

Lorenzen, J. M., & Thum, T. (2016). Long noncoding RNAs in kidney and cardiovascular diseases. *Nature Reviews.Nephrology, 12*(6), 360.

Martens-Uzunova, E. S., Böttcher, R., Croce, C. M., Jenster, G., Visakorpi, T., & Calin, G. A. (2014). *Long noncoding RNA in prostate, bladder, and kidney cancer* doi:https://doi.org/10.1016/j.eururo.2013.12.003

POTENTIAL WAYS TO MAKE YOUR QUESTION KNOWN TO THE PUBLIC AT LARGE (*e.g.* TO YOUR NON-BIOLOGIST FAMILY AND FRIENDS):

1. Present this project at MURC
2. Look for other research conferences that I could potentially present the project at via school clubs e.g UBC Undergraduate Research Club
3. Just talk about it in daily conversation ☺!

ANY OTHER PARTS OF THE PROJECT COMPLETED SO FAR:

ANYTHING YOU WOULD LIKE SPECIFIC FEEDBACK ON:

1. During my research, I found an interesting result in which the offspring of mother mice that were diabetic, exhibited a reduced overall expression of H19 throughout development until it was undetectable for the postnatal period. As H19 still remains a critical player after birth, especially in the heart and the skeletal muscle, there could be an increased vulnerability to acquiring diseases. It would be interesting to look at this, but this could be perhaps beyond the scope of our course?

2. Definitely on my hypothes(es)! And whether they are too simple or too much!

3. I kind of like my 4th question the best; however, I am concerned that the question is too broad and too simple for the scope of this project. I want to keep in mind that the lessons/objectives learned in the course should be woven throughout the project; however, at the same time, as this project is centered around a "novel" question, I'm concerned that I am not meeting this.
    a. Following this, in the guideline, it was mentioned that the question should be able to be answered with one experiment. E.g the example that "a conditional knockout of the insulin gene" is a good way to test a

prediction, but it is not doable. I'm wondering what "doable/feasible" means exactly in this course. In my head, I would have thought that a knock-out would be a logical experiment for many types of questions. I hope this makes sense!