Introduction

The Kidney Foundation of Canada reports that 1 in 10 Canadians have kidney disease and that it is the 10th leading cause of death in Canada. As of the moment, there is no cure for kidney disease. Research has shown that many kidney disease (adult-formation or pediatrics) has early onset in abnormal or suboptimal kidney development. A step to understanding our treatment option is to understand the big-picture role for the formation of the organs in development. One way to better understand the big-picture, is by looking at long non-coding RNAs (lncRNAs) in the context of development.

It has been shown that in the field of developmental biology, long non-coding RNAs play a critical role in many biological processes. Emerging evidence reveals that lncRNAs play essential roles in gene regulation: this ranges from chromatin modification in the nuclei to mRNA translation in the cytoplasm (Liu, Li, Zhang 2017). Although the function of most lncRNAs is unknown, the number of characterized lncRNAs is growing and many publications suggest that they play roles in negatively or positively regulating gene expression in development, differentiation and human disease (Kornienko 2013). __In the context of kidney development, one player that shows promise in elucidating kidney formation is the lncRNA H19. Its presence, and subsequent activity, during kidney development remains to be explained in detail. This project will aim to elucidate H19 in relation to important morphogens in embryogenesis and development from the bone morphogenetic protein (BMP) family. The remainder of the introduction will outline for the reader an exposition and understanding of H19 and its relevance to kidney disease, an exposition of BMP and its relevance to kidney disease, and the importance of looking at the interaction between H19 and BMP.

H19 is an imprinted gene that regulates embryonic growth. Due to diverse biological functions of long non-coding RNA, it has been shown that H19 can mediate multi-differentiation by serving as a miRNA sponge or interacting with the target genes, and activating/inactivating the downstream signaling pathway (Loi, Li, Zhang 2017). Furthermore, it has been implicated in a series of genetic disease which includes a pediatric kidney cancer known as Wilm's tumor. H19 encodes two conserved micro-RNA, a class of small, non-coding RNAs that can negatively regulate gene expression by translation repression and target mRNA degradation, in its first exon: mir-675-3p and mir-675-5p. Typical of microRNAs, the aforementioned microRNAs are involved in biological processes that include development, differentiation, proliferation and apoptosis (Callis et al. 2007). It has been documented that for skeletal muscles, miR-675-3p and miR-675-5p function by directly targeting and down-regulating the anti-differentiation Smad transcription factors critical for the bone morphogenetic protein (BMP) pathway and the DNA replication initiation factor Cdc6. The H19 lncRNA promotes myogenesis by generating a microRNA to inhibit the negative regulator of muscle differentiation. Paradoxically, H19 sponges let-7, a marker of kidney cancer, in the 2993T kidney cell line (Su, Chen, 2012). This suggests that H19 inhibits CDC12 myoblast differentiation by sponging let-7. However, in the case of myoblast differentiation and muscle differentiation, there is data to suggest it being a pro-myogenic factor

It is important to note the interaction of the microRNA's interaction with members of the BMP family as the BMP family are a group of signaling molecules that is ubiquitous in all organ systems for its role in embryogenesis and development (Wang et al. 2014). Many organ systems have one or more BMPs that are critical for development. Subsequently, a depletion in certain BMP expression or a reduction in functionality results in an abnormal phenotype.

Initiation of mature kidney development involves the ureteric bud, which originates from the caudal end of the mesonephric duct, invaginating into metanephric mesenchyme. The metanephric mesenchyme ultimately gives rise to structures from the glomerulus to distal convoluted tubule, while the ureteric bud is the precursor to all structures distal to the collecting duct. BMP signaling is one mediator of the interaction between ureteric bud and metanephric mesenchyme. Recent research has shown that the presence of BMP signaling may be protective against renal disease. Progression of chronic kidney disease (CKD) is mainly determined by renal fibrosis, which is characterized by an accumulation of extracellular components that leads to loss of renal parenchyma and function. BMP7, highly expressed in podocytes, distal tubules, and collecting ducts, has been shown to be protective against CKD (Wetzel et al. 2006). Exogenous administration of BMP7 or transgenic overexpression of BMP7 reduces overall renal fibrosis and nephrocyte apoptosis (Mitu and Hirschberg, 2008). Additionally, BMP7 signaling has been shown to be protective against hypertensive nephrosclerosis, another major cause of CKD. Therefore, given the introduction, I hope to further investigate H19, and the interaction of its microRNA with prominent members of the BMP family.

Renal hypodysplasia (RHD) is characterized by reduced kidney size or maldevelopment of the renal tissue. BMP4 mutations were identified in RHD patients, consistent with BMP4 being mainly expressed in the mesenchyme surrounding the branching ureter.

Question

The questions that I have are in two parts:

1. What is the developmental effect on kidney, of H19 microRNA products, mir-675-3p and mir-675-5p on the following transcription factors from the BMP family: BMP7, BMP11, and BMP4?
2. Will the mir-675-3p and mir-675-5p produce the same results observed in skeletal muscles for Smad1 and Smad5 as they would in kidney cells in a transplantation experiment? In other words, are the microRNA products pre-programmed or can they adjust to their surroundings via environmental cues.

Relevance, Potential Impact, Importance

Hypothesis

Based off of evidence present in literature I hypothesize that

1) both mir-675-3p and mir-675-5p will positively regulate BMP7, BMP11, and BMP4.

2) mir-675-3p and mir-675-5p, when transplanted from skeletal cells to kidney cells, we will observe a downregulation of Smad1 and Smad5 transcription factors in the kidney cells.

Prediction and Experimental Plan

Signaling Molecule	Phenotype Observed in Mice (Found in Literature)
BMP7	Die after birth, defect in kidney development, defect in skeletal pattern, precocious differentiation of kidney progenitor cells; limb: no effect; podocyte: defective kidney development
BMP11	Kidney agenesis
BMP4	Mutations were linked to reduced kidney size or maldevelopment of renal tissue
Smad1	Die mid-gestation, defects in allantois formation, no PGCs; chondrocyte: delayed calvarial bone development; osteoblast: osteopenia; lung epithelium: severe neonatal respiratory failure
Smad5	Die mid-gestation, multiple embryonic and extraembryonic defects; defective primordial germ cell formation

Predictions for Hypothesis 1 and Hypothesis 2 [In Revision]

microRNA	Target	Predicted Phenotype
mir-675-3p	BMP7	Lethal phenotype
mir-675-3p	BMP11	Abnormal Development of kidney
mir-675-3p	BMP4	Abnormal development of kidney
mir-675-3p	Smad1	Abnormal development of kidney
mir-675-3p	Smad5	Abnormal development of kidney

microRNA	Target	Predicted Phenotype
mir-675-5p	BMP7	Lethal phenotype
mir-675-5p	BMP11	Abnormal Development of kidney
mir-675-5p	BMP4	Abnormal development of kidney
mir-675-5p	Smad1	Abnormal development of kidney
Mir-675-5p	Smad5	Abnormal development of kidney

Experimental Plan

General outline (Hypothesis 1)

1. Find a cell line to study kidney cells in vitro
    1. Knock down H19 with siRNA via transfection
    2. Genome-wide microarray to measure the expression of BMD 7, 11, 4, and SMAD1 and 5
    3. Co-transfect the cells with mir-675-3p and mir-675-5p and observe its effect
2. Employ a mouse model and observe mir-675-3p and mir-675-5p's effect for kidney development