Background

Imprinted genes are expressed depending on parent of origin (Santoro et al., 2013). The *Airn* long non-coding RNA (lncRNA) regulates imprinted gene expression during embryogenesis (Nagano et al., 2008). The *Airn* gene is located on mouse chromosome 17 and is antisense to *Igf2r* (Sleutels, Zwart & Barlow, 2002). There are 4 imprinted genes at this locus of chromosome 17 including *Airn*, *Igf2r*, *Slc22a2* and *Slc22a3* (Santoro et al., 2013). Of these genes, *Airn* is the only one expressed on the paternal chromosome. On the maternal chromosome, the *Airn* gene is repressed via methylation marks. On the paternal chromosome, *Airn* expression results in *Igf2r*, *Slc22a2* and *Slc22a3* repression and these genes demonstrate monoallelic expression during embryogenesis.

Airn acts in cis to silence *Igf2r*, *Slc22a2* and *Slc22a3* genes (Nagano et al., 2008). Interestingly, the mechanism by which Airn silences the *Igf2r* is different from *Slc22a2* and *Slc22a3* gene repression (Santoro et al., 2013). Airn represses *Igf2r* expression in a transcription-dependent manner in all developmental tissues early in development. DNA methylation of the *Igf2r* promoter at a later stage of embryogenesis results in stable gene repression (Santoro et al., 2013). In contrast, *Slc22a2* and *Slc22a3* are only expressed in extraembryonic tissues and regulation of their expression is transcrip-dependent. The *Airn* transcript acts in cis to silence *Slc22a2* and *Slc22a3* (Nagano et al., 2008). A recent study was able to demonstrate that the *Airn* transcript coats the *Slc22a3* promoter and recruits histone methyltransferase G9a.

Basis for Research, Research Question, Hypothesis and Predictions:

Studies have investigated the mechanism by which *Airn* regulates *Slc22a2*, *Slc22a3* and *Igf2r* expression (Nagano et al., 2008; Santoro et al., 2013). In particular, research has been done to elucidate the mechanism of *Igf2r* repression by *Airn*. Early during embryogenesis, *Igf2r* expression is regulated by *Airn* transcription however, later on during development *Airn* transcription is not necessary to repress paternal *Igf2r* expression (Santoro et al., 2013). At approximately day 13.5 of development, DNA methylation machinery is recruited to the *Igf2r* promoter, and repressive epigenetic marks stably silence gene expression. The maternal *Igf2r* gene is always active during development and is not targeted by DNA methylation machinery. My research question is: does *Airn* transcript play a role in recruiting DNA methyltransferase machinery to the *Igf2r* promoter for stable gene repression? DNMT3b is a DNA methyltransferase that has be shown to methylate the *Igf2r* promoter. I hypothesize that the Airn transcript associates with DNMT3b in order to recruit DNA methylation machinery for stable gene repression (Santoro et al., 2013).

To test this hypothesis I will perform a Fluorescent In Situ Hybridization (FISH) co-localization assay to determine if Airn RNA interacts with DNMT3b. We know that Airn RNA has been shown to interact with proteins to recruit them to genes (Nagano et al., 2008). I expect that *Airn* RNA will interact with DNMT3a thus providing evidence that *Airn* RNA recruits DNMT3a to the *Igf2r* gene.

Relevance and importance of Work

Understanding the mechanism used by *Airn* to recruit epigenetic modifier machinery to chromatin can give us some insight on how lncRNA in other imprinted gene clusters silence gene expression. Xist and Kq0Not1 are both imprinted lncRNAs that regulate gene expression of imprinted genes by recruiting epigenetic modifiers to genes. Furthermore, if we are able to show that *Airn* RNA recruits DNMT3b to *Igf2r*, then we can demonstrate that imprinted lncRNA can interact with various cellular components. Thus, this experiment will also be able to shed light on secondary roles imprinted lncRNA may play in the cell.

Recently, *Igf2r* was implicated in Non-Small Cell Lung Cancer (NSCLC) development (Tian et al., 2014). *Igf2r* is currently being studied as a molecular target for NSCLC drug treatment therefore understanding *Igf2r* regulation will be crucial in order to develop a drug to target *Igf2r* expression (Tian et al., 2014)

Experiment

These experiments will be carried out using the mouse *Igf2r* imprinted gene cluster as a model to study imprinted lncRNA functions in the cell. We will mate Balb/c mice mice together. Embryos will be removed from pregnant mice and tissue will be frozen and stored at -80 degrees Celsius. For the FISH co-localization assay, 14 um-thick frozen section will be made fromt the frozen tissues on a cryostat and mounted on silane-coated glass slides (Nagano et al., 2008).

References:

Nagano, T., Mitchell, J. A., Sanz, L. A., Pauler, F. M., Ferguson-Smith, A. C., Feil, R., & Fraser, P. (2008). The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science*, *322*(5908), 1717-1720.

Santoro, F., Mayer, D., Klement, R. M., Warczok, K. E., Stukalov, A., Barlow, D. P., & Pauler, F. M. (2013). Imprinted Igf2r silencing depends on continuous Airn lncRNA expression and is not restricted to a developmental window. *Development*, *140*(6), 1184-1195.

Sleutels, F., Zwart, R., & Barlow, D. P. (2002). The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature*, *415*(6873), 810-813.

Tian, Z., Yao, G., Song, H., Zhou, Y., & Geng, J. (2014). IGF2R expression is associated with the chemotherapy response and prognosis of patients with advanced NSCLC. *Cellular Physiology and Biochemistry*, *34*(5), 1578-1588.