Chromatin-Associated lncRNAs in the Cerebral Cortex During Stroke

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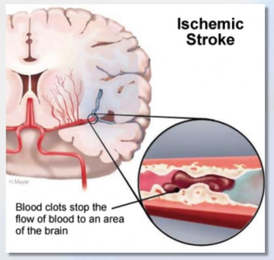
ABSTRACT

An ischemic stroke occurs when there is a blockage of blood flow to part of the brain, resulting in oxygen and nutrient deprivation, and leading to cell death and neurological dysfunction. These outcomes are associated with widespread gene expression changes.

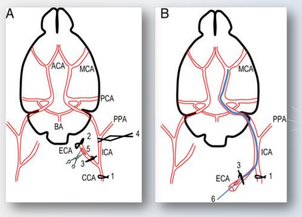


Regulatory molecules called **long noncoding RNAs** (**lncRNAs**) that are localized to the chromatin are known to regulate such gene expression changes.

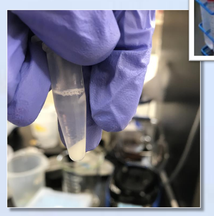
In this research project conducted at JFK Neuroscience Institute, our goal was to study stroke-induced lncRNAs in order to evaluate whether they are localized at the chromatin and may therefore participate in mediating post-stroke gene expression outcomes.

PROTOCOL

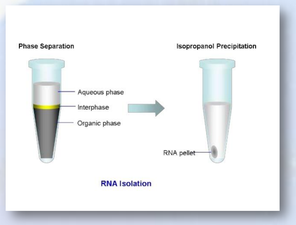
1. We induced ischemia in mice by performing middle cerebral artery occlusion (MCAO) for 1 hour followed by reperfusion for 6 hours. For the MCAO, a silicone coated filament is inserted through the internal cerebral artery and pushed up to occlude the middle cerebral artery.



2. Infarcted cortical tissues were then dissected and fractionated into cytoplasm, nucleoplasm and chromatin via centrifugation and homogenization.



3. RNA was then isolated from each fraction via homogenization in an acidified phenol-containing solution and centrifugation. Upper aqueous phase was then recovered and RNA was collected by alcohol precipitation, converted to cDNA and quantified using real-time polymerase chain reaction (qPCR).

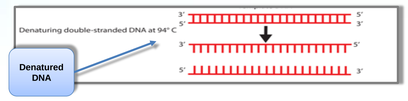


4. Polymerase chain reaction protocol consisted of:

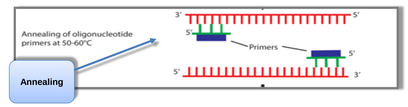
- Calculating master mix, cDNA, water, and primer needed for each sample
- Adding master mix, cDNA, and water to Eppendorf tubes
- Aliquot PCR tubes and add primer
- Place PCR tubes in thermocycler and run for 35 cycles

5. The qPCR amplified a small amount of DNA using three stages:

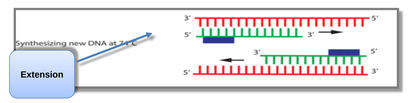
- **Denaturation**



- **Annealing**



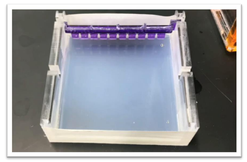
- **Elongation**

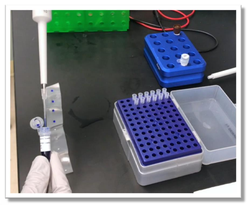


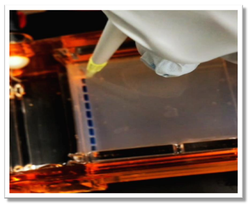
6. The qPCR data was cross-compared across the three independent fractions to determine relative enrichment of the lncRNAs. The qPCR products were visualized using agarose gel electrophoresis.

When the PCR run was finished, agarose gel was prepared by:

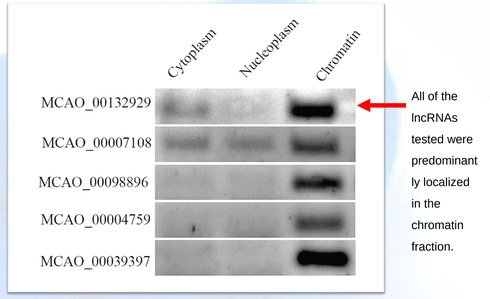
- Adding 3uL of loading dye to 10uL of a 1:1 PCR sample/ RNAase free water
- Adding 5uL of DNA ladder
- Loading 13uL into gel wells, and running gel at 100V for 20-40 minutes.







RESULTS



CONCLUSIONS

Our results showed that all of the lncRNAs tested were predominantly localized in the chromatin fraction. These results were confirmed by agarose gel electrophoresis. We, thus, conclude that the lncRNAs studied in this project are chromatin-enriched lncRNAs, which suggests their potential role in regulating gene expression during stroke.

Future work will attempt to identify the precise mechanistic roles of these lncRNAs by performing functional studies using *in vitro* and *in vivo* models of ischemic stroke.

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