CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

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Project Number

36862

Project Title

Examining Coding and Non-Coding Regions of Enhancer Landscapes in Vascular Cells (VSMCs) Stimulated with Angiotensin II

Abstract

Objectives/Goals

Activation of aortic vascular smooth muscle cells (VSMCs) by the pro-inflammatory hormone Angiotensin II (Ang II) is a critical event in the development of atherosclerosis and hypertension. In addition, enhancers play crucial roles in cell-type-specific transcription and gene expression via interaction with transcription factors (TFs) and cooperation with long non-coding RNAs (lncRNAs). Ang II-induced gene expression in VSMC is unknown and was therefore examined in this study.

Methods/Materials

Basal and Ang II-regulated enhancer repertoires were identified by ChIP-seq with antibodies to key enhancer marks (namely H3K4me1 and H3K27Ac), in rat VSMCs before and after Ang II stimulation. Data showed putative active enhancers were associated with the expression of 873 nearby genes in. The validation of enhancers' effect on gene expression was conducted in vitro and ex vivo. RNA was isolated from VSMCs treated with or without Ang II (0.1 uM) at zero (control), 1, 3, and 6 hours. cDNA was synthesized using 1 µg of RNA with reverse transcriptase. I then: (i) validated the expression of nearby genes including lncRNAs regulated by Ang II by RT-qPCR (with SYBR Green reagent), (ii) cloned enhancer fragments into pGL4-luc2 reporter plasmids with endogenous CCl2 promoter to demonstrate enhancer responsiveness to Ang II, and (iii) performed de novo motif analysis to identify transcription factor binding sites (with JASPER database, UCSC Genome Browser, and a developed Java parse-code). Motifs located within enhancer regions were of key interest, and their corresponding transcription factors (as well as frequency of motifs within the enhancer region) were noted.

Results

Results showed altered activity states in several nearby genes and lncRNAs, in cultured VSMC (in vitro) and in rat aortas (ex vivo). Lnc-Ang26 and lncAng1B4, which overlap with enhancer regions, showed a fold-over-control increase in gene expression of 20.94 and 8.42 in vitro, and 7.86 and 1.59 ex vivo. With the Jasper Database, it was found that active enhancers were enriched with binding sites for several key TFs including c-Fos and c-Jun (AP1), as well as ETS-1, both known to be involved in Ang II-mediated gene transcription.

Conclusions/Discussion

These results provide novel information about VSMC-specific enhancers, TF motifs in Ang II-regulated enhancers, and their functional roles in the regulation of genes relevant to cardiovascular disease.

Summary Statement

I found how the hormone Angiotensin II (high in diabetics and hypertensive individuals) severely alters the activities of coding and non-coding portions of the genome

Help Received

I carried out my project in the lab of Dr. Rama Natarajan of City of Hope, while consulting with her on the design and progress of my work.