Angiotensin II actions on distal regulatory elements promote vascular dysfunction and hypertension

Cardiovascular diseases such as hypertension (high blood pressure) and atherosclerosis (blocked blood vessels) lead to heart attacks and strokes, and are highly prevalent worldwide. Furthermore, these complications are significantly accelerated in patients with diabetes. Angiotensin II (AngII) is a hormone that promotes hypertension and atherosclerosis and its actions are augmented in diabetes. AngII can promote these pathologies by inducing the synthesis of potent and harmful factors that cause inflammation, aberrant growth and behavior of cells in the blood vessel called vascular smooth muscle cells (VSMC). It is still not well understood how AngII exerts such pathological effects on VSMC. We hypothesize that AngII alters the levels and modifications of key nuclear proteins and DNA that result in changes to the structure around cellular genes (a process known as “Chromatin remodeling”). These novel epigenetic changes can lead to the activation of several harmful factors. Notably, increasing evidence suggests that environmental factors can contribute to diseases via such epigenetic mechanisms. However, very little is known about the role of epigenetics (changes occurring in gene expression without affecting the DNA sequence) in AngII actions related to cardiovascular diseases.

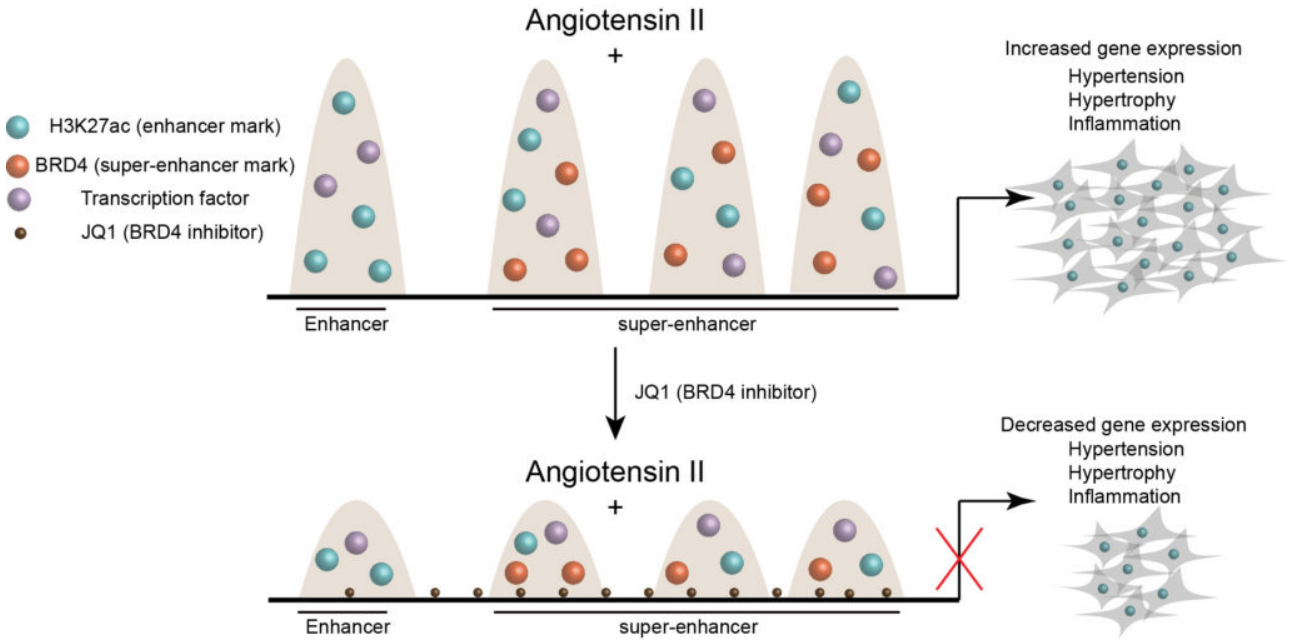

Fig. 1. Angiotensin II induces enhancer and super-enhancer formation along with transcription factor activation, and leads to increases in the expression of genes associated with vascular dysfunction. The super-enhancer inhibitor (JQ1) reduces Angiotensin II-induced super-enhancer formation also decreases pathological gene expression, hypertension, hypertrophy and inflammation.

Gene expression is usually under the tight control of DNA regulatory elements or sequences called enhancers, which are situated in a chromosome far away from the target gene that they regulate. In addition, large regulatory elements called super-enhancers, consisting of clusters of closely spaced enhancers, can control gene expression even more profoundly than enhancers, and are mostly cell-specific. Enhancers and super-enhancers are regulated by interactions with proteins called transcription factors, which bind to DNA at specific sites and control gene expression.

Therefore, dissecting the role of enhancers and super-enhancers, key genomic regulatory elements, in VSMC, is important to gain a better understanding of how AngII promotes cardiovascular diseases.

In our recently published article in Nature Communications (2017), we utilized several state-of-the-art techniques to examine how AngII regulates the enhancer and super-enhancer landscape in VSMC. AngII treated VSMC were profiled for enhancer mark H3-lysine-27-acetylation (H3K27ac), and super-enhancer mark transcriptional coactivator bromodomain protein 4 (BRD4) using a technique called chromatin-immunoprecipitation linked to high-throughput sequencing. We found AngII treatment increases enrichment of H3K27ac (enhancer activation) in cultured VSMC. AngII recapitulated similar effects on enhancers in rat aortas *in vitro* and in mouse aortas *in vivo*. AngII-induced enhancers and super-enhancers are enriched in binding sites for signal-dependent transcription factors like activator protein (AP-1), and are dependent on AngII receptor (AT_1R) activation and key downstream signaling proteins. Moreover, we employed CRISPR-Cas9 mediated gene editing approach to delete candidate enhancers and super-enhancers in order to demonstrate their involvement in the expression of the related AngII-induced genes. An inhibitor of the super-enhancer binding protein BRD4, JQ1, was able to block expression of AngII-induced genes associated with growth-factor signaling and atherosclerosis. We also found interesting cross talk among key enhancers, super-enhancers and long noncoding RNAs. Furthermore, JQ1 attenuates AngII-induced formation of enhancers and expression of inflammatory genes in VSMC. Importantly, treating mice with JQ1 ameliorates AngII-induced hypertension, medial hypertrophy and inflammation. Moreover, we observed that human genomic regions that share similar DNA sequence with AngII-regulated rat enhancers harbor genetic variations (SNPs) known to predispose individuals to cardiovascular diseases/traits.
Together our results demonstrate that AngII-induced signals integrate events at enhancer and super-enhancers to promote the expression of genes involved in vascular dysfunction (Fig. 1). These data could form the basis for the development of novel therapeutic targets for cardiovascular diseases.

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Publication

Regulation of angiotensin II actions by enhancers and super-enhancers in vascular smooth muscle cells.

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