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Epigenetic Mechanisms of Angiogenesis in the Ischemic Heart Diseases with Acupuncture Treatment

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ABSTRACT

Objective: Epigenetics, including DNA methylation, histone modification, and posttranscriptional regulation of microRNAs, is the study of heritable changes in gene expression that do not include DNA-sequence alterations. Epigenetics has become a new strategy for basic and clinical research on acupuncture in the last decade. The aim of this research update was to summarize the **e**pigenetic mechanisms of angiogenesis induced by acupuncture treatment in ischemic heart diseases.
Materials and Methods: The current authors' group has been working to illustrate the mechanism of acupuncture from an epigenetics perspective, which has shed new lights on the mechanisms and applications of acupuncture in cardiovascular diseases. This article summarizes the group's new findings in animal models as well as in patients with chronic stable angina. Progress since 2011 in other teams' research in this field is also discussed in this article.
Conclusions: Acupuncture could regulate histone modifications and could rescue patients who sustain ischemic injuries. This treatment could possibly work through promoting angiogenesis.

Keywords: acupuncture, histone modification, angiogenesis, ischemic disease, PC 6

INTRODUCTION

ISCHEMIC HEART DISEASES (IHD) are an ongoing challenge managed primarily through surgery and medications.[1] Acupuncture, as one of the most widely used strategies in traditional medicine, has recently been reported to treat chronic stable angina effectively as an adjuvant.[2] Acupuncture not only reduced angina symptoms and electrocardiogram (ECG) results in patients,[2,3] but also relieved associated anxiety and depression.[4] Understanding the mechanisms involved in protection against ischemic myocardial events will help promote application of acupuncture for clinical treatment for IHD.

Evidence has shown that acupuncture can protect the myocardium against ischemia/reperfusion (I/R) injuries in both animal experiments and in clinical research,[3,5] and the *Neiguan* (PC 6) acupoint has been mostly used for treating IHD, although other acupoints, such as *Lieque* (LU 7) and *Zusanli* (ST 6) are also effective in combination with PC 6 or in single uses.[6] To understand how acupuncture can be effective for treating IHD, mechanistic studies have been gaining increasing attention. Previous studies have focused on cardiac electrophysiology,[7] biochemistry, neurotransmitters,[8] and expression of certain molecules.[9] With the development of newer technologies, studies have probed more deeply and found more-interesting mechanisms by which acupuncture treatment can protect the heart against myocardial ischemia (MI) and injuries. Many signaling pathways have been discovered, including hypoxia, an inflammatory immune response, endothelial function, endoplasmic reticulum stress, autophagy, and angiogenesis.[10–13]

Epigenetics is the study of changes produced in gene expression caused by mechanisms other than changes in the underlying DNA sequence. It has been known that

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epigenetics can regulate gene expression through DNA methylation, histone modification, chromatin remodeling, and microRNAs.[14]

MATERIALS AND METHODS

To investigate if acupuncture reduces MI and promotes angiogenesis through epigenetic regulation, the current authors' group conducted a series of studies in the past decade by carrying out a clinical study and using a permanent ligation of the left anterior descending coronary artery (LAD) animal model.[15,16] Some research progress that this group has made since 2011 is summarized in this article.

RESULTS

Animal Research

The current group's studies in animals with ischemia or I/R injuries have demonstrated that electroacupuncture (EA) treatment at PC 6 reversed the S-T segment change; reduced the Q-wave area; decreased creatine kinase (CK), CK-MB, and lactate dehydrogenase (LDH) levels; mitigated myocardial remodeling; and promoted microvessel formation in the MI heart. Increased histone 3 lysine acetylation (H3K9 and H3K27) was observed according to the increased expression of vascular endothelial growth factor (VEGF)[13] and antiapoptotic proteins, such as caspase 3/cleaved caspase 3, Xiap, Bcl-xL, and Bcl2.[5] A chromatin immunoprecipitation (ChIP) assay confirmed that EA treatment could notably stimulate recruitment of H3K9ace at the *Vegf* promoter, having 5–12 times higher binding peaks of H3K9ace at the potential binding sites, compared with what occurred in an untreated group. It demonstrated for the first time that acupuncture can effectively upregulate *Vegf* expression through H3K9 acetylation modification directly at the *Vegf* promoter and hence activate VEGF-induced angiogenesis in rat MI models.[13]

High-throughput sequencing was applied in these studies, and genome-wide gene-expression profiles were generated both in the rat MI and Machine Intelligence Research Institute (MIRI) models, and evaluated effects of acupuncture treatment from genetic and molecular levels. RNA-seq analysis showed that genes of multiple functional pathways—such as VEGF-induced angiogenesis signaling, apoptosis signaling, and cytokine and leukocyte pathways—were altered by EA intervention in the rat MI model.[13] In the MIRI model, the data indicated that gene expressions in the I/R hearts might be responsible for the cardioprotective effects delivered by EA pretreatment. Many pathologic genes in the I/R condition were regulated differentially, while the injury of cardiomyocytes was attenuated effectively—such as lipocalin 2 (Lcn2), which is related to restoration of mitochondrial function and phospholipids' remodeling, and nuclear-receptor subfamily-4 group a-member 1 (Nr4a1), an immediate-early responsible gene—and associated with cardiomyocyte apoptosis during I/R injury.[17]

The comparison between pretreatment at PC 6 and at non-acupoints showed that stimulation on PC 6 specifically regulated cardiac-muscle contraction, vascular smooth-muscle contraction, hypertrophic cardiomyopathy, oxidative phosphorylation, inflammation and immune response, and apoptosis pathways, while the non-acupoints group only showed very mild or no regulation for the functional genes.[17]

Furthermore, applying acupuncture together with a medicinal compound (tanshinone IIa) to the animals with MI, this study demonstrated that the combination therapy's effectiveness was much stronger than acupuncture or tanshinone IIa single therapy (data not published). This combination of acupuncture and tanshinone IIa promoted angiogenesis in the heart tissue; improved serologic CK, CK-MB, and LDH and ECG scores; and elevated VEGF and CD151 mRNA level more profoundly than the single use of acupuncture or tanshinone IIa. These improvements were correlated with the increased H3K27ace expression in the heart tissue. These data provided reliable experimental basis for the theory that a ''combination of acupuncture and medication exerts a more effective role in treating diseases than single therapy.''[18]

Epigenetic regulation is a dynamic process *in vivo*, especially in disease states.[19] Histone acetylation is associated with gene expression,[20] while histone methylations can be associated with gene activation or repression depending on the different residues or positions where the modifications occur.[21] Usually, corresponding transcription factors, such as C/EBPα or C/EBP β, are corecruited to the enhancer regions of certain genes and promote histone methylation (formed by H3K4me1/2); then the acetyltransferase substrate P300 of H3K27 passes to bind and activate cell-specific enhancers through enhancer–promoter interactions of gene expression, or another unknown mechanism.[22] Both *Utx* (the ubiquitously transcribed tetratricopeptide repeat on chromosome X-UTX, identified as a lysine 27 of histone H3 demethylase, H3K27me2/3) and *Mll*4 (mixed-lineage leukemia 4, a specific methyltransferase of histone 3 lysine 4, H3K4me1/2) have been well-investigated in heart development and the occurrence of disease.[23,24] Germline deletion of either of them caused embryonic death in mice.[25,26]

To identify further if and what histone modification contributes to cardioprotection against MI injury, *Utx* or *Mll*4 cardiac-specific knockout mice (*Utx*-cKO or *Mll*4-cKO) were generated and applied to the MI model. It has been observed that the myocardial-protective effect induced by acupuncture was weakened in the absence of *Utx* or *Mll*4, suggesting that histone methyltransferase/demethylase might contribute to the protection that acupuncture treatment confers against MI (data not published). Further mechanisms are still being studied.

Clinical Research

A protocol of histone modification–based mechanistic study of acupuncture in patients with stable angina pectoris was set up during the current clinical research.[15] The protocol for this clinical study was approved by the Chinese Clinical Trial Registry (ChiCTR) on November 9, 2012 (registration no.: ChiCTR-TRC-12002668*).

The study follows the principles of the Declaration of Helsinki (Version: Edinburgh 2000). In patients with chronic stable angina, EA at PC 6 reduced the attacks of angina, visual analogue scale (a parameter for assessment of pain) scores, and dosages of nitroglycerin (a classic medicine for dilating blood vessels and increasing blood flow to the heart) used. RNA-seq from these patients' blood samples showed their deferentially expressed genes (DEGs), and ChIP-seq confirmed that the bindings of H3K9ac on the functional genes, including *Il8*, *Cxcr1* and *Cxcr2* genes were enhanced by EA treatment (data not published).

DISCUSSION

In the animal research, the current authors' group chose permanent ligation of the LAD coronary artery as a MI model, which is widely accepted in the field. After evaluating acupuncture therapeutic effects systemically, genome-wide gene-expression profiles in rats were achieved by using RNA-seq and bioinformatics analysis for the first time. It was found that VEGF, an important molecule involved in the angiogenic pathway, was activated by acupuncture treatment, together with regulation of histone modification H3K9ace. This research team was also first to introduce ChIP analysis technology to acupuncture research. This technology identified and confirmed that acupuncture treatment can obviously increase H3K9ace enrichment on the promoter of *Vegf*. This also confirmed that acupuncture can promote transcription of the *Vegf* gene to mediate angiogenesis in the ischemic myocardium through H3K9 acetylation regulation. The result was eventual myocardial protection.

For the first time, the clinical research revealed that, as regulators of gene transcription, epigenetic modifications through acupuncture should be considered important fundamental parameters for evaluating the clinical effects of such treatment. What is more, indepth epigenetic studies will provide more substantial and reliable experimental evidence for understanding and development of traditional Chinese acupuncture.

During this research, the team discovered the integrity and multitargeting roles of acupuncture, which is in good agreement with epigenetic theory,[27] and which will help illustrate the complex mechanism of acupuncture for treating IHD. Although histone modification has been given increasing attention in the study of MI-related diseases, it is still hard to generalize clearly about the contribution of methylation or acetylation to MI injury and to find the specific genes or functional pathways that are regulated directly by histone modification. This calls more attention for the field to use transgenic animals whose epigenetic marks are mutated. The current authors' ongoing projects, which focus on histone methyltransferase/demethylase, are aimed at revealing the potential true mechanism through which acupuncture works on MI, although the findings will need to be clarified in clinical studies. A limitation in the research performed so far is the limited timepoints after acupuncture treatment. Only rapid effects for some days rather than a relatively longer period were observed, so the timeframe needs to be extended in future research.

*For more information, visit the www.chictr.org.cn/showproj.aspx?proj=6884 website.

CONCLUSIONS

The current authors' research has demonstrated, for the first time, that epigenetics can be modified by acupuncture and then gene expression can be regulated in the MI or I/R condition. This is now recognized to offer a new perspective and research direction for the fields of acupuncture and moxibustion.

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DISCLOSURE STATEMENT

The authors have nothing to disclose.

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