Novel Heart Failure Therapy Targeting Transcriptional Pathway in Cardiomyocytes by a Natural Compound, Curcumin

Tatsuya Morimoto, MD, PhD*; Yoichi Sunagawa**,†; Masatoshi Fujita, MD, PhD**; Koji Hasegawa, MD, PhD†

Hypertensive heart disease and post-myocardial-infarction heart failure (HF) are leading causes of cardiovascular mortality in industrialized countries. To date, pharmacological agents that block cell surface receptors for neurohumoral factors have been used, but despite such conventional therapy, HF is increasing in incidence worldwide. During the development and deterioration process of HF, cardiomyocytes undergo maladaptive hypertrophy, which markedly influences their gene expression. Regulation of histone acetylation by histone acetyltransferase (eg, p300) and histone deacetylase plays an important role in this process. Increasing evidence suggests that the excessive acetylation of cardiomyocyte nuclei is a hallmark of maladaptive cardiomyocyte hypertrophy. Curcumin inhibits p300-mediated nuclear acetylation, suggesting its usefulness in HF treatment. Clinical application of this natural compound, which is inexpensive and safe, should be established in the near future. (Circ J 2010; 74: 1059–1066)

Key Words: Curcumin; Heart failure; Histone acetyltransferase; p300; Pharmacological therapy

The heart, an organ that repetitively contracts and relaxes, has advanced differential development. Its principal cells, the cardiomyocytes, actively divide and proliferate in the embryonic phase by maintaining their cell-dividing capacity after differentiation. However, after birth, their regenerative/dividing capacity rapidly reduces. Cardiomyocytes show maladaptive hypertrophy in response to various stimuli, such as hemodynamic overload, leading to heart dysfunction (ie, heart failure [HF]). HF is a terminal clinical feature common among various heart diseases, such as hypertensive, valvular, and ischemic heart diseases, so it is clinically important to overcome this.

Hypertension- or myocardial infarction-related stress on the heart causes the onset/deterioration of HF via neurohumoral factors of the sympathetic nervous and renin–aldosterone systems. To treat HF, agents that block signal transmission routes on the cell surface, such as β-blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin-receptor antagonists, have been used. Although therapy with these agents has improved the prognosis, the 5-year survival rate in patients with severe HF remains at less than 50%. There are a large number of complex signal transmission routes involved in cardiac remodeling, so for radical HF treatment, therapy targeting common downstream intranuclear signals may be more effective. Recent studies indicate the importance of histone acetylation-regulating enzymes as stress response-regulating factors in cardiac gene expression.

We investigated curcumin, the principal component of turmeric, which is used as a dietary compound. This substance has been used as a spice/coloring agent for curry in India and a traditional herbal medicine in China. In the United States, it was approved as a safe food material. In Japan, it has also been used to prepare mustard, pickled radish, and traditional Japanese sweets, in addition to supplements and curry.

In this study, we examined the relationship between the acetylation of a transcription factor in the nuclei of cardiomyocytes and cellular remodeling, which has recently been emphasized, and evaluated the usefulness of a new HF treatment with curcumin targeting that same factor.

Nuclear Acetylation-Regulating Mechanism

Relationship Between Histone Acetylation and Transcription

It has been previously shown that histone acetylation is closely involved in transcription control. In 1996, Allis et al initially reported histone-acetylating molecules. They found that Gcn5, a coactivator bridging transcription factors with basal transcription factors, exhibited histone acetyltransferase (HAT) activity. p300 was identified as an adenovirus E1A-binding protein, and CREB binding protein (CBP) as a
cAMP-responsive transcription factor CBP. The primary structure of p300 resembles that of CBP, and both these proteins possess the KIX domain, Bromodomain, and 3 C/H domains in addition to the HAT domain (Figure 1). They are conserved in Drosophila to the Mammalia, and target a similar transcription factor. Both their structural and biochemical features are similar. p300/CBP shows inherent HAT activity, modifying chromatin and transcription-regulating factor, and promoting gene expression via the unwinding of the chromatin structure. Nakatani identified p300/CBP-
associated factor (PCAF) as a p300/CBP-binding factor, based on the human EST database, and reported that it was homologous to Gcn5, suggesting that PCAF is a HAT. Therefore, various molecules with HAT activity were identified: 9 HATs in yeasts and 16 in the Mammalia. These are classified into at least 5 groups (Table 1). HATs transfer the acetyl group from acetyl coenzyme A (CoA) to the ε-amino group of the residual lysine group in the intranuclear histone tail, neutralizing the electric charge of positively charged amino acids. The changes in the histone tail’s electric charge may be involved in the unwinding of 3D aggregating chromatin, creating an environment to recruit proteins involved in transcription on DNA. Thus, histone acetylation may be associated with transcriptional activation.

Concerning the histone-mediated suppression of transcription, De Rubertis et al reported Rpd3 as a histone deacetylase (HDAC) in 1996. In 1997, Nagy et al found that the NcoR-SMRT repressor complex acting as a transcription-suppressing factor contained an HDAC. These findings suggest that histone acetylation contributes to transcription activity: when a HAT enhances acetylation, transcription is positively controlled, and when HDAC enhances deacetylation, transcription is negatively controlled (Figure 2).

Acetylation of Transcription Factors

Previous studies hypothesized that histone acetylation might be involved in transcriptional activation, and that histone deacetylation may suppress transcription. However, it has remained unclear how acetylation is involved in the activation of specific transcription, as well as in the increase of general transcription.

Accumulating evidence suggests that a large number of proteins other than histone, including various transcription factors, are acetylated. For example, p53, GATA1, and E2F1 are regulated via acetylation. Post-translational modifications, such as phosphorylation, acetylation, ubiquitination, and SUMOylation, play an important role in the activation, stabilization, decomposition, and nuclear transport of transcription factors. With respect to the acetylation of transcription factors, a tumor suppressor gene, p53, has been the most frequently investigated. It has been shown that 3 HATs (p300, CBP, and PCAF) are involved in p53 acetylation.

Although the sites of acetylation differ among these HATs, they are located in the C terminal of p53. The p53 acetylation-related neutralization of the positive electric charge of the C terminal may influence the structure of p53, enhancing its DNA-binding capacity. Among HATs, PCAF/GCN5, MOZ, Tip60, and p300/CBP reportedly promote the acetylation of transcription factors (Table 1).

Auto-Acetylation Mechanism of p300

Although for the past 10 years or more, it has been known that the HATs, p300/CBP, are acetylated, their roles have remained unclear. However, in 2004, Cole et al reported that auto-acetylation was involved in the activation of p300. Briefly, the auto-acetylation of a proteolytically sensitive loop region in the HAT domain of p300 enhances p300 activity. Although this is interesting, it is unclear whether other HAT molecules, such as PCAF and GCN5, regulate p300 activity via p300 acetylation.

Figure 2. Mechanism involved in transcriptional control via acetylation/deacetylation. HAT, histone acetyltransferase; HDAC, histone deacetylase.
HATs and Cardiogenesis/Cardiovascular Differentiation

The HATs involved in transcription, p300/CBP, play an important role in cardiogenesis/cardiovascular differentiation. Various types of congenital heart disease are observed in approximately one-third of patients with Rubinstein-Taybi syndrome, which may be associated with p300/CBP gene deletion or mutation, showing primary symptoms such as mental retardation, growth retardation, and a particular dysmorphology, suggesting that p300/CBP are involved in the development/proliferation of cardiomyocytes. Various studies have compared the role of p300 with that of CBP, and indicated that p300 activity is essential for muscular differentiation. Differences in the HATs’ roles in the heart should be investigated.

To examine the roles of p300 and CBP in cardiogenesis, gene-modified mice were prepared. The p300 knock-out mice died between 9 and 11.5 days of gestation, in the embryonal phase. The fetuses showed cardiac enlargement and retention of pericardial fluid, suggesting fetal heart dysfunction. In addition, congenital heart diseases, such as atrial/ventricular septal defects and valvular hypoplasia, were detected. Thinning of the ventricular wall and a decrease in the expression of proteins comprising the myocardium, such as β-MHC and β-actin, were observed, suggesting that p300 plays an important role in intracellular transcription associated with the development, differentiation, and proliferation of cardiomyocytes. Furthermore, a p300 HAT mutant was generated by substituting 2-amino acid WY of the 1,466th and 1,467th amino acids on the p300-HAT domain for AS. This p300 HAT mutant showed complete loss of HAT activity. In mice in which the above HAT domain, with the disappearance of HAT activity, was knocked into p300 using the gene knock-in approach, embryonic mortality or fatal HF early after birth was also noted, as demonstrated in the p300 knock-out mice. In this model, congenital heart diseases, a decrease in the expression of constitutional proteins, and cardiac aplasia were also observed. These findings suggest that the HAT domain of p300 is essential for normal cardiogenesis.

On the other hand, CBP knock-out mice also died between 10.5 and 12.5 days of gestation. In that model, the fetuses showed exencephaly, with deletion of cranial neural tube closure, as well as abnormalities in the mesenchymal region. Macroscopically, there were no marked abnormalities other than developmental retardation before 9.5 days of gestation. However, microscopic observation revealed massive cerebral hemorrhage related to the inhibition of angiogenesis at 10.5 to 11.5 days of gestation. This resulted in embryonic mortality. In this model, hematopoiesis was also inhibited. However, an abnormal heart structure, as observed in the p300 knock-out mice and patients with Rubinstein-Taybi syndrome, was not noted.

Based on a review of studies using genetically modified mice, p300 may play a more important role in cardiogenesis (at least in the initial phase) than CBP, although the roles of p300 and CBP, which have similar structures, have been considered to be common and alternative.

Activation of Transcription Factors in the Deterioration/Exacerbation of HF

In the deterioration/exacerbation process of HF, various signals reach the nuclei via information transmission in the cytoplasm of cardiomyocytes, influencing the pattern of gene expression by activating transcription-regulating factors. It has been reported that, in addition to cardiomyocyte hypertrophy, changes in the expression of various myocardial genes are involved in the deterioration of HF. Previous studies indicated that ventricular expression of β-myosin heavy-chain genes and atrial expression of diuretic peptides was enhanced. Furthermore, an increase in the expression of myocardial endothelin-1 plays an important role in the deterioration of HF. These changes are mainly regulated at the transcription level; if the transcription-regulating mechanism is clarified, it will be useful for clarifying the mechanism of HF deterioration/exacerbation.

In the presence of hypertrophic response stimulation, myocardium-specific transcription factors are activated via post-translational modifications, such as phosphorylation and acetylation, and not via an increase in the protein level. In particular, recent studies have shown that the acetylation-related activation of transcription factors is important. As transcription factors involved in cardiac hypertrophy, myocyte enhancer factor 2 (MEF2), serum response factor, and the zinc finger protein GATA4, 37-38 have been reported. The activities of these factors are regulated by both HATs and HDACs, at least in part, through acetylation. Class II HDAC directly or indirectly binds to these factors and inhibits their activities. In contrast, the binding of these hypertychoresponsive transcription factors to p300 promotes their activities. The role of HDACs in HF has been described precisely in previously published papers. Concerning the acetylation of transcription factors in cardiomyocytes, we and others have intensively investigated GATA4. p300 promotes GATA4 acetylation, increasing its DNA-binding capacity and transcriptional activity. The residual lysine group of GATA4 is concentrated in 2 zinc finger domains of the C terminal, which play an important role in DNA-binding capacity and binding to other factors. However, structural changes related to the acetylation of the residual lysine group of GATA4 may be involved in its transcriptional activity, as reported for p53. We analyzed the sites of p300-related GATA4 acetylation and found that 4 residual lysine groups, K311, K318, K320, and K322, were acetylated in the presence of p300, indicating that mutant GATA4 contributing to the mutation of these residual groups inhibited cardiomyocyte hypertrophy as a dominant-negative mutant.

It is also known that transcription factors are deacetylated in the presence of HDAC, as demonstrated for histone. In muscles, the HDAC-related deacetylation of MEF2 reduces the DNA-binding capacity, inhibiting transcriptional activity. In the heart, HDAC also inhibits transcriptional activity. However, no study has investigated its influence on the acetylation of transcription factors.

The intracellular protein level of transcription cofactor p300 is strictly limited. It is known that DNA-binding factors compete with each other to bind to this cofactor. In contrast, when the p300 protein level decreases, cardiomyocytes show apoptosis. Briefly, the administration of an anticancer agent, doxorubicin hydrochloride, to mice causes the apoptosis of cardiomyocytes, leading to HF. In this process, the p300 protein level in the heart is decreased. Concerning the mechanism, ubiquitinization-mediated degradation may be involved, however, the ligase remains to be clarified. Doxorubicin hydrochloride inhibits muscle-specific transcriptional expression, inducing the apoptosis of cardiomyocytes and causing HF via cardiomyopathy.
However, the cardiac overexpression of p300 prevents the deterioration of doxorubicin hydrochloride-related acute HF. Therefore, p300 is also necessary for myocardial survival, and its quantitative decrease may result in cardiomyocyte death.

Thus, control of the level of cardiomyocyte p300 protein may be important for maintaining the homeostasis of cardiomyocytes.

**Target of a New HF Treatment: HAT**

As excessive nuclear acetylation has been observed in the left ventricular cardiomyocytes after myocardial infarction, we assumed that HAT activity might play an important role in the onset of HF, that is, the overexpression of p300 and CBP in cardiomyocytes induces hypertrophy of these cells in a HAT-activity-dependent manner. Conversely, a dominant-negative form of p300 blocks the hypertrophic stimulation-related hypertrophy of cardiomyocytes. In addition, cardiac remodeling after myocardial infarction in transgenic mice with cardiac overexpression of p300 was markedly enhanced. However, in mice with the cardiac overexpression of mutant p300 lacking HAT activity, the enhancement of remodeling was inhibited to the same level as observed in wild-type mice. In this remodeling process, acetylation of the GATA4/DNA-binding capacity in residual cardiomyocytes with increased stress is enhanced. In mice showing overexpression of intact p300, these were markedly enhanced. However, in mice with cardiac overexpression of mutant p300, these were inhibited to the same level as observed in normal mice. The data showed that, when the acetylation of a myocardium-specific transcription factor, GATA4, was inhibited, post-myocardial-infarction remodeling was suppressed, suggesting that HF treatment should target p300 activity in the heart.

Several agents that inhibit HAT activity have been reported over the past few years (Table 2). Firstly, Lys-CoA and H3-CoA-20 are synthetic HAT inhibitors, which specifically inhibit p300 and PCAF, respectively. However, these agents are not easily able to permeate cells. Secondly, natural HAT inhibitors, anacardic acid and garcinol, are purified from cashew nuts and Garcinia indica, respectively. These agents are able to permeate cells, but they nonspecifically inhibit all HATs, including p300/CBP and PCAF. The roles of HATs other than p300 in the heart remain to be clarified, so the usefulness of nonspecific inhibitors in the treatment of cardiac hypertrophy/HF is unclear. A recent study reported that curcumin, the principal component of turmeric, which is used as a dietary compound, specifically inhibits p300. Curcumin

**Figure 3.** Curcumin, a p300-specific histone acetyltransferase inhibitor, prevents the development of cardiomyocyte hypertrophy.
has been used as a spice/coloring agent for curry in India and a traditional herbal medicine in China. In the United States, it has been approved as a safe food material. In Japan, it has also been used to prepare mustard, pickled radish, and traditional Japanese sweets, in addition to supplements and curry. We investigated whether this inexpensive, safe, natural substance can be used for HF treatment.

**Possibility of HF Treatment Using Curcumin**

Initially, we examined the effects of curcumin treatment in cultured primary cardiomyocytes from neonatal rats in which the hypertrophy of cardiomyocytes was induced in the presence of phenylephrine stimulation.\(^{59}\) Curcumin inhibited the increase in cardiomyocyte diameter and enhancement of the transcriptional activity of promoters of hypertrophic response genes such as ANF and \(\beta\)-MHC. It also reduced the hypertrophic responses of cardiomyocytes related to the overexpression of p300. In addition, curcumin inhibited the phenylephrine-related acetylation of GATA4 (myocardial transcription factor), GATA4 binding to p300, DNA-binding capacity, and enhanced nuclear/histone acetylation. These results suggest that curcumin inhibits p300 activity, preventing cardiomyocyte hypertrophy (Figure 3).

Secondly, we investigated the effects of curcumin using 2 rat models of HF.\(^{59}\) In a hypertensive heart disease model, we administered 50 mg/kg of curcumin orally to salt-sensitive Dahl rats, every day for 7 weeks from the compensatory hypertrophic phase. Echocardiography revealed significant curcumin-related improvement in left ventricular fractional shortening (FS), a parameter of cardiac contractility. Furthermore, curcumin inhibited thickening of the left ventricular wall and cardiomyocyte hypertrophy. In addition, this agent inhibited an increase in the cardiac p300 protein level and the enhancement of GATA4 acetylation/binding to p300. We similarly examined effects of curcumin also in another rat model of myocardial infarction. Curcumin (50 mg/kg) or a control agent was administered orally every day for 6 weeks starting from 1 week after myocardial infarction was induced. In this model, curcumin also improved FS, and inhibited the myocardial infarction-related hypertrophy of cardiomyocytes (Figure 4).

Thus, curcumin, a component of a natural substance, turmeric, inhibited the hypertrophy of cultured cardiomyocytes, preventing the onset of HF in rat models of hypertensive heart disease and myocardial infarction.

In an experiment using salt-sensitive Dahl rats, we found that curcumin inhibited not only the deterioration of hypertensive cardiac hypertrophy to HF, but also the onset of cardiac hypertrophy related to hypertension. This agent prevented the onset of cardiac hypertrophy by directly inhibiting cardiomyocyte hypertrophy without exhibiting hypotensive effects. For the clinical application of curcumin therapy, we compared the efficacy of this agent with that of a conventional, standard agent for HF, an ACE inhibitor (Enalapril), using a rat myocardial infarction model. The effects of monotherapy with curcumin on HF were similar to those of the ACE inhibitor. Combination therapy with curcumin and hypotensive agents may be more effective for cardiac hypertrophy/HF. These findings suggest the clinical usefulness of curcumin in humans.
Clinical Application
Curcumin is known to exhibit antitumor, antioxidant, and anti-inflammatory actions. In the United States, Taiwan, and Japan, clinical trials involving patients with cancer, rheumatoid arthritis, cystic fibrosis, inflammatory colitis, psoriasis, pancreatitis, or Alzheimer's disease are being conducted. In those trials, few side-effects have been reported. Curcumin, which is used as a dietary compound, may become a safe, inexpensive agent for HF. However, previously, the administration of an agent that was effective in rodents was ineffective in humans, so further examination is needed for clinical application.

Conclusion
It has been shown that the HAT-related acetylation of histone or transcription factors is involved in cardiomyocyte hypertrophy, myocardial remodeling, and the onset/deterioration of HF. In contrast, the deacetylation of histone reduces cardiomyocyte hypertrophy by inhibiting chromatin aggregation and transcription. Physiological hypertrophy is a compensatory mechanism of the heart in response to stress. However, excessive stress affects this adaptability, leading to non-compensatory HF. Briefly, balanced control between acetylation and deacetylation may be important for inhibiting acute/chronic stress-responsive myocardial hypertrophy and regulating gene expression.

Curcumin, a component of a natural substance, turmeric, has inhibited cardiomyocyte hypertrophy in cultured cardiomyocytes, as well as the onset of HF in rat models of hypertensive heart disease and myocardial infarction. The results suggest the usefulness of this inexpensive, safe, crude drug as an inexpensive agent for HF. However, previously, the administration of this compound, which is used as a dietary compound, may become a safe, inexpensive agent for HF. In those trials, few side-effects have been reported. Curcumin, known to exhibit antitumor, antioxidant, and anti-inflammatory actions. In the United States, Taiwan, and Japan, clinical trials involving patients with cancer, rheumatoid arthritis, cystic fibrosis, inflammatory colitis, psoriasis, pancreatitis, or Alzheimer's disease are being conducted. In those trials, few side-effects have been reported. Curcumin, which is used as a dietary compound, may become a safe, inexpensive agent for HF. However, previously, the administration of an agent that was effective in rodents was ineffective in humans, so further examination is needed for clinical application.

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