Topic: 3. Heart Failure and Cardiomyopathy**A Novel Target Molecule of Nobiletin Derived from Citrus Peels has a Therapeutic Potency Against the Development of Heart Failure**

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Introduction and Objectives

Maladaptive hypertrophy is being recognized as a critical event during the development of heart failure. In a previous study, we demonstrated that a natural compound, curcumin, prevented cardiomyocyte hypertrophy and the development of heart failure in two different model animals.¹⁻⁴ Recently, we screened a natural compound library and found that nobiletin, a poly-methoxy flavonoid derived from *Citrus unshu*, repressed phenylephrine (PE)-induced hypertrophic responses in cardiomyocytes and prevented the deterioration of systolic function and LV pathological hypertrophy in animals with myocardial infarction and pressure-overload. Thus, nobiletin is expected as an attractive pharmacological agent for the treatment of heart failure. However, the target molecule of nobiletin in cardiomyocytes is still unclear. To solve this problem, we attempted to identify novel nobiletin-binding proteins by a proteomics approach and investigate its functional mechanism in mice.

Materials and Methods

To purify NBPs, biotin-conjugated nobiletin (Bio-nobiletin) was synthesized. Protein extracts from rat hearts were incubated with Bio-nobiletin or biotin alone. Nobiletin binding proteins (NBP) were precipitated with streptavidin beads and analyzed by LC/LC-MS/MS. *In vitro* binding assay and enzyme activity assay were performed using recombinant NBP1. Neonatal rat cardiomyocyte expressing NBP1 or transducing siRNA-NBP1 were treated with nobiletin and then stimulated with PE for 48 hours. These cells were stained with β -MHC antibody and cell size were measured. TG mice carrying NBP1 gene under the CAG promoter (NBP1-TG)

and WT mice were subjected to sham or transaortic constriction (TAC). After 8 weeks, echocardiography, histological analysis, and quantification of hypertrophic responses gene transcription were performed.

Results

162 NBPs were identified by LC-LC/MS-MS. One of them, nobiletin-binding protein 1 (NBP1) which related to cellular metabolic pathway, physically bound to biotin-conjugated nobiletin. Nobiletin also activated enzyme activity of NBP1 *in vitro*. In cardiomyocyte, knockdown of NBP1 failed to nobiletin-mediated anti-hypertrophic effect, and overexpression of NBP1 inhibited phenylephrine-induced cardiomyocyte hypertrophy. The results of echocardiography showed that NBP1-TG mice resisted TAC induced cardiac hypertrophy and systolic dysfunction compared with WT mice. NBP1 overexpression prevented TAC-induced myocardial cell hypertrophy and perivascular fibrosis, and hypertrophy-responses genes activation such as ANF and BNP.

Conclusions

Proteomics analysis on molecular targets of nobiletin is useful to delineate nobiletin-mediated signaling pathways and anti-hypertrophic effect in cardiomyocytes. These findings suggest that nobiletin inhibits cardiomyocyte hypertrophy and the development of heart failure through the functional regulation of NBP1 activity. A natural compound, nobiletin, might be a candidate for heart failure agent in human. ■

1. Sunagawa Y, Sono S, Katanasaka Y, et al. Optimal dose-setting study of curcumin for improvement of left ventricular systolic function after myocardial infarction in rats. *J Pharmacol Sci* 2014;**126**(4):329–36. DOI: 10.1254/jphs.14151FP. PMID: 25409899
2. Sunagawa Y, Wada H, Suzuki H, et al. A novel drug delivery system of oral curcumin markedly improves efficacy of treatment for heart failure after myocardial infarction in rats. *Biol Pharm Bull* 2012;**35**(2):139–44. PMID: 22293342
3. Sunagawa Y, Morimoto T, Wada H, et al. A natural p300-specific histone acetyltransferase inhibitor, curcumin, in addition to angiotensin-converting enzyme inhibitor, exerts beneficial effects on left ventricular systolic function after myocardial infarction in rats. *Circ J* 2011;**75**(9):2151–9. PMID: 21737953
4. Morimoto T, Sunagawa Y, Kawamura T, et al. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. *J Clin Invest* 2008;**118**(3):868–78. DOI: 10.1172/JCI33160. PMID: 18292809