Tao He

Dr. med.

Disruption of the interaction between Ca^{2+} /Calmodulin-Dependent Protein Kinase II and Histone Deacetylase 4 protects from pathological cardiac remodeling: a translational study towards epigenetic therapy of heart failure

Fach/Einrichtung: Innere Medizin

Doktorvater: Prof. Dr. med. Johannes Backs

Zusammenfassung/ Summary:

In response to diverse hemodynamic and neurohormonal insults, the heart undergoes adverse remodeling which is characterized by cardiac hypertrophy, fibrosis, myocytes apoptosis, and culminates to heart failure. Notably, this process is accompanied by activation of the MEF2 transcription factor and reprogramming of cardiac gene expression. Class IIa histone deacetylases (HDACs), including isoform 4, 5, and 9, act as signal-responsive repressors of in the nucleus. A substantial body of evidence from our lab and other researchers has revealed that Calcium/calmodulin-dependent protein kinase II (CaMKII) contributes to pathological cardiac remodeling at least in part by phosphorylating HDAC4. CaMKII specifically signals to HDAC4 by binding to a unique docking site that is absent in other Class IIa HDACs. Phosphorylation of HDAC4 by CaMKII promotes cytosolic accumulation of HDAC4, with consequent derepression of MEF2. Moreover, HDAC4 confers CaMKII-responsiveness to HDAC5, which does not directly interact with CaMKII, by oligomerization with HDAC5. Thus, the CaMKII-HDAC4 interaction represents a point of convergence for upstream kinase signaling to downstream MEF2-regulated genes. The present study aimed at translating the molecular biology characterizations of HDAC4/CaMKII interaction to the novel therapeutic approach that protects against adverse cardiac remodeling.

In the first study, we generated a CaMKII-nonresponsive HDAC4 mutant mice by mutating the 598th arginine to phenylalanine. In vitro analysis has determined that the 598th Arginine is essential for CaMKII-HDAC4 interaction. Point mutagenesis of HDAC4 598F most efficiently disrupts CaMKII docking, diminishes 14-3-3 binding and consequently prevents CaMKII-induced cytosolic accumulation of HDAC4. Compared with wild type littermates,

HDAC4 mutant mice exhibited protective effects against cardiac dysfunction, hypertrophy and fibrosis in response to pressure overload, which were accompanied by the diminished CaMKII binding to HDAC4, the decreased CaMKII-induced phosphorylation of HDAC4, as well as the attenuated MEF2 transcriptional activity. This is the first in vivo evidence suggesting that phosphorylation of HDAC4 by CaMKII is a central mechanism in the progression of cardiac remodeling and dysfunction. Meanwhile these data established that disrupting the interaction of CaMKII and HDAC4 acts as a potential therapeutic target.

Next, we sought to develop the small molecules that sufficiently inhibit HDAC4/CaMKII interaction, acting as therapeutic target-specific CaMKII inhibitors. Based on the findings that the existing of CaMKII pseudo substrate motif in the CaMKII binding domain of HDAC4 which is critical for the interaction of CaMKII and HDAC4, we developed a HDAC4-derived peptide with high extensive homology to the CaMKII binding domain of HDAC4. We identified that this peptide could compete with HDAC4 for the binding at the CaMKII binding site so that it partially abrogated CaMKII signals specifically to HDAC4, as evidenced by the decreased CaMKII-induced phosphorylation of HDAC4. Notably, this competition peptide exerted the potency of repression of FCS-induced cardiomyocytes hypertrophy. However, the in vivo application of this peptide was pending mainly because of a complete lack of cell specificity. Alternatively, identifying compounds that selectively prevent or disrupt the CaMKII and HDAC4 interaction would be a viable approach. Here, we described the first assay designed for high-throughput screening to identify the inhibitors of CaMKII-HDAC4 interaction. Based on Alphascreen technology, we established a sensitive, homogeneous beads-based proximal assay format and validated its specificity by using the acknowledged inhibitory peptide. Pilot screen of small molecules using this screen format showed good assay quality, as evidenced by the high absolute signal, excellent Z-prime values, and good reproducibility of inhibitions values between two independent runs. To date, High-throughput screening of approximately 78000 compounds has been accomplished, and several hits were identified. Efforts at validating such target-specific inhibitors are underway.

Taken together, we determined that CaMKII-HDAC4 interaction contributes to the progression of cardiac remodeling and dysfunction, and disrupting the interaction of CaMKII and HDAC4 is a potential therapeutic target. Based on these findings, we further made initial efforts to identify the drug-like molecules (peptide and compound) that interrupt specifically this protein-protein interaction, which pave the way to translate the basic molecular

characterization of HDAC4/CaMKII interaction to novel therapeutic approach against heart failure.

HDAC4 and HDAC5 have been identified to mediated cyclic AMP (cAMP)-induced repression of MEF2 by two different mechanisms, while the role of HDAC9, another important HDAC IIa isoform, is unknown. In the second study, we described that likewise HDAC5, cAMP impairs 14-3-3 binding to MITR (the homolog of HDAC9) in cardiomyocytes via diminished phosphorylation of MITR at 14-3-3 binding sites, with consequence of association with chromatin. Using pharmacological agonists or inhibitors, we identified that cAMP-induced hypo-phosphorylation of MITR is due to PKA-dependent PKD inhibition. Strikingly, we further found that not HDAC9 but HDAC5 is required for cAMP-induced MEF2 inhibition. These findings complement our previous publication in which selective repression of MEF2 activity by PKA-dependent proteolysis of HDAC4 has been described, providing a comprehensively understanding of crosstalk between cAMP/PKA signaling and diverse stress signaling that ultimately converge on Class IIa HDACs and the influence on MEF2 -governed cardiac gene expression.