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Inhibition of GATA4 dimerization suppress hypertrophic responses

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Introduction: Hypertrophic signals eventually reach the nuclei of cardiomyocytes, change patterns of gene expression, and cause the development of heart failure. During the development of heart failure, intrinsic histone acetyltransferase called p300 induce GATA4 acetylation. Acetylated GATA4 increases its DNA binding, up-regulates cardiac hypertrophic response genes, and lead to heart failure. A zinc finger protein, GATA4 is the transcription factor that expression level is high in heart. It has been reported that GATA1, the same GATA family, regulates transcriptional activity through its homo-dimerization. However, GATA4 homo-dimerization and its relationship to hypertrophic responses are still unknown.

Purpose: To clarify the relationship between GATA4 homo-dimerization and transcriptional activity and investigate whether inhibition of this homo-dimerization become therapeutic target for cardiac hypertrophy.

Methods: GST pull-down and DNA pull-down assay were performed using GST fusion full length and deletion mutants of GATA4 and biotin-conjugated ET-1 promoter probe including a GATA element. Recombinant C-zinc finger domain (256–326), including C-zinc finger motif (256–295) and acetylation site (308–326) was cross-linked using glutaraldehyde and subjected to silver staining. An expression plasmid with three GATA4-acetylation site mutant-conjugated with nuclear localization sequence (3xG4D) was constructed. Immunoprecipitation and western blotting were performed using nuclear extract from HEK293T cells expressing p300, GATA4, and 3xG4D. Luciferase assay was using ANF and ET-1 promoter sequences. Neonatal rat cultured cardiomyocyte expressed 3xG4D and then stimulated with phenylephrine (PE) for 48 hours. Next cardiomyocytes stained with α-actinin antibody and measured the cell surface area.

Results: The acetylation site of GATA4 was required for the dimerization of GATA4. But, C-zinc finger motif (256–295) and the acetylation site were required for the DNA binding. Recombinant C-zinc finger domain formed not only a homo-dimer but also a multimer. Co-expression of p300 increased the formation of homo-dimer as well as the acetylation of GATA4 in HEK293T cells. The GATA4 homo-dimer was disrupted by acetyl-deficient GATA4 or HAT-deficient p300 mutant. Overexpression of 3xG4D prevented the dimerization of GATA4, but not acetylation of GATA4. The result of luciferase assay showed that overexpression of 3xG4D prevented p300/GATA-induced ANF and ET-1 promoter activities. Furthermore, overexpression of 3xG4D inhibited phenylephrine-induced cardiomyocyte hypertrophy.

Conclusions: These results suggest that GATA4 dimerization may play an important role in hypertrophy-response gene activation. Thus, it is likely that inhabitation of GATA4 dimerization become therapeutic target for cardiac hypertrophy.