ABSTRACTS

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Regulation of myogenesis via kinase driven activation of DPF3a, a BAF complex member and its interaction with transcription repressor HEY1

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Purpose: Interactions between chromatin-associated proteins and transcription factors play an important role in chromatin activation and gene regulation. They can be modulated by extracellular kinases, which are frequently activated by hormones and can phosphorylate chromatin-associated proteins to induce gene transcription. DPF3 is a member of the BAF (brahma-associated factor) chromatin remodeling complex and functions as an epigenetic factor to initiate gene transcription during heart and muscle development. Previous studies identified two isoforms of DPF3. DPF3b contains two PHD (plant homeo domain) fingers that recognize acetylated and methylated lysine residues of histone H3 and H4. However, very little is known of DPF3a, which contains a half-PHD finger. Here, we present the molecular mechanism of DPF3a regulation and its signaling cascade in myogenesis.

Methods and Results: Using co-immunoprecipitation, we found that HEY proteins (HEY1, HEY2 and HEYL) are associated with BAF complex via the specific interaction with DPF3a. Further experiments showed that the posttranslational modification of DPF3a is essential for the interaction, which is mediated by the half-PHD finger of DPF3a. Using mass spectrometry, we identified three phosphorylation sites within DPF3a and in Tandem Affinity Purification we observed an interaction of DPF3a with all three subunits of the serine/threonine kinase CKII (casein kinase II). In vitro kinase assay showed that DPF3a is phosphorylated by CKII and inhibition of CKII by RNAi significantly reduces the DPF3a phosphorylation in vivo. DPF3a and HEY1 have common genomic targets; however, DPF3a is not capable of binding DNA directly. HEY1 knockdown in C2C12 skeletal muscle cells, combined with ChIP-qPCR demonstrated that the association of DPF3a with its targets is significantly reduced. Thus, HEY1 may represent the linker between DPF3a and its genomic targets. Interestingly, we found that the C-term of DPF3a shows high transcriptional activity in GAL4 transactivation assay and DPF3a significantly reduces HEY1 mediated repression of its targets (e.g. ANF, HEY1 and JAG1) using luciferase reporter assays.

Conclusion: Our findings demonstrate an unprecedented mechanism of CKII-depending release of HEY1 from its genomic targets mediated by DPF3a and reveal a link between kinase activity and gene transcription mediated by a member of the BAF complex.