Differential Association of Chromatin Proteins Identifies BAF60a/SMARCD1 as a Regulator of Embryonic Stem Cell Differentiation.

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Abstract:
Embryonic stem cells (ESCs) possess a distinct chromatin conformation maintained by specialized chromatin proteins. To identify chromatin regulators in ESCs, we developed a simple biochemical assay named D-CAP (differential chromatin-associated proteins), using brief micrococcal nuclease digestion of chromatin, followed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Using D-CAP, we identified several differentially chromatin-associated proteins between undifferentiated and differentiated ESCs, including the chromatin remodeling protein SMARCD1. SMARCD1 depletion in ESCs led to altered chromatin and enhanced endodermal differentiation. Gene expression and chromatin immunoprecipitation sequencing (ChIP-seq) analyses suggested that SMARCD1 is both an activator and a repressor and is enriched at developmental regulators and that its chromatin binding coincides with H3K27me3. SMARCD1 knockdown caused H3K27me3 redistribution and increased H3K4me3 around the transcription start site (TSS). One of the identified SMARCD1 targets was Klf4. In SMARCD1-knockdown clones, KLF4, as well as H3K4me3 at the Klf4 locus, remained high and H3K27me3 was abolished. These results propose a role for SMARCD1 in restricting pluripotency and activating lineage pathways by regulating H3K27 methylation.

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