Embryonic Stem Cell-specific Phosphorylation Shapes Splicing During Differentiation

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Background: Alternative pre-mRNA splicing expands proteomic diversity and plays a central role in regulating cell fate transitions. Dysregulation of splicing contributes to developmental disorders and disease. Emerging evidence suggests that phosphorylation of splicing regulators influences their subcellular localization, protein–protein interactions, and splicing outcomes. However, the mechanistic basis of phosphorylation-dependent splicing regulation during human embryonic stem cell (hESC) differentiation remains poorly understood. Method: We employed hESC differentiation as a model to investigate global changes in splicing factor phosphorylation during developmental transitions. We identified phosphorylation changes in key splicing regulators and focused on SF3B2, a core component of the U2 snRNP complex involved in branch-point recognition, which showed hyperphosphorylation at serine 362 (S362) upon exit from pluripotency. An endogenous phospho-deficient SF3B2-S362A hESC variant was generated to investigate the functional consequences of this modification on lineage commitment. Changes in SF3B2 protein interactions during differentiation were further characterized using co-immunoprecipitation followed by mass spectrometry (Co-IP-MS). Results: Global profiling revealed widespread changes in the phosphorylation of splicing regulators during hESC differentiation. SF3B2 was markedly hyperphosphorylated at S362 upon exit from pluripotency. Functional characterization of SF3B2-S362A hESC revealed significantly attenuated induction of embryonic neuroectoderm, accompanied by downregulation of SOX2 and SOX17. Furthermore, interactome profiling demonstrated that phosphorylation of SF3B2 at S362 alters its protein–protein interactions within the U2 snRNP complex, suggesting that this modification regulates the assembly or functional state of the splicing machinery during differentiation. Conclusion: Our findings identify SF3B2 phosphorylation at S362 as a regulatory switch linking post-translational modification of a core splicing factor to lineage-specific cell fate decisions. These results provide mechanistic insight into phosphorylation-dependent regulation of the splicing machinery during hESC differentiation and expand our understanding of how RNA-processing pathways contribute to developmental transitions.