Spring 2017

Site-Specific Epigenetic Manipulation in Stem Cells

Gregory Fishberger
Washington University in St. Louis

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Mentor: Grant Challen

Epigenetic abnormalities, such as aberrant DNA methylation, have been implicated to play a causal role in the development of many genetic diseases and forms of cancer. DNA methylation has long been thought to be involved in transcriptional repression; however, the direct relationship is not well characterized. Furthermore, the cause and effect relationship between aberrant epigenetic marks at distinct loci and certain genetic diseases is not well established. In this study, we utilized the CRISPR-Cas9 targeting system with a catalytically inactive Cas9 endonuclease fused to the catalytic domain of hDNMT3a to directly investigate the influence of DNA methylation on gene expression. With multiple guide RNAs, we localized the fusion construct to a discrete locus on the *Cdkn1a* gene promoter region in order to induce targeted DNA methylation. Following treatment with dCas9-3a, we observed site-specific DNA methylation at the designated locus and a corresponding decrease in *Cdkn1a* gene expression. The induced methylation and *Cdkn1a* repression remained stable across multiple cell passages indicating the stability of the technique. This novel approach of site-specific epigenetic modulation has the potential to provide highly specialized treatment options for human diseases, as well as the ability to regulate cell differentiation to control cell fate.