Review

UHN Microarray Centre

Regulation of genomic DNA methylation by Vezf1

Review of: Gowher H, Stuhlmann H, Felsenfeld G. Vezf1 regulates genomic DNA methylation through its effects on expression of DNA methyltransferase Dnmt3b. Genes Dev 2008, 22:2075-2084

DNA methylation is one form of epigenetic modification that is responsible for the regulation of gene expression. DNA methylation is maintained throughout a cell's lifespan by de novo DNA methyltransferases Dnmt3a and Dnmt3b and the maintenance methyltransferase Dnmt1 (1). Vascular endothelial zinc finger 1 (Vezf1) is a recently identified zinc finger transcription factor that is expressed in endothelial cells during vascular development in the mouse embryo (2). Vezf1, and its human homologue, DB1, bind in a sequence-specific manner to GC-rich sequences of several genes including metallothionein 1 (2), interleukin-3 (3), endothelin-1 (4), and stathmin/oncoprotein18 (5). Although the mechanism by which Vezf1 regulates the expression of the DNA methyltransferase *Dnmt3b* is not completely understood, this study shows that the absence of Vezf1 results in a depletion of Dnmt3b, which in turn affects global genomic methylation and the epigenetic regulation of gene expression (6).

Using a mouse embryonic stem cell line in which both copies of *Vezf1* have been deleted (7), Gowher *et al*. present evidence that the absence of *Vezf1* causes major loss of genomic methylation at specific sites, including certain repeat elements, some imprinted loci, and many CpG islands. Using methylated DNA immunoprecipitation (MeDIP), the genome-wide methylation patterns of *Vezf1*$^{-/-}$ embryonic stem cells and wild-type (WT) cells were compared. Following the hybridisation of immunoprecipitated product to UHNMAC Mouse 4.6k CGI arrays, the genome-wide loss of methylation at more than 1300 CpG islands was revealed for *Vezf1*$^{-/-}$ cells. Among these, 76 CGIs were located within 3 Kb upstream of promoters and 71 CGIs were within 3Kb downstream of genes. Many of these CGIs were associated with testis-specific, neuronal-specific, and homeobox genes and genes involved in tumourigenesis. For 14 of these genes, the expression levels between WT and *Vezf1*$^{-/-}$ were validated using real time-PCR.

Although this study found no significant difference in the overall DNA methylation activity of *Vezf1*$^{-/-}$ and WT cells, differences in the levels of different methylating enzymes were revealed. The expression levels of three known active DNA methyltransferases were compared in *Vezf1*$^{-/-}$ and WT cells. The levels of Dnmt3a and Dnmt1 transcripts were relatively similar, however, Dnmt3b expression was considerably reduced in *Vezf1*$^{-/-}$ cells compared with WT. To complicate matters, Dnmt3b transcripts are known to undergo alternative splicing and more than eight splice variants have been identified (8). Dnmt3b1 (full length) and Dnmt3b6 variants are the major variants of Dnmt3b found in normal embryonic stem cells. The mechanism by which Vezf1 regulates the expression of *Dnmt3b* has not yet been determined, however three binding motifs for Vezf1 in the introns or 3' UTR of the *Dnmt3b* gene have been identified (6).

This study shows that the partial loss of *Dnmt3b* expression in *Vezf1*$^{-/-}$ embryonic stem cells causes methylation defects similar to those found in ICF (immunodeficiency, centromere instability, facial anomalies) syndrome patients (9,10). ICF syndrome is a genetic disease directly related to a genomic methylation defect that mainly affects classical satellites 2 and 3, both components of constitutive heterochromatin (10). This study shows that *Vezf1*$^{-/-}$ cells display widespread loss of DNA methylation and that this loss correlates with an approximately fourfold reduced expression in of the de novo DNA methyltransferase Dnmt3b, compared with WT embryonic stem cells (6).

References

1. Goll MG & Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem 2005, 74:481
2. Miyashita H & Sato Y. Metallothionein 1 is a downstream target of vascular endothelial zinc finger 1 (VEZF1) in endothelial cells and participates in the regulation of angiogenesis. Endothelium 2005, 12(4):163
3. Koyano-Nakagawa N *et al*. Molecular cloning of a novel human cDNA encoding a zinc finger protein that binds to the interleukin-3 promoter. Mol Cell Biol 1994, 14(8):5099
4. Aitsebaomo J *et al*. Vezf1/DB1 is an endothelial cell-specific transcription factor that regulates expression of the endothelin-1 promoter. J Biol Chem 2001, 276(42):39197
5. Miyashita H, *et al*. Vascular endothelial zinc finger 1 is involved in the regulation of angiogenesis: possible contribution of stathmin/OP18 as a downstream target. Arterioscler Thromb Vasc Biol 2004, 24(5):878
6. Gowher H, *et al*. Vezf1 regulates genomic DNA methylation through its effects on expression of DNA methyltransferase Dnmt3b. Genes Dev 2008, 22:2075
7. Kuhnert F, *et al*. Dosage-dependent requirement for mouse Vezf1 in vascular system development. Dev Biol 2005, 283(1):140
8. Ostler KR, *et a*l. Cancer cells express aberrant DNMT3B transcripts encoding truncated proteins. Oncogene 2007, 26:5553
9. Hansen RS, *et al*. Escape from gene silencing in ICF syndrome: Evidence for advanced replication time as a major determinant. Hum Mol Genet 2000, 9:2575
10. Jiang YL, *et al*. DNMT3B mutations and DNA methylation defect define two types of ICF syndrome. Human Mutation 2005, 25(1):56