

Personal Opinion

Methylation of CpG islands: potential relevance for hypertension and kidney diseases

Felix J. Frey

Department of Nephrology and Hypertension, Inselspital, University of Berne, Switzerland

DNA methylation

Methylation of DNA is an epigenetic process which modulates gene expression [1]. In the mammalian genome, methylation is almost exclusively observed at cytosines 5' to guanines, i.e. in the CpG dinucleotides. Many of these CpG islands are associated with promoters [2,3]. Methylation of CpGs in the promoter region has the potential to silence gene expression. Mechanisms accounting for diminished or abrogated gene expression following CpG methylation comprise blocking of the binding of sequence-specific *trans*-activating proteins or binding of proteins that interfere with transcription [3,4]. Prominent examples of gene silencing induced by CpG methylation comprise X-chromosome inactivation in females, developmental transcriptional regulation, genomic imprinting, carcinogenesis and tissue-specific expression of a gene [5–8].

Inhibition of DNA methyltransferase activity

A family of DNA methyltransferases (DNMTs) that can catalyse cytosine methylation in different sequence contexts has been identified. Inhibition of DNMT enzymes by xenobiotics, antisense or small interfering RNA resulted in lower steady-state methyltransferase activity, global or gene-specific methylation and, most interestingly, in re-expression of silenced genes [9,10]. Such a reactivation of genes by inhibitors of DNMTs, including 5-aza-2'-deoxycytidine (decitabine) and 5-azacytidine (azacitidine), has been shown to be clinically useful for the treatment of tumours attributable to repressed tumour suppressor genes or in subjects with β -thalassaemia or sickle cell anaemia by inducing hypomethylation of the γ -globin chain

gene with effectively increased production of fetal haemoglobin [10,11]. In studies of normal healthy volunteers it is interesting to observe that the anti-arrhythmic drug procainamide reverses CpG island hypermethylation [7,12].

Examples of gene silencing by CpG methylation in the field of hypertension and nephrology

A fascinating observation is the distinct cell type-specific expression of the native erythropoietin gene in infrequent interstitial peritubular cells [8]. In this gene, the promoter and 5'-untranslated region comprise a CpG island; methylation of the CpG island correlates inversely with expression and, furthermore, methylation of this CpG island was shown to induce recruitment of a methyl-CpG-binding protein to the promoter and to block the association of nuclear proteins with consecutive inhibition of expression [8,13].

Similarly to erythropoietin, the 11β -hydroxysteroid dehydrogenase enzyme (11β -HSD2) exhibits a remarkable cell-specific constitutive expression in mineralocorticoid target tissues such as epithelial cells from the renal cortical collecting duct [14,15]. The main function of the 11β -HSD2 enzyme is to protect the non-selective mineralocorticoid receptor (MR) from activation by 11β -hydroxyglucocorticoids, such as cortisol in humans or corticosterone in rodents. A reduced activity of 11β -HSD2 leads to overactivation of the MR by cortisol, with renal sodium retention, hypokalaemia and a salt-sensitive increase in blood pressure. Recently, CpG islands covering the promoter and exon 1 of 11β -HSD2 were found to be densely methylated in tissues and cell lines with low expression but not those with high expression of 11β -HSD2. Demethylation induced by 5-aza-2'-deoxycytidine and procainamide enhanced the transcription and activity of the 11β -HSD2 enzyme in human cells *in vitro* and in rats *in vivo*. Methylation of 11β -HSD2 promoter-luciferase constructs decreased transcriptional activity, and methylation of recognition sequences of transcription factors known to be relevant

Correspondence and offprint requests to: Felix J. Frey, MD, Department of Nephrology and Hypertension, Inselspital, University of Berne, Freiburgstrasse 10, CH-3010 Berne, Switzerland. Email: felix.frey@insel.ch

for the expression of this enzyme diminished their binding activity, indicating a role for the epigenetic mechanism of DNA methylation for the expression of this gene causally linked with hypertension [7].

A second gene possibly linked with blood pressure regulation, endothelin-converting enzyme (ECE-1c), recently has been shown to exhibit CpG islands in the promoter [16]. *In vitro* methylation of these islands reduced the activity of the ECE-1c promoter. These observations made in cell cultures must be clarified by investigations *in vivo*, because methylation is a phenomenon that can occur in cell cultures as a result of the inability of cell lines to express all of the functions typical of the tissue from which they were derived.

Outlook

The prevalence of neoplastic diseases increases with age. For many of the genes associated with carcinogenesis, altered CpG methylation has been described to be initiated during the course of ageing [17]. Similarly, the prevalence of hypertension increases with age and it is conceivable that the same fundamental molecular mechanism, CpG methylation, accounts at least in part for the age-dependent appearance of both disease states.

The demonstration that the degree of CpG methylation in the promoter of the 11 β -HSD2 gene determines activity and tissue-specific expression of this enzyme provides a kind of proof of principle for the relevance of the methylation status of pivotal genes for blood pressure control. It is difficult to predict which of the many candidate genes involved in the regulation of blood pressure should be investigated with respect to their relevance to DNA methylation, because most housekeeping genes and ~40% of genes with tissue-specific expression contain CpG islands [2,3] and many of these genes might change their methylation status as a function of age or in the presence of disease states. Furthermore, such changes might not be relevant due to compensatory mechanisms abrogating the overall effect on blood pressure.

Promising candidates worth investigating for CpG methylation-dependent effects are those genes for which exonic mutations have been shown to induce a disease state, because these mutations unambiguously indicate that a reduced or an enhanced expression of the corresponding protein cannot be compensated by other mechanisms. The vast majority of monogenic diseases causing arterial hypertension are linked to renal sodium handling in the cortical collecting duct [18]. Thus, in a first approach, these genes are reasonable targets to be analysed with respect to regulation by the epigenetic mechanism of CpG methylation, an attractive contention in the light of the known age-dependent sodium sensitivity in humans [19].

Conflict of interest statement. None declared.

References

1. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; 3: 415–428
2. Larsen F, Gundersen G, Lopez R, Prydz H. CpG islands as gene markers in the human genome. *Genomics* 1992; 13: 1095–1107
3. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J Mol Biol* 1987; 196: 261–282
4. Taddei A, Maison C, Roche D, Almouzni G. Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. *Nat Cell Biol* 2001; 3: 114–120
5. Razin A, Shemer R. DNA methylation in early development. *Hum Mol Genet* 1995; 4 Special issue: 1751–1755
6. Rand E, Cedar H. Regulation of imprinting: a multi-tiered process. *J Cell Biochem* 2003; 88: 400–407
7. Alikhani-Koopaei R, Fouladkou F, Frey FJ, Frey BM. Epigenetic regulation of 11 beta-hydroxysteroid dehydrogenase type 2 expression. *J Clin Invest* 2004; 114: 1146–1157
8. Yin H, Blanchard KL. DNA methylation represses the expression of the human erythropoietin gene by two different mechanisms. *Blood* 2000; 95: 111–119
9. Robert MF, Morin S, Beaulieu NA *et al.* DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. *Nat Genet* 2003; 33: 61–65
10. Goffin J, Eisenhauer E. DNA methyltransferase inhibitors—state of the art. *Ann Oncol* 2002; 13: 1699–1716
11. Charache S, Dover G, Smith K, Talbot CC Jr, Moyer M, Boyer S. Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the gamma-delta-beta-globin gene complex. *Proc Natl Acad Sci USA* 1983; 80: 4842–4846
12. Lin X, Asgari K, Putzi MJ *et al.* Reversal of GSTP1 CpG island hypermethylation and reactivation of pi-class glutathione S-transferase (GSTP1) expression in human prostate cancer cells by treatment with procainamide. *Cancer Res* 2001; 61: 8611–8616
13. Wenger RH, Kvietikova I, Rolfs A, Camenisch G, Gassmann M. Oxygen-regulated erythropoietin gene expression is dependent on a CpG methylation-free hypoxia-inducible factor-1 DNA-binding site. *Eur J Biochem* 1998; 253: 771–777
14. Escher G, Vogt B, Beck T, Guntern D, Frey BM, Frey FJ. Reduced 11beta-hydroxysteroid dehydrogenase activity in the remaining kidney following nephrectomy. *Endocrinology* 1998; 139: 1533–1539
15. Frey FJ, Odermatt A, Frey BM. Glucocorticoid-mediated mineralocorticoid receptor activation and hypertension. *Curr Opin Nephrol Hypertens* 2004; 13: 451–458
16. Funke-Kaiser H, Thomas A, Bremer J *et al.* Regulation of the major isoform of human endothelin-converting enzyme-1 by a strong housekeeping promoter modulated by polymorphic microsatellites. *J Hypertens* 2003; 21: 2111–2124
17. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* 1994; 7: 536–540
18. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell* 2001; 104: 545–556
19. Luft FC, Fineberg NS, Weinberger MH. The influence of age on renal function and renin and aldosterone responses to sodium-volume expansion and contraction in normotensive and mildly hypertensive humans. *Am J Hypertens* 1992; 5: 520–528

Received for publication: 12.11.04

Accepted in revised form: 25.1.05