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Analysis of tissue-specific differentially methylated regions (TDMs) in humans

Eiko Kitamura [a], Jun Igarashi [a], Aiko Morohashi [a], Naoko Hida [a], Toshinori Oinuma [b], Norimichi Nemoto [b], Fei Song [d], Srimoyee Ghosh [e], William A. Held [d], Chikako Yoshida-Noro [a], Hiroki Nagase [a,c,e,*]

[a] *Life Science, Advanced Research Institute for the Sciences and Humanities, Nihon University School of Medicine, Tokyo 173-8610, Japan*
[b] *Department of Pathology, Nihon University School of Medicine, Tokyo 173-8610, Japan*
[c] *Division of Cancer Genetics, Department of Advanced Medical Science, Nihon University School of Medicine, Tokyo 173-8610, Japan*
[d] *Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*
[e] *Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*

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Abstract

Alterations in DNA methylation have been implicated in mammalian development. Hence, the identification of tissue-specific differentially methylated regions (TDMs) is indispensable for understanding its role. Using restriction landmark genomic scanning of six mouse tissues, 150 putative TDMs were identified and 14 were further analyzed. The DNA sequences of the 14 mouse TDMs are analyzed in this study. Six of the human homologous regions show TDMs to both mouse and human and genes in five of these regions have conserved tissue-specific expression: preferential expression in testis. A TDM, *DDX4*, is further analyzed in nine testis tissues. An increase in methylation of the promoter region is significantly associated with a marked reduction of the gene expression and defects in spermatogenesis, suggesting that hypomethylation of the *DDX4* promoter region regulates *DDX4* gene expression in spermatogenic cells. Our results indicate that some genomic regions with tissue-specific methylation and expression are conserved between mouse and human and suggest that DNA methylation may have an important role in regulating differentiation and tissue-/cell-specific gene expression of some genes.

Keywords: DNA methylation; Tissues; CpG islands; Bisulfites; DDX4; Gene expression; Testis; Spermatocytes; Gene expression regulation; Human genome

Introduction

DNA methylation of cytosine at position C5 in CpG dinucleotides in the mammalian genome is an inheritable modification of DNA that does not alter the nucleotide sequence [1,2]. It is important to understand the global genome-wide DNA methylation patterns and to understand the role of methylation in diverse genomic processes such as chromosomal stability, gene regulation, and parental imprinting. Many of these processes and their relationship to genome-wide methylation patterns are still unknown. This is partly due to a lack of high-throughput methods for scanning quantitative changes in DNA methylation status at each CpG dinucleotide in the genome. Generally, cytosine residues in the CpGs are methylated in the genome, especially within noncoding DNA, introns, repetitive sequences, and potentially active transposable elements resulting in long-term silencing [3,4]. Most CpG clusters, called CpG islands, frequently found in the proximal promoter regions of many genes, are unmethylated during normal cell development, with the exception of imprinted genes, genes on the inactive x chromosome, and tissue-specific differentially methylated genes [1,5–7]. Studies indicate that aberrant DNA methylation patterns of gene promoters impair normal transcription, causing abnormal development associated with various diseases such as cancer [8–11].

* Corresponding author. Life Science, Advanced Research Institute for the Sciences and Humanities, Cancer Genetics, Nihon University School of Medicine, 30-1 Oyaguchi, Kami-cho, Itabashi-ku, Tokyo 173-8610, Japan.
E-mail address: nagase-hiroki@arish.nihon-u.ac.jp (H. Nagase).

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Table 1
Conserved regions of TDMs in human

TDM loci identified in mice					Human orthologues					
Mouse loci [a]	UCSC location (August 2005) (Mb) [b]	*Not*I site location	CpG island	Gene	Human loci	UCSC location (March 2006) (Mb) [b]	Homologous region	CpG island	Gene	Function
Pvu2	chr11:115.57	Exon 2	No	AK037416	hPvu2	chr17:70.60	Exon 5	No	SLC16A5	Solute carrier family 16 (monocarboxylic acid transporters), member 5
Pvu4	chr7:11,55	Exon 4	cpgi 32	zfp324	hPvu4-1	chr19:63.67	Exon 4	cpgi 109	ZNF324	Zinc finger protein 324
					hPvu4-2	chr19:63.66	Exon 4	cpgi 84	ZNF324B	Hypothetical protein LOC388569
Pvu6	chr17:45.80	Exon3 [c]	Close to cpgi 56	Usp49	hPvu6	chr6:41.88	Exon 4	Close to cpgi 46	USP49	Ubiquitin-specific peptidase 49
Pvu8	chr4:59.04	5′ Promoter	cpgi 76	AK136359	hPvu8	chr9:113.43	5′ Promoter	cpgi 89	bA16L21.2.1	DnaJ-like protein (predicted gene)
Pvu29	chr14:51.53	5′ Promoter	cpgi 65	4930548G07Rik	hPvu29	chr13:19.10	5′ Promoter	cpgi 71	HSMPP8	M-phase phosphoprotein, mpp8
Pvu42	chr1:16.80	5′ Promoter	cpgi 107	6130401J04Rik	hPvu42	chr8:74.95	5′ Promoter	cpgi 109	UBE2W	Ubiquitin-conjugating enzyme E2W
Pvu66	chr11:43.73	Exon 2	cpgi 46	Adra1b	hPvu66	chr5:159.33	Exon 2	cpgi 95	ADRA1B	Andrenergic receptor, α 1b
Pst3	chr13:109.80	Exon 1	cpgi 68	Ddx4	hPst3	chr5:55.06	5′ Promoter	cpgi 30	DDX4	DEAD box polypeptide 4, RNA helicase
Pst6	chr17:33.07	Exon 2	cpgi 33	Hspa1l	hPst6	chr6:31.88	Exon 2	No	HSPA1L	Heat shock 70-kDa protein 1-like
Pst32	chr12:69.26	Exon 4	cpgi 74	Dact1	hPst32	chr14:58.18	Exon 4	Close to cpgi 50	DACT1	Dapper, antagonist of β-catenin, homologue 1
Pst33	chr7:41.96	Exons	No	Lmtk3	hPst33	chr19:53.69	Exon 12	Close to cpgi 93	LMTK3	Lemur tyrosine kinase 3
Pst61	chr6:88.26	5′ Promoter	cpgi 101	Gata2	hPst61	chr3:129.69	5′ Promoter	cpgi 514	GATA2	GATA-binding protein 2
Pst21	chr19:6.40	Intron	No	Nrxn2	hPst21	chr11:64.15	Intron	No	NRXN2	Neurexin 2 isoforms
Pst46	chr17:17.10	No [c]	No	No [c]	Not conserved					

[a] Locus names are quoted from Song et al. [23].
[b] The BLAT, UCSC Genome Bioinformatics website was used for the search.
[c] The differences between the October 2003 assembly and the August 2005 assembly at the University of California, Santa Cruz, Bioinformatics Database.