

The Differential DNA Methylation in Genomic DNA from Tissues: Approach of Genomic Imprinting Genes Isolation.

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ABSTRACT

DNA methylation is one of the mechanisms of gene regulation and has broadly been found in cell tested, both of the prokaryotes or eukaryotes. This DNA methylation is also the only marker which reflects genomic imprinting, genes expressed from only one allele inherited from parent. These genes express in certain tissues and developmental stages. It was estimated that there are 100 to 200 imprinting genes in whole human genome. However, only around 40 of them have been found. We take the advantages of those characters mentioned before and try to isolate de novo imprinting genes by combination of characters mentioned. Methods include digested genomic DNA from tissues with methylation-sensitive restriction enzyme and the digested DNA subjected to arbitrary primed PCR. Our preliminary data showed that through these methods we could isolate some distinguish bands, termed AP101、AP102、AP103、AP104、AP105、AP201、AP301、AP501 and AP801 from mice cortex. The differential methylation and genes expression will be confirmed by Southern hybridization and RT-PCR.

Keywords : DNA Methylation ; Genomic Imprinting ; Genomic DNA ; Arbitrary Primed PCR

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REFERENCES

吳岱珍。2002。肝細胞癌內FAP-1及其染色體鄰近區域之分子遺傳學研究。國立陽明大學遺傳學研究所。碩士論文。何康潔。1999。基因印刻。當代醫學。26(11):924-927。李中芬。1993。DNA cytosine甲基化和基因表現的關係。中國文化大學生命科技研究所。碩士論文。林秋宏。2000。DNA甲基化異常與肝細胞腫瘤的關係。私立長庚大學基礎醫學研究所。碩士論文。柯順耀。1999。口腔癌基因甲基化異常之鑑定。國立陽明大學口腔生物研究所。碩士論文。蘇家興。2001。利用Alu-PCR指紋分析及Bisulfite定序尋找及選殖小鼠組織基因體DNA中的甲基化的片段:p53啟動子到s27基因之間組織特異性的CpG甲基化的分析。國立陽明大學生物化學研究所。碩士論文。

Antequera, F., and Bird, A. 1993. Number of CpG islands and genes in human and mouse. Proc. Natl. Acad. Sci. USA. 90: 11995-11999. Bagwe, A.N., Kay, P.H., Spagnolo, and D.V. 1997. Evidence that DNA methylation imbalance is not involved in the development of malignant mesothelioma. Anticancer Res. 17: 3341-3344. Bartolomei, M.S., Webber A.C., Brunkow M.F., and Tilghman S. 1994. The search for imprinted genes. Nature Genet 6:220-221. Baylin, S.B., Herman, J.G., Graff, J.R., Vertino, P.M., and Issa, J.P. 1998. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res. 72:141-196. Baylin, S.B., Makos, M., and Wu, J. 1991. Abnormal patterns of DNA methylation in human neoplasia: Potential consequence for tumor progression. Cancer Cell 3:383-390. Bhattacharya, S.K., Ramchandani, S., Cervoni, N., and Szyf, M. 1999. A mammalian protein with specific demethylase activity for mCpG DNA. Nature 397:579-583. Bird, A.P. 1995. Gene number, noise reduction and biological complexity. Trends Genet. 11:94-100. Bird, A. 1992. The essentials of DNA methylation. Cell 70:5-8. Bird, A.P., and Southern, E.M. 1978. Use of restriction enzymes to study eukaryotic DNA methylation: I. The methylation pattern in ribosomal DNA from *Xenopus laevis*. J. Mol. Biol. 118:27-47. Cervoni, N., Bhattacharya, S., and Szyf, M. 1999. DNA demethylase is a processive enzyme. J. Biol. Chem. 274:8363-8366. Christman, J.K., Sheikhejad, G., Marasco, C.J., and Sufrin, J.R. 1995. 5-Methyl-2'-deoxycytidine in single-stranded DNA can act in cis to signal de novo DNA methylation. Proc. Natl. Acad. Sci. USA. 92:7347-7351. Clark, S.J., Harrison, J., and Frommer, M. 1995. CpNpG methylation in mammalian cells. Nat. Genet. 10:20-27. Counts, J.L., and Goodman, J.I. 1995. Alteration in DNA methylation may play a variety of roles in carcinogenesis. Cell 83:13-15. Cravo, M., Pinto, R., Fidalgo, P., Chaves, P., Gloria, L., Nobre-Leitao, C., and Mira, F. 1996. Global DNA hypomethylation occurs in the early stages of intestinal type gastric carcinoma. Gut 39:434-438. Denissenko, M.F., Chen, J.X., Tang, M.S., and Pfeifer, G.P. 1997. Cytosine methylation determines hot spots of DNA damage in the human P53 gene. Proc. Natl. Acad. Sci. USA. 94:3893-3898. Dieguez, M.J., M, Bellotto, K., Afsar, O., Mittelsten, Scheid., and J, Paszkowski. 1997. Methylation of cytosine in nonconventional methylation acceptor sites can contribution to reduced gene expression. Mol. Gen. Genet. 253:581-588. Ferguson, A.T., Vertino, P.M., Spitzner, J.R., Baylin, S.B., Muller, M.T., and Davidson, N.E., 1997. Role of estrogen receptor gene demethylation and DNA methyltransferase DNA adducts formation in 5-aza-2'-deoxycytidine-induced cytotoxicity in human breast cancer cells. Biol. Chem. 272:32260-32266. Ferguson, A.T., Lapidus, R.G., Baylin, S.B., and Davidson, N.E. 1995. Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression. Cancer Res. 55:2279-2283. Fremont, M., Siegmann, M., Gaulis, S., Matthies, R., Hess, D., and Jost, J.P. 1997. Demethylation of DNA by purified chick embryo 5-methylcytosine-DNA glycosylase requires both protein and RNA. Nucleic Acids Res. 25:2375-2380. Frommer, M., McDonald, L.E., Millar, D.S., Collis, C.M., Watt, F., Grigg, G.W., Molloy, P.L., and Paul, C.L. 1992. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc. Natl. Acad. Sci. USA. 89:1827-1831. Gonzalez-Zulueta, M., Bender, C.M., Yang, A.S., Nguyen, T., Beart, R.W., Van Tornout, J.M., and Jones, P.A. 1995. Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissue correlates with gene silencing. Cancer Res. 55:4531-4535. Gonzalgo, M.L., Bender, C.M., You, E.H., Glendening, J.M., Flores, J.F., Walker, G.J., Hayward, N.K., Jones, P.A., and Fountain, J.W. 1997. Low frequency of p16/CDKN2A methylation in sporadic melanoma: comparative approaches for methylation analysis of primary tumors. Cancer Res. 57:5336-5347. Gonzalgo, M.L., Liang, G., Spruck III, C.H., Zingg, J.M., Rideout, III, W.M., and Jones, P.A. 1997. Identification and characterization of differentially methylated regions of genomic DNA by methylation-sensitive arbitrary primed PCR. Cancer Res. 57:594-599. Greenblatt, M.S., Bennett, W.P., Hollstein, M., and Harris, C.C. 1994. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res. 54:4855-4878. Herman, J.G., Merlo, A., Mao, L., Lapidus, R.G., Issa, J-P., Davidson, N.E., Sidransky, D., and Baylin, S.B. 1995. Inactivation of the CDKN2/p16/MTS 1 gene is frequently associated with aberrant DNA methylation in all common human cancer. Cancer Res. 55:4525-4530. Hess, J.F., Fox, M., Schmid, C., and Shen, C.K. 1983. Molecular evolution of the human adult alpha-globin-like gene region: insertion and deletion of Alu family repeats and non-Alu DNA sequences. Proc. Natl. Acad. Sci. USA. 80:5970-5974. Hiltunen, M.O., Alhonen, L., Koistinaho, J., Myohanen, S., Paakkonen, M., Marin, S., Kosma, V.M., and Janne, J. 1997. Hypermethylation of the APC (Adenomatous polyposis coli) gene promoter region in human colorectal carcinoma. Int. J. Cancer 70:644-648. Holliday, R., and Grigg, G.W. 1993. DNA methylation and mutation. Mutat. Res. 285:61-67. Hollstein, M., Shomer, B., Greenblatt, M., Soussi, T., Hovig, E., Montesano, R., and Harris, C.C. 1996. Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. Nucleic Acids Res. 24:141-146. Hollstein, M., Rice, K., Greenblatt, M.S., Soussi, T., Fuchs, R., Sorlie, T., Hovig, E., Smith-Sorensen, B., Montesano, R., and Harris, C.C. 1994. Database of p53 gene somatic mutations in human tumors and cell lines. Database of p53 gene somatic mutations in human tumors and cell lines. Nucleic Acids Res. 22:3551-3555. Huang, T. H-M., Laux, D.E., Hamlin, B.C., Tran, P., Tran, H., and Lubahn, D.B. 1997. Identification of DNA methylation markers for human breast carcinomas using the methylation-sensitive restriction fingerprinting technique. Cancer Res. 57: 1030-1034. Huynh, L., Alpert, L., and Pollak, M. 1996. Silencing of the mammary-derived growth inhibitor (MDGI) gene in breast neoplasms is associated with epigenetic changes. Cancer Res. 56: 4865-4870. Issa, J.P., Zehnauer, B.A., Civin, C.I., Collector, M.I., Sharkis, S.J. Davidson, N.E., Kaufmann, S.H., and Baylin, S.B. 1996. The estrogen receptor CpG island is methylated in most hematopoietic neoplasms. Cancer Res. 56:973-977. Issa, J.P., Vertino, P.M., Wu, J., Sazawal, S., Celano, P., Nelkin, B.D., Hamilton, S.R.,

and Baylin, S.B. 1993. Increased cytosine DNA-methyltransferase activity during colon cancer progression. *J. Natl Cancer Inst.* 85:1235-1240.

Jones, P.A., Wolkowiz, M.J., Rideut III, W.M., Gonzales, F.A., Marziasz, C.M., Coetze, G.A., and Tapscott, S.J., 1990. De novo methylation of the Moy D, CpG island during the establishment of immortal cell lines. *Proc. Natl. Acad. Sci. USA.* 87:6117-6121.

Kautiainen, T.L., and Jones, P.A., 1986. DNA methyltransferase levels in tumorigenic and nontumorigenic cells in culture. *J. Biol. Chem.* 261:1594-1598.

Keshet, I., Lieman-Hurwitz, J., and Cedar, H. 1986. DNA methylation affects the formation of active chromatin. *Cell* 44:535-543.

Keverne, E.B. 1997. Genomic imprinting in the brain. *Curr. Opin. In Neurobiology* 7:463-468.

Keverne, E.B., Fundele, R., Narasimha, M., Barton, S.C., and Surani, M.A. 1996. Genomic imprinting and the differential roles of parental genomes in brain development. *Devel. Brain Res.* 92:91-100.

Klein, C.B., and M, Costa. 1997. DNA methylation and gene expression: Introduction and overview. *Mut. Res.* 286:103-105.

Kondo, M., Suzuki, H., Ueda, R., Osada, H., Takagi, K., Takahashi, T., and Takahashi, T. 1995. Frequent loss of imprinting of the H19 gene is often associated with its overexpression in human lung cancer. *Oncogene* 10:1193-1198.

Kono, T., Obata, Y., Wu, Q., Niwa, K., Ono, Y., Yamamoto, Y., Park, E.S., S, J-S., and Oagawa, H. 2004. Birth of parthenogenetic mice that can develop to adulthood. *Nature Publishing Group* 428:860-864.

Laird, P.W., and Jaenisch, R. 1996. The role of DNA methylation in cancer genetics and epigenetics. *Ann. Rev. Genet.* 30:441-464.

Laird, P.W., Jackson-Grusby, L., Fazeli, A., Dickinson, S.L., Jung, W.E., Li, E., Weinberg, R.A., and Jaenisch, R. 1995. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* 81:197-205.

Laird, P.W., and Jaenisch, R. 1994. DNA methylation and cancer. *Hum. Mol. Genet.* 3:1487-1495.

Larsen, F., Gundersen, G., Lopez, R., and Prydz, H., 1992. CpG island as gene markers in the human genome. *Genomics* 13:1095-1107.

Lee, W-H., Morton, R.A., Epstein, J.I., Brook, J.D., Campbell, P.A., Boca, G.S., Hsieh, W.S., Issacs, W.B., and Nelson, W.G. 1994 Cytidine methylation of regulatory sequences near the -class glutathione S-transferase gene accompanies human prostatic carcinogenesis. *Proc. Natl. Acad. Sci. USA.* 91:11733-11777.

Liu, C., Xu, D., Sjoberg, J., Forsell, P., Bjorkholm, M., and Claesson, H.E. 2004 Transcriptional regulation of 15-lipoxygenase expression by promoter methylation. *Cell Res.* 297:61-67.

Mancini, M.A., Shan, B., Nickerson, J.A., Penman, S., and Lee, W.H. 1994. The retinoblastoma gene product is cell cycle-dependent nuclear matrix-associated protein. *Proc. Natl. Acad. Sci. USA.* 91:418-422.

Mannens, M., Hoovers, J.M., Redeker, E., Verjaal, M., and Feinberg, A.P. 1994. Parental imprinting of human chromosome region 11p15.3 involved in the Beckwith-Wiedemann syndrome and various human neoplasia. *Eur. J. Hum. Genet.* 2:3-23.

McGhee, J.D., and Ginder, G.D. 1979. Specific DNA methylation sites in the vicinity of the chicken beta-globin genes. *Nature* 280:419-420.

Meehan, R.R., Lewis, J.D., and Bird, A.P. 1992. Characterization of MeCP 2, a vertebrate DNA binding protein with affinity for methylated DNA. *Nucleic Acids Res.* 20:5085-5092.

Meehan, R.R., Lewis, J.D., McKay, S., Kleiner, E.L., and Bird, A.P. 1989. Identification of a mammalian protein that binds specifically to DNA containing methylated CpGs. *Cell* 58:499-507.

Merlo, A., Herman, J.G., Mao, L., Lee, D.J., Gabrielson, E., Burger, P.C., Baylin, S.B., and Sidransky, D. 1995. 5'CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDK /MTS1 in human cancers. *Nat. Med.* 1:686-692.

Nan, X., Campoy, F.J., and Bird, A. 1997. MeCP 2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell* 88:471-481.

Nan, X., Tate, P., Li, E., and Bird, A. 1996. DNA methylation specifies chromosomal localization of MeCP 2. *Mol. Cell. Biol.* 16:414-421.

Ohtani-Fujita, N., Dryja, T.P., Rapaport, J.M., Fujita, T., Matsumura, S., Ozasa, K., Watanabe, Y., Hayashi, K., Maeda, K., Kinoshita, S., Matsumura, T., Ohnishi, Y., Hotta, Y., Takahashi, R., Kato, M.V., shizaki, K., Sasaki, M.S., Horsthemke, B., Minoda, K., and Sakai, T. 1997. Hypermethylation in the retinoblastoma gene is associated with unilateral, sporadic retinoblastoma. *Cancer Genet. Cytogenet.* 98:43-49.

Panning, B., and Jaenisch, R. 1996. DNA hypomethylation can activate Xist expression and silence X-linked genes. *Genes Dev.* 10:1991-2002.

Poirier, F., Chen, C.T.J., Timmons, D.M., Robertson, E.J., Evans, M.J., and Rigby, P.W.J. 1991. The murine H19 gene is activated during embryonic stem cell differentiation in vitro and at the time of implantation in the developing embryo. *Dev.* 113:1105-1114.

Prowse, A.H., Webster, A.R., Richards, F.M., Richard, S., Olschwang, S., Resche, F., Affara, N.A., and Maher, E.R. 1997. Somatic inactivation of the VHL gene in Von Hippel-Lindau disease tumors. *Am. J. Hum. Genet.* 60:765-771.

Pulford, D.J., Falls, J.G., Killian, J.K., and Jirtle, R.L. 1999. Polymorphism, genomic imprinting and cancer susceptibility. *Mut. Res.* 436:59-67.

Razin, A., and Cedar, H. 1991. DNA methylation and gene expression. *Microbiol. Rev.* 55:451-458.

Razin, A. 1988. Assay for studying DNA methylation. *Plant Mol Biol. Manual.* B3:1-28.

Razin, A., and Riggs, A.D. 1980. DNA methylation and gene function. *Science* 210:604-610.

Recillas-Targa, F., 2002. DNA methylation, chromatin boundaries, and mechanisms of genomic imprinting. *Archi. Med. Res.* 33:428-438.

Rice, J.C., Massey-Brown, K.S., and Futscher, B.W. 1998. Aberrant methylation of the BRCA 1 CpG island promoter is associated with decreased BRCA 1 mRNA in sporadic breast cancer cells. *Oncogene* 17:807-812.

Robertson, K.D., and Jones, P.A. 2000. DNA methylation: past, present and future directions. *Carcinogenesis* 21:461-467.

Sakai, T., Toguchida, J., Ohtani, N., Yandell, D.W., Rapaport, J.M., and Dryja, T.P. 1991. Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. *Am. J. Hum. Genet.* 48:880-888.

Shen, J.C., Rideout, W.M., and Jones, P.A. 1994. The role of hydrolytic deamination of 5-methylcytosine in double-stranded DNA. *Nucleic Acids Res.* 22:972-976.

Singer-Sam, J., Grant, M., LeBon, J.M., Okuyama, K., Chapman, V., Monk, M., and Riggs, A.D. 1990. Use of a Hpa II-polymerase chain reaction assay to study DNA methylation in the Pgk-1 CpG island of mouse embryos at the time of X-chromosome inactivation. *Mol. Cell. Biol.* 10:4987-4989.

Stopper, H., Pechan, R., and Schiffman, D. 1992. 5-Azacytidine induces micronuclei and morphological transformation of Syrian hamster embryo fibroblasts in the absence of unscheduled DNA synthesis. *Mut. Res.* 283:21-28.

Tate, P., Skarnes, W. and Bird, A. 1996. The methyl-CpG binding protein MeCP2 is essential for embryonic development in the mouse. *Nat. Genet.* 12:205-208.

Tate, P.H. and Bird, A.P. 1993. Effects of DNA methylation on DNA-binding protein and gene expression. *Curr Opin Genet Dev* 3:226-231.

Tollefsbol, T.O., and Hutchison, C.A. 1997. Control of methylation spreading in synthetic DNA sequences by the murine DNA methyltransferase. *J. Mol. Biol.* 269:494-504.

Tornaletti, S., and Pfeifer, G.P. 1995. Complete and tissue-

independent methylation of CpG sites in the p53 gene: implications for mutations in human cancers. *Oncogene* 10:1493-1499. Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J.G., Baylin, S.B., and Issa, J-P.J. 1999. CpG island methylation phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA.* 96:8681-8686. Trinh, B.N., Long, T.I., and Laird, P.W. 2001. DNA methylation analysis by methyligh technology. *Methods* 25:456-462. Vachtenheim, J., Horakova, I., and Novotna, H. 1994. Hypomethylation of CCGG sites in the 3' region of H-ras protoonco gene is frequent and is associated with H-ras allele loss in non-small cell lung cancer. *Cancer Res.* 54:1145-1148. Van der Ploeg, L.H., and Flavell, R.A. 1980. DNA methylation in the human gamma delta beta-globin locus in erythroid and nonerythroid tissues. *Cell* 19:947-958. Waalwijk, C., and Flavell, R.A. 1978. Mspl, an isoschizomer of Hpa II which cleaves both unmethylated and methylated Hpa II sites. *Nucleic Acids Res.* 5:3231-3236. Yoder, J.A., Walsh, C.P., and Bestor, T.H. 1997. Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet.* 13:335-340. Yoder, J.A., Walsh, C.P., and Bestor, T.H. 1997. Cytosine methylation and the ecology of intragenomic parasites. *Trends in Genet.* 13:335-340. Yoshiura, K., Kanai, Y., Ochiai, A., Shimcyama, Y., Sugimura, T., and Hirohashi, S. 1995. Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas. *Proc. Natl. Acad. Sci. USA.* 92:7416-9419. Zemel, S., Bartolomei, M.S., and Tilghman, S.M. 1992. Physical linkage of two mammalian imprinting genes, H19 and insulin-like growth factor 2. *Nature Genet.* 2:61-65. Zingg, J-M., and Jones, P.A. 1997. Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. *Carcinogenesis* 18:869-882. Zrihan-Licht, S., Weiss, M., Keydar, I., and Wreschner, D.H. 1995. DNA methylation status of the MUC 1 gene coding for a breast- cancer-associated protein. *Int. J. Cancer* 62:245-251.