

Hypermethylation of Multiple Genes in Pancreatic Adenocarcinoma¹

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Abstract

Hypermethylation of CpG islands is a common mechanism by which tumor suppressor genes are inactivated. We studied 45 pancreatic carcinomas and 14 normal pancreata for aberrant DNA methylation of CpG islands of multiple genes and clones using methylation-specific PCR (MSP) and bisulfite-modified sequencing. Using MSP, we detected aberrant methylation of at least one locus in 60% of carcinomas. The genes analyzed included *RARβ* (methylated in 20%), *p16* (18%), *CACNA1G* (16%), *TIMP-3* (11%), *E-cad* (7%), *THBS1* (7%), *hMLH1* (4%), *DAP kinase* (2%), and *MGMT* (0%). In addition, aberrant methylation was found in three CpG islands (MINT31, -1, and -2) in 38, 38, and 14% of carcinomas, respectively. Hypermethylation was largely confined to the carcinomas with only three loci (*E-cad*, *DAP kinase*, and *MINT2*) harboring methylation in some normal pancreata (36, 21, and 14%, respectively). Simultaneous methylation of at least four loci was observed in 5 of 36 (14%) pancreatic adenocarcinomas. We defined this subgroup of pancreatic adenocarcinomas as “CpG island-methylator-phenotype positive (CIMP+).” Two of four carcinomas with microsatellite instability harbored promoter hypermethylation of *hMLH1*, and both cases were CIMP+. Thus, we conclude that many pancreatic carcinomas hypermethylate a small percentage of genes, whereas a subset displays a CIMP+ phenotype.

Introduction

Both DNA hypomethylation (1) and the more focal DNA hypermethylation have been well documented in cancers (2). Aberrant DNA hypermethylation in carcinomas usually occurs at CpG islands, which are regions of the DNA rich in CpG dinucleotides. CpG islands are located mainly in 5' regulatory regions of genes (2). The CpG islands of tumor suppressor genes that normally are unmethylated are potential targets of aberrant hypermethylation during tumorigenesis. DNA hypermethylation can inhibit transcription of tumor suppressor and mismatch repair genes (*e.g.*, *p16*, *Rb*, *VHL*, and *hMLH1*), providing an epigenetic mechanism of selection during tumorigenesis (2–4).

Pancreatic carcinoma is a relatively well-characterized genetic disease. Multiple tumor suppressor pathways are abrogated in most pancreatic carcinomas. For example, the Rb/p16 pathway is inactivated in >95% of pancreatic cancers, and the transforming growth factor- β /DPC4 pathway is inactivated in >50% (5, 6). Similarly, *p53* is inactivated by mutation in ~75%, and the *K-ras* oncogene is mutationally activated in ~90% of pancreatic cancers (6). A subset of

pancreatic adenocarcinomas harbor MSI³ and can be recognized by their distinct pathological phenotype (7). In addition, numerous other genes are targeted for genetic inactivation at low frequency (~5% or less). These include *STK11*, *TGFBR2*, *MKK4*, and *ALK5* (6).

The identification of genes targeted by hypermethylation may provide insights into tumor-suppressive pathways inactivated in pancreatic cancer. In addition, hypermethylated genes may serve as targets for the development of novel screening tests for cancer (8). In this study, we examined a large series of pancreatic cancers for DNA hypermethylation using a panel of 13 genes and CpG islands. The panel includes several tumor suppressor genes, such as *p16*, and *E-cad* (3) and several cancer-associated genes, including *TIMP-3* (9), *DAP kinase*, *MGMT* (10), *RARβ* (11), *THBS-1* (12), *CACNA1G* (13), and the mismatch repair gene, *hMLH1* (4, 14). Four methylated clones originally recovered from a colorectal carcinoma cell line by methylated CpG island amplification (15) were also analyzed.

Materials and Methods

Patient Population and Tissue Samples. Normal and neoplastic tissues were obtained from pancreatic adenocarcinomas resected at The Johns Hopkins Hospital. The institutional review committee on clinical investigation reviewed and approved the collection of the tissue samples for genetic analysis. Pancreatic cancer xenografts were established from the primary carcinomas as described previously, and carcinoma and normal tissues were stored at -70°C (16). Thirty-two xenografts were selected at random from a total of 90 xenografts. In addition, three MSI+ carcinoma xenografts reported previously (7) and another MSI+ primary carcinoma were added to the panel of pancreatic carcinomas. Genomic DNA was prepared from these 35 xenografts, the primary pancreatic carcinoma, and from 9 pancreatic adenocarcinoma cell lines. The pancreatic carcinoma cell lines were BxPc3, Capan1, Capan2, Panc1, CFPAC1, MiaPaca2, Hs766T (all from American Type Culture Collection, Rockville, MD); Colo357 (from ECACC, Salisbury, United Kingdom); and PL45, a low-passage cell line established in our laboratory. Where available, DNA was obtained from fresh-frozen (-70°C) pancreatic cancer and normal pancreatic tissues for comparison with the DNA methylation obtained from the pancreatic cancer xenografts. Frozen tissues were microdissected to obtain DNA from normal pancreatic tissue and the pancreatic carcinoma.

Patient records were reviewed to determine each patient's history of cigarette smoking, and diabetes mellitus; the patient's status at last follow-up; and the patient's family history of pancreatic cancer. These data were then correlated with the DNA methylation data.

Bisulfite Modification and Genomic Sequencing. The bisulfite treatment was carried out for 16 h at 50°C on 1 μg of genomic DNA, according to the procedure of Herman *et al.* (3). Usually CpG islands are uniformly methylated at each CpG. To rule out variations in methylation patterns that could prevent optimal design of MSP primers, sequencing was performed on promoter regions of *RARβ*, *THBS1*, *CACNA1G*, and *hMLH1* genes and the CpG islands MINT1, -2, -31, and -32. For each locus, DNA from ~14 pancreatic tissues (8 carcinomas and 6 normal pancreata) were sequenced to screen for methylation.

³ The abbreviations used are: MSI, microsatellite instability; E-cad, E-cadherin; TIMP, tissue inhibitor of metalloproteinase-3; DAP, death-associated protein; MGMT, O⁶-methylguanine DNA methyltransferase; RAR β , retinoic acid receptor β ; THBS-1, thrombospondin-1; CACNA1G, T-type calcium channel; MSP, methylation-specific PCR; CIMP, CpG island methylator phenotype.

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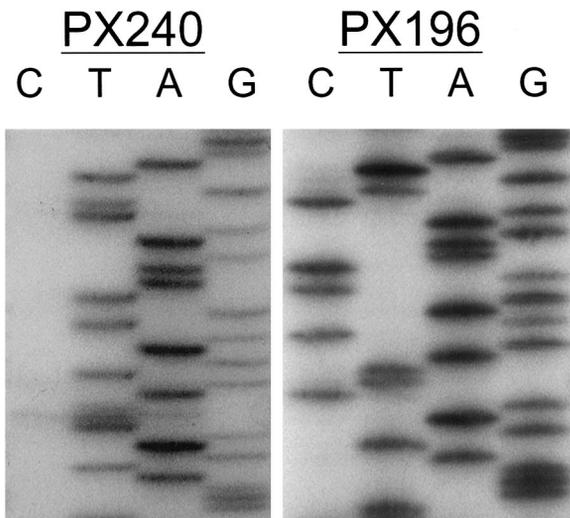


Fig. 1. Bisulfite-modified sequencing of *RARβ*. The sequence demonstrated was the 5' region of *RARβ* gene, beginning at +100 with respect to a transcription start site (GenBank accession no. X57849). All cytosines in the *PX240* have been converted to thymines, whereas all of cytosines located in CpG dinucleotides in *PX196* remain as C. Two CpG sites at the top of these sequence were selected for the sense primer of *RARβ* MSP.

Modified DNA was purified and eluted into 50 μ l of LoTE buffer. The primers used for genomic sequencing of bisulfite-treated DNA are available at our website.⁴ PCR was performed on 1–2 μ l of bisulfite-treated DNA; prior to sequencing, PCR reactions were incubated with exonuclease I and shrimp alkaline phosphatase (Amersham), according to the manufacturer's recommendations. Sequencing of PCR products was performed in microtiter plates as recommended by the manufacturer (Sequitum Excel; Epicentre Technologies, Madison, WI).

MSP Assay. The methylation status of each gene was determined by MSP as described by Herman *et al.* (3), in which the sequence difference of bisulfite-treated DNA was detected by PCR using primers specific for either the methylated or for the unmethylated DNA. Primer sequences and the specific annealing temperatures for the 13 CpG islands are available at our website.⁴ MSP was performed on 1 μ l of bisulfite-treated DNA under following conditions: 95°C for 3 min; 35–40 cycles of 95°C for 30 s, the specific annealing temperature for 30 s, and 72°C for 30 s; and a final extension of 4 min at 72°C. Five μ l of each PCR product were directly loaded onto 3% agarose gels or 10% acrylamide gels, stained with ethidium bromide, and visualized under UV illumination. All PCR reactions were performed with positive controls for both unmethylated and methylated alleles, and no DNA-loaded control. Finally, three to six CpG sites were included in each primer pair, and the specific annealing temperatures were used for each gene to obtain optimal specificity.

Results

We sequenced bisulfite-treated DNA from the promoter regions of *RARβ*, *THBS1*, *CACNA1G*, and *hMLH1* genes and the CpG islands MINT1, -2, -31, and -32. Methylation of each CpG site of these genes was determined by the presence of unconverted cytosines (Fig. 1). Partial methylation at CpG sites of some CpG islands was identified in normal tissue, *e.g.*, *hMLH1* and MINT32 (data not shown). Loci with CpG methylation specific to pancreatic carcinoma cell lines or xenografts were selected for MSP to provide specific amplification of methylated alleles.

The results of MSP of these genes are summarized in Table 1. MINT32 was methylated in 67% of the 35 xenografts, the 1 primary carcinoma, and 100% of cell lines. In colorectal carcinomas, methylation of this clone occurred frequently in normal colonic mucosae

and was thus characterized as aging-specific (17). Twenty-seven of the 45 carcinomas (60%) showed methylation at ≥ 1 of the remaining 12 loci, and the frequency of methylation ranged from 0 to 38%. As shown in Fig. 2, the methylation patterns of each gene were usually clonal. For example, whereas PX26 contained only methylated *THBS1*, PX29 contained both methylated and unmethylated templates (Fig. 2A). The presence of methylated templates in primary carcinomas was confirmed when frozen tissues were available. Almost all of the primary carcinomas displayed amplification of methylated templates of all genes that were detected in corresponding xenografts (PX26, -196, -198, -240, -248, -281, and -287; Fig. 2D). Xenograft PX287 had weak amplification of methylated templates of MINT31, but the corresponding primary carcinoma did not.

MSP of *E-cad*, *DAP kinase*, and MINT2 revealed weak amplification of methylated templates in 36, 21, and 14% of normal pancreata, respectively. No methylated templates of the other 11 genes were detected by MSP in all 14 normal pancreata.

Hypermethylation of these CpG islands did not occur randomly in these carcinomas but instead clustered in specific carcinomas (Fig. 3), suggesting that there is a CpG island hypermethylator phenotype (CIMP+) in a subset of pancreatic carcinomas (17, 18). In defining a CIMP+, we excluded genes that were either not methylated in any pancreatic cancer or methylated at some frequency in histologically normal tissue sections. Thus, we excluded *MGMT* (not methylated in pancreatic cancer), and *E-cad*, *DAP kinase*, MINT2 (methylated in normal tissue at low frequency), and MINT32 (evidence for age-related methylation in normal tissue; Ref. 17). Carcinomas having methylation at four or more of eight loci were defined as CIMP+, those showing methylation at two or three loci were defined as CIMP-I (intermediate), whereas those showing only one or no methylation were classified as CIMP-. Of the 35 xenografts and 1 primary carcinoma, 5 (14%) were CIMP+, 4 (11%) were CIMP-I, and 26 (72%) were CIMP-. When we compared the observed distribution of methylated loci in each group [CIMP- ($n = 27$), CIMP-I ($n = 4$), and CIMP+ ($n = 5$)] to the expected distribution if the frequency of gene methylation were randomly distributed around the mean number of methylated loci in our pancreatic carcinomas [CIMP- ($n = 23$), CIMP-I ($n = 12$), and CIMP+ ($n = 1$)], a statistically significant difference in the proportions in each group was observed ($P < 0.0005$; χ^2 goodness-of-fit analysis; Ref. 18). The cell lines harbored more methylated CpG islands than the pancreatic cancer xenografts/primary cancers. Three of the nine cell lines (33%) were CIMP+. In the xenograft panel, 11.6% of loci were methylated, whereas 28% of loci in the cell lines were methylated. ($\chi^2 = 18$; $P < 0.001$).

Four carcinomas included in this study were not chosen at random, but instead were included because they showed MSI. *hMLH1* methylation was found in two of four of them. Notably, whether or not we included *hMLH1* methylation as a marker of CIMP status in these cancers did not affect their status as a CIMP+ or CIMP- cancer. The two MSI+ carcinomas that were methylated at *hMLH1* were CIMP+. All of the MSI+ pancreatic cancers lacked *hMLH1* expression by immunohistochemistry (not shown). Despite a lack of *hMLH1* promoter methylation, the other two MSI+ carcinomas also harbored aberrant methylation of other CpG islands; one case was classified as CIMP+, and the other case, as CIMP-. None of 32 microsatellite-stable carcinomas showed hypermethylation of *hMLH1* promoter region. The CIMP+ pancreatic carcinoma with MSI that lacked *hMLH1* methylation by MSP was from a 34-year-old male who also developed a synchronous right-sided colon carcinoma that was MSI+ and lacked *hMLH1* expression.⁵ These features suggest that the patient harbors a

⁵ R. Wilentz *et al.* Genetic, immunohistological, and clinical features of medullary carcinoma of the pancreas: a newly described and characterized entity, submitted for publication.

⁴ <http://www.path.jhu.edu/gogginlab/primers>.

Table 1 Methylation of multiple genes in pancreatic cancers and normal pancreatic tissue

Samples	<i>n</i> ^a	<i>p16</i>	<i>RARβ</i>	<i>CACNAIG</i>	<i>TIMP3</i>	<i>THBS1</i>	<i>hMLH1</i>	<i>E-cad</i>	<i>DAPK</i>	<i>MGMT</i>	<i>MINT1</i>	<i>MINT2</i>	<i>MINT31</i>	<i>MINT32</i>
Pancreatic carcinomas ^b	36	14%	11%	11%	11%	6%	6%	3%	0%	0%	33%	8%	28%	67%
Pancreatic cell lines	9	33%	56%	33%	11%	11%	0%	22%	11%	0%	56%	22%	78%	100%
Total	45	18%	20%	16%	11%	7%	4%	7%	2%	0%	38%	11%	38%	73%
Normal pancreata	14	0%	0%	0%	0%	0%	0%	36%	21%	0%	0%	14%	0%	0%

^a *n*, number examined.^b Thirty-five pancreatic cancer xenografts and 1 primary carcinoma.

germline defect in a mismatch repair gene. Sequencing of the promoter region of *hMLH1* in the pancreatic cancer from this patient identified methylation in regions of the promoter often methylated in normal tissues but no methylation in the region of the promoter thought to require methylation to silence *hMLH1* expression (Ref. 14; and data not shown). The methylation pattern of *hMLH1* in this carcinoma raises the possibility that methylation outside the *hMLH1* promoter region identified by Deng *et al.* (14), can inactivate transcription. Alternatively, the presence of the CIMP+ phenotype in this cancer despite an apparent lack of *hMLH1* methylation is consistent with the mismatch repair defect predating the CIMP+ defect. Observations made by others suggests that the CIMP phenotype usually predates the mismatch repair defect (4).

There was no significant correlation between CIMP status and clinicopathological parameters among the 36 patients with pancreatic carcinomas with respect to age, sex, survival, smoking, presence of a family history of pancreatic cancer, the size of the tumor, lymph node status, or concomitant diabetes mellitus.

Discussion

In this study, 60% of pancreatic carcinomas harbored CpG island methylation of at least 1 of 12 cancer-related markers. Aberrant promoter methylation was detected in several genes in the panel in pancreatic carcinoma (*p16*, *RARβ*, *CACNAIG*, *TIMP-3*, *THBS1*, and *hMLH1*), and occurred at a rate of ~5–20% for each gene. These data suggest that aberrant hypermethylation may be a common mechanism of tumor suppressor gene inactivation in pancreatic carcinoma. Promoter methylation of these genes has been shown to be associated with loss of their expression (4, 9, 11–14). Aberrant methylation of the four CpG islands studied (*MINT1*, -2, -31, and -32) occurred at a higher rate (11–73%). Presently, it is not known whether these CpG islands are part of genes (15).

Current evidence supports the notion that subsets of cancers (*e.g.*, bladder, prostate cancer, colorectal carcinoma, leukemia, and gastric cancer) harbor the CIMP+ phenotype (17–20). Colorectal

carcinomas with *p16* methylation are more often right-sided (21) and have different patterns of genetic inactivation (22). Among an unselected group of both pancreatic and stomach carcinomas, the proportion of methylated genes appears to nonrandomly cluster to a subset of cases (18). We believe that classifying carcinomas that are particularly prone to methylate tumor suppressor genes may help to identify a subset of carcinomas that have a different etiology, natural history, and/or response to treatment. All of the CIMP+ pancreatic carcinomas had methylation of *p16* as well as one or more other cancer-related genes (*RARβ*, *CACNAIG*, *THBS1*, *TIMP-3*, or *hMLH1*). The three CIMP+ cases with MSI histologically displayed the medullary phenotype as reported previously (7), and one other CIMP+ case was an adenosquamous carcinoma. Three of the CIMP+ cases previously have been well characterized genetically, and two of these had an unusual pattern of genetic alterations. These two carcinomas (PX26 and PX29) harbored genetic alteration of only one of the four genes commonly mutated in pancreatic carcinoma (*K-ras*, *p16*, *p53*, and *DPC4*; Ref. 16), providing supportive evidence that at least some CIMP+ pancreatic carcinomas may evolve as a result of predominantly epigenetic rather than genetic events. Ultimately, confirmation of the validity of such a classification and refinement of a definition of CIMP+ carcinomas will require additional studies into the causes of the CIMP phenotype.

MSP can detect 1 methylated allele among 1000 unmethylated ones (3), suggesting that hypermethylated loci could ultimately serve as targets in screening tests for the early detection of pancreatic carcinomas in clinical samples such as stool, blood, duodenal fluid, or pancreatic juice (8, 10). The percentage of methylated loci in the pancreatic cancers in this study was lower than that found for colorectal cancers (17). This may reflect the fact that additional hypermethylated genes that provide a growth advantage for pancreatic cancers remain to be identified. Techniques such as methylated CpG island amplification, restriction landmark fragment scanning, and methylation-sensitive arbitrarily primed PCR can be used for this purpose (17, 19, 23). Another explanation for the lower rate of

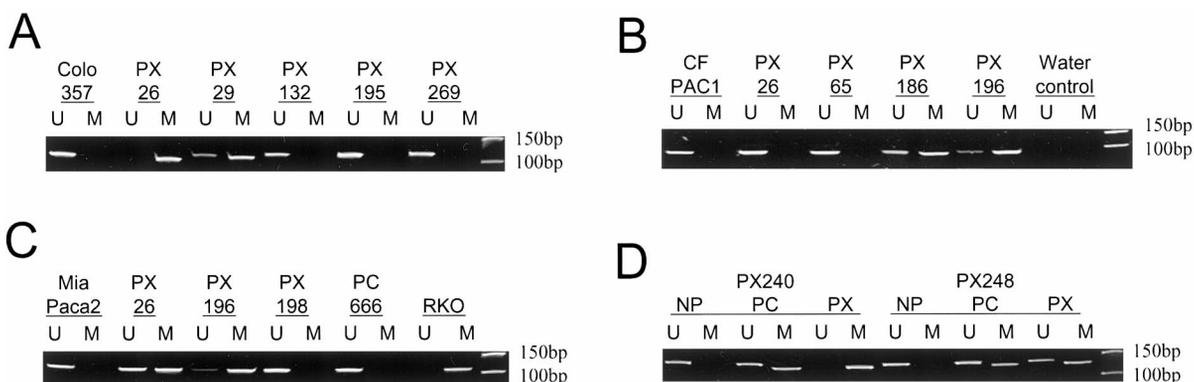


Fig. 2. MSP analysis of *THBS1* (A), *RARβ* (B), *hMLH1* (C), and *MINT32* (D) in pancreatic cancer. The PCR products in the lanes marked *U* show the presence of unmethylated templates of each gene, whereas the products in the lanes marked *M* indicate the presence of methylated templates. *NP*, normal pancreas; *PC*, primary carcinoma; *PX*, pancreatic cancer xenograft; *RKO*, colonic cancer cell line (positive control for *hMLH1* MSP).

Sample	MSI	p16	RARβ	CACNA1G	TIMP3	THBS1	hMLH1	MINT1	MINT31	E-Cad	DAPK	MGMT	MINT2	MINT32	
PC666	+														CIMP+
PX26	+														
PX196	+														
PX17	-														CIMP-I
PX29	-														
PX198	-														
PX132	-														CIMP-
PX186	-														
PX240	-														
PX30	-														
PX102	-														
PX108	-														
PX125	-														
PX185	-														
PX195	-														
PX248	-														
PX281	-														
PX287	+														
PX65	-														
PX143	-														
PX147	-														
PX184	-														
PX188	-														
PX191	-														
PX192	-														
PX194	-														
PX208	-														
PX247	-														
PX269	-														
PX280	-														
PX282	-														
PX289	-														
PX303	-														
PX321	-														
PX328	-														
PX348	-														
MiaPaca2	-														CIMP+
Colo357	-														
PL45	-														CIMP-I
Capan2	-														
Capan1	-														CIMP-
CFPAC1	-														
BxPC3	-														
Hs766T	-														
Panc1	-														

Fig. 3. Methylation profile of pancreatic cancer. Methylation status of 13 loci in 36 pancreatic carcinomas (top group) and 9 cell lines (bottom group). Filled boxes, methylated loci; open boxes, unmethylated loci. MSI+, tumor with microsatellite instability; MSI-, tumor without microsatellite instability. CIMP+, methylated at four or more loci; CIMP-I, methylated at two or three loci; CIMP-, methylated at one or no loci. (The CIMP classification excluded *E-cad*, *DAP kinase*, *MGMT*, *MINT2*, and *MINT32*; see text.) The methylated loci were nonrandomly distributed among the 36 pancreatic carcinomas: $P < 0.0005$.

methylated loci in pancreatic carcinoma is that the carcinogens associated with pancreatic cancer target genetic pathways more often than epigenetic pathways. Support for this possibility comes from association between a smoking history of patients and a low incidence of methylation of the estrogen receptor gene (*ER*) in lung cancer and

association between radiation and a high incidence of *ER* methylation (24).

As described previously for other cancer cell lines (25), pancreatic cell lines in this study had higher rates of DNA methylation (28% of loci) than the pancreatic cancer xenografts and primary carcinomas (11.6% of loci;

$\chi^2=18$; $P < 0.001$; Table 1). These findings may reflect the small number of cell lines studied, or they may signify the selection of subclones of tumor cells with DNA hypermethylation throughout the establishment of an immortal cell line. Alternatively, carcinomas that are CIMP+ may be more likely to be established as cell lines.

This study represents our ongoing attempts to refine the molecular classification of pancreatic carcinoma. Additional studies are needed to refine our understanding of the role of DNA hypermethylation in pancreatic cancer biology.

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References

- Goelz, S. E., Vogelstein, B., Hamilton, S. R., and Feinberg, A. P. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science (Washington DC)*, 228: 187–190, 1985.
- Baylin, S. B., Herman, J. G., Graff, J. R., Vertino, P. M., and Issa, J. P. Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv. Cancer Res.*, 72: 141–196, 1998.
- Herman, J. G., Graff, J. R., Myohanen, S., Nelkin, B. D., and Baylin, S. B. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc. Natl. Acad. Sci. USA*, 93: 9821–9826, 1996.
- Herman, J. G., Umar, A., Polyak, K., Graff, J. R., Ahuja, N., Issa, J. P., Markowitz, S., Willson, J. K., Hamilton, S. R., Kinzler, K. W., Kane, M. F., Kolodner, R. D., Vogelstein, B., Kunkel, T. A., and Baylin, S. B. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc. Natl. Acad. Sci. USA*, 95: 6870–6875, 1998.
- Schutte, M., Hruban, R. H., Geradts, J., Maynard, R., Hilgers, W., Rabindran, S. K., Moskaluk, C. A., Hahn, S. A., Schwarte-Waldhoff, I., Schmiegel, W., Baylin, S. B., Kern, S. E., and Herman, J. G. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res.*, 57: 3126–3130, 1997.
- Goggins, M., Kern, S. E., Offerhaus, J. A., and Hruban, R. H. Progress in cancer genetics: lessons from pancreatic cancer. *Ann. Oncol.*, 10: 4–8, 1999.
- Goggins, M., Offerhaus, G. J., Hilgers, W., Griffin, C. A., Shekher, M., Tang, D., Sohn, T. A., Yeo, C. J., Kern, S. E., and Hruban, R. H. Pancreatic adenocarcinomas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology. Poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+. *Am. J. Pathol.*, 152: 1501–1507, 1998.
- Belinsky, S. A., Nikula, K. J., Palmisano, W. A., Michels, R., Saccomanno, G., Gabrielson, E., Baylin, S. B., and Herman, J. G. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. *Proc. Natl. Acad. Sci. USA*, 95: 11891–11896, 1998.
- Bachman, K. E., Herman, J. G., Corn, P. G., Merlo, A., Costello, J. F., Cavenee, W. K., Baylin, S. B., and Graff, J. R. Methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene suggest a suppressor role in kidney, brain, and other human cancers. *Cancer Res.*, 59: 798–802, 1999.
- Esteller, M., Sanchez-Cespedes, M., Rosell, R., Sidransky, D., Baylin, S. B., and Herman, J. G. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res.*, 59: 67–70, 1999.
- Cote, S., Sinnott, D., and Momparler, R. L. Demethylation by 5-aza-2'-deoxycytidine of specific 5-methylcytosine sites in the promoter region of the retinoic acid receptor beta gene in human colon carcinoma cells. *Anticancer Drugs*, 9: 743–750, 1998.
- Li, Q., Ahuja, N., Burger, P. C., and Issa, J. P. Methylation and silencing of the thrombospondin-1 promoter in human cancer. *Oncogene*, 18: 3284–3289, 1999.
- Toyota, M., Ho, C., Ohe-Toyota, M., Baylin, S. B., and Issa, J. P. Inactivation of *CACNA1G*, a T-type calcium channel gene, by aberrant methylation of its 5' CpG island in human tumors. *Cancer Res.*, 59: 4535–4541, 1999.
- Deng, G., Chen, A., Hong, J., Chae, H. S., and Kim, Y. S. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. *Cancer Res.*, 59: 2029–2033, 1999.
- Toyota, M., Ho, C., Ahuja, N., Jair, K. W., Li, Q., Ohe-Toyota, M., Baylin, S. B., and Issa, J. P. Identification of differentially methylated sequences in colorectal cancer by methylated CpG island amplification. *Cancer Res.*, 59: 2307–2312, 1999.
- Rozenblum, E., Schutte, M., Goggins, M., Hahn, S. A., Panzer, S., Zahurak, M., Goodman, S. N., Sohn, T. A., Hruban, R. H., Yeo, C. J., and Kern, S. E. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res.*, 57: 1731–1734, 1997.
- Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J. G., Baylin, S. B., and Issa, J. P. CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. USA*, 96: 8681–8686, 1999.
- Toyota, M., Ahuja, N., Suzuki, H., Itoh, F., Ohe-Toyota, M., Imai, K., Baylin, S. B., and Issa, J. P. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res.*, 59: 5438–5442, 1999.
- Liang, G., Salem, C. E., Yu, M. C., Nguyen, H. D., Gonzales, F. A., Nguyen, T. T., Nichols, P. W., and Jones, P. A. DNA methylation differences associated with tumor tissues identified by genome scanning analysis. *Genomics*, 53: 260–268, 1998.
- Melki, J. R., Vincent, P. C., and Clark, S. J. Concurrent DNA hypermethylation of multiple genes in acute myeloid leukemia. *Cancer Res.*, 59: 3730–3740, 1999.
- Wiencke, J. K., Zheng, S., Lafuente, A., Lafuente, M. J., Grudzen, C., Wrensch, M. R., Miike, R., Ballesta, A., and Trias, M. Aberrant methylation of p16INK4a in anatomic and gender-specific subtypes of sporadic colorectal cancer. *Cancer Epidemiol. Biomark. Prev.*, 8: 501–506, 1999.
- Toyota, M., Ohe-Toyota, M., Ahuja, N., and Issa, J. P. Distinct genetic profiles in colorectal tumors with or without the CpG island methylator phenotype. *Proc. Natl. Acad. Sci. USA*, 97: 710–715, 2000.
- Hirotsune, S., Hatada, I., Komatsubara, H., Nagai, H., Kuma, K., Kobayakawa, K., Kawara, T., Nakagawara, A., Fujii, K., Mukai, T., and Hayashizaki, Y. New approach for detection of amplification in cancer DNA using restriction landmark genomic scanning. *Cancer Res.*, 52: 3642–3647, 1992.
- Issa, J. P., Baylin, S. B., and Belinsky, S. A. Methylation of the estrogen receptor CpG island in lung tumors is related to the specific type of carcinogen exposure. *Cancer Res.*, 56: 3655–3658, 1996.
- Antequera, F., Boyes, J., and Bird, A. High levels of de novo methylation and altered chromatin structure at CpG islands in cell lines. *Cell*, 62: 503–514, 1990.

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