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Human Breast and Colon Cancers Exhibit Alterations of DNA Methylation Patterns at Several DNA Segments on Chromosomes 11p and 17p

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Abstract In breast and colon adenocarcinomas methylation patterns at CCGG sites of several loci located on the short arm of chromosome 11 were determined by Southern blot analysis. Results obtained indicate that all tumor samples (20/20) exhibit DNA methylation changes when compared to their normal counterparts. In colon tumors, γ-globin gene is usually hypomethylated (9/10), whereas Ha-ras gene, which is located in the same region, retains an unmodified DNA methylation pattern. Hypomethylation of parathyroid hormone (5/10) and catalase genes (4/10) are also frequently detected in colon tumor specimens. For the catalase gene the region around exon 2 is the only one which is affected by these changes. In breast adenocarcinoma, modifications of the methylation patterns are less frequently observed. However, hypomethylation of the γ-globin gene is a very common event in these tumors (8/10), and it is also detected (2/2) in lobular carcinoma in situ which is an early step in breast tumorigenesis. In addition, hypermethylation of a CpG island is also observed at the locus 17p13.3 in both colon (5/5) and breast (4/9) adenocarcinomas. In the tumoral tissues analyzed these hypermethylations are not associated with the hypermethylation of the 5′ flanking sequences which contain a limited amount of CpG. Some of these alterations seem, therefore, to be tumor and sequence specific.

Key words: methylation, DNA, chromosomes 11p and 17p13.3, human breast adenocarcinoma, human colon adenocarcinoma, γ-globin, Ha-ras, parathyroid hormone, catalase, calcitonin

In mammalian, the methylation of DNA is accomplished by enzymes which catalyze the transfer of methyl groups from S-adenosylmethionine to the carbon-5 of deoxycytidine at CpG sites [for review, see Adams, 1990]. The amount and the distribution of 5-methyldeoxycytidine are dependent on the type of organism as well as on the tissue of origin [Razin and Szyf, 1984]. The determination of the base composition of DNA and the analysis by Southern blotting of specific DNA segments have most frequently shown a reduced level of DNA methylation in human tumors [for review, see Jones and Buckley, 1990]. Analysis of DNAs extracted from identically paired samples of human colon carcinoma or benign adenoma and adjacent normal mucosa have shown that hypomethylation is also detected in the early steps of carcinogenesis [Goelz et al., 1985; Feinberg et al., 1988].

Although genomic hypomethylation seems to be very common in human tumors, total genomic hypermethylation has been reported in pediatric tumors [Flatau et al., 1983] and hypermethylation of CpG island at tissue specific genes in transformed or immortalized cell lines has been also observed [Jones et al., 1990; Antequera et al., 1990]. Hypermethylation of several GCGGCCGC sites on the short arm of chromosome 17 (at p13.3) is very frequent in human neural tumors, colon carcinomas, and lung carcinomas [Makos et al., 1992, 1993a]. It had been suggested that methylation changes at the 17p13.3 locus may mark and precede the loss of heterozygoty (LOH) frequently detected in these tumors at this locus [Makos et al., 1993b].

The analysis of different genes located within the same chromosomal region have shown in some human tumors a regional modification of

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the methylation of DNA. In leukemia and small cell lung carcinomas despite a widespread genomic hypomethylation an hypermethylation of the short arm of the chromosome 11 was observed [de Bustros et al., 1988]. In addition regional hypomethylation was also found on the chromosome 3p [Makos et al., 1992]. However in five randomly selected colon tumors, a selective hypomethylation was observed between ten genes located on six different chromosomes [Feinberg and Vogelstein, 1983; Goelz et al., 1985]. Taken together these results suggest that the modification of the DNA methylation pattern is a very specific event dependent on the tumor type.

In order to gain further insight into this question the methylation pattern of several loci located on chromosome 11p and 17p13 were investigated in breast and colon cancers, two human epithelial adenocarcinomas. These two chromosomal regions were chosen because, in breast carcinoma, these two regions seem to be involved in tumorigenesis. In 50 to 70% of sporadic breast carcinomas [Mackay et al., 1988; Sato et al., 1990] and at least in 80% of colon carcinomas LOH at the 17p13.3 locus were detected [Fearon and Volgelstein, 1990]; LOH at 11p15.5 and 11p15.4 were also detected in about 30% of breast tumors [Winquist et al., 1993]. Furthermore, for colon tumors, normal adjacent mucosa was available with each tumor and, therefore, a direct comparison between identically paired samples was possible.

MATERIALS AND METHODS

Preparation of Tissues and Cells

A total number of ten cases of primary breast adenocarcinomas, two lobular carcinomas in situ, one normal breast sample, and ten paired colonic samples (adenocarcinoma and normal adjacent mucosa from the same patient) were analyzed. Both normal and tumor samples were diagnosed using routine histopathological techniques. Immediately after surgery, breast and colonic specimens were frozen in liquid nitrogen and stored at −80°C. High molecular weight DNA was extracted from frozen tissue by standard procedures [Sambrook et al., 1989].

Southern Blot Analysis

In a typical experiment, 30 μg of DNA was cleaved with an 8-fold excess of TaqI for 10–12 h at 65°C. Two thirds of the sample was then digested overnight at 37°C with *Msp*I and *Hpa*II (10 units/μg). To ascertain cleavage patterns obtained, some DNAs samples were first digested with 10 units/μg of DNA, extracted again with proteinase K and phenol and then digested again with 10 units of enzyme per μg of DNA. Simple digestions with *Not*I were carried out in the same conditions. Restriction endonuclease digests were fractionated by electrophoresis through 1.2% (*Taq*I, *Taq*I-*Msp*I/*Hpa*II digests), or 0.8% agarose gel (*Not*I digests), and transferred to Hybond N^+ membranes (Nylon; Amersham, France). Molecular weight markers used were *Eco*RI SSPI DNA digests and *Eco*RI λDNA digests. Membranes were hybridized overnight at 42°C to randomly primed ^{32}P-labeled probes (Random primed DNA labelling kit, Boehringer, Mannheim, France), in the hybridization solution (Amersham hybridization buffer, RPN 1518, in 50% formamide). Membranes were washed at a final stringency of 0.2 × SSPE (1 × SSPE is 0.18 M NaCl, 10 mM Sodium phosphate, and 1 mM EDTA), SDS 1%, at 68°C and exposed to Amersham Hyper Film-MP for 2–5 days, using an intensifying screen. Nylon membranes could be used for rehybridizations after two successive treatments with SDS 1% at 80°C for 30 min. The same filters were successively hybridized to several probes.

Genes Probes

The probes used for the short arm of chromosome 11 were, from telomeric to centromeric region: 11p 15.5, a *Hind*III 3.3 kb genomic fragment excised from the plasmid pA-B7 containing the human γ-globin gene; 11p 15.5, a *Bam*HI 6.6 kb cloned genomic human Ha-ras gene [Shih and Weinberg, 1982]; 11p 15.2/1 a cloned *Pst*I-*Eco*RI 2.5 kb genomic fragment containing the 3′ end of the human parathyroid hormone sequence [Vasicek et al., 1983]; 11p 15.2/1 a *Pst*I 0.59 kb cloned genomic fragment of the 3′ end of the human calcitonin gene [Craig et al., 1982]; and 11p 13, an *Eco*RI 2.4 kb cloned cDNA of human catalase gene coding sequences excised from the plasmid pCAT10 [Quan et al., 1986. ATCC] and an *Eco*RI-*Hind*III 0.8 kb genomic fragment, portion of the intron 1 of catalase gene, excised from the plasmid pINT-800 [Quan et al., 1985, ATCC]. On chromosome 17: a *Bam*HI 1.7 kb genomic fragment excised from the plasmid pYNZ22 at 17p 13.3 [Nakamura et al., 1988, ATCC].

TABLE I. Methylation Patterns of DNA Extracted From Colonic and Breast Adenocarcinomas*

Regional assignment		11p									17p	
		15.5	15.5		15.2/1	15.2/1	13				13.3	
			Ha-ras				CATALASE				D17S5	
Loci	HBG1	promoter region	gene	PTH	CALCA	5′ region + exon 1	intron 1	exon 2	exons 3 to 13	Taq I digests	Not I digests
Normal mucosa	M+/−	M−	M+/−	M+	M−	M−	M+	M+	M+	M−	M−
Colonic Ade.C. 1	m−	=	=	m−	=	=	=	=	=	=	/
2	m−	=	=	m−	=	=	=	=	=	=*	/
3	m−	=	=	m−	m+	=	=	m−	=	=*	/
4	m−	=	=	=	=	=	=	m−	=	m+	m+
5	m−	=*	=*	=	=	=	=*	=	=	=	m+
6	=	=	m+	=	=	=	m−	m−	=	=	m+
7	m−	=	=	=	=	=	=	m−	=	=	/
8	m−	=	=	m−	=	=	=	=	=	=*	m+
9	m−	=	=	m−	=*	=	=	=	=	=*	m+
10	m−	=	=	=	=	=	=	=	=	=	/
Normal breast	M+/−	M−	M+/−	M+	M−	M−	M+	M+	M+	M−	M−
Breast Ade.C. 1	m−	=	=	=	=	=	=	=	=	=	m+
2	=	=	=	=	=	=	=	=	=	m+	=
3	m−	=	=	=	=	=	=	=	=	m+	=
4	=	=	=	=	=	=	m−	m−	=	=	m+
5	m−	=	=	=	=	=	=	=	=	=	=
6	m−	=	=	=	=	=	=	=	=	=	m+
7	m−	=	=	=	=	=	=	=	=	=	/
8	m−	=	=	m−	=	=	=	=	=	=	m+
9	m−	=	=	=	=	=	=	=	=	m+	=
10	m−	=	=	=	=	=	=	=	=	=	=

*In normal mucosa, for each locus the methylation status of the CCGG sites is indicated by M+ (methylated sites) and M− (unmethylated sites). Compared with those of the normal tissue, in adenocarcinoma modified patterns of methylation are designated by m+ (Hypermethylation) or m− (Hypomethylation), and unmodified patterns of methylation by =. *, Loss of heterozygosity; /, Not done; Ade.C, adenocarcinomas; HBG1, A-γ-globin; PTH, parathyroid hormone; CALCA, calcitonin.

RESULTS

Methylation patterns were determined using the pair of isoschizomeric restriction endonucleases, *Hpa*II and *Msp*I. *Msp*I yielded fragments resulting from cleavage of all CCGG sites, whereas *Hpa*II only cleaved CCGG with unmethylated internal cytosine. In order to map these sites all DNAs were also cleaved with *Taq*I, a methylation insensitive endonuclease. To control the efficiency of *Hpa*II digestion, several samples were once and twice digested with a 10-fold excess of this enzyme. Southern blot experiments have shown that the pattern of *Hpa*II digested DNAs was not modified in the double digested samples. In addition, the CpG island of Ha-ras gene and the 5′ region of catalase gene (see Fig. 5B, lanes H) were never found methylated in colonic and breast samples, which indicates that partial cleavage observed for other sequences are not due to an artifactual inhibition of this enzyme.

The patterns of methylation obtained with the probes used were identicals in the normal breast tissue and all normal mucosa. However, in order to avoid misinterpretations, particularly of the methylation patterns of the breast tumors (the normal counterpart for each tumor was not available), only samples exhibiting methylation changes in a consistent range of the tumoral cellular population were scored. Results obtained are summarized in Table I.

A-γ Globin Gene (HBG1), 11p15.5

DNA methylation patterns of the A-γ globin gene were analyzed in normal and tumoral tissues using a cloned genomic DNA probe (Fig. 1B) which spans the γ-globin gene nucleotide 7591 to 9012 [Shen et al., 1981]. *Taq*I digestion

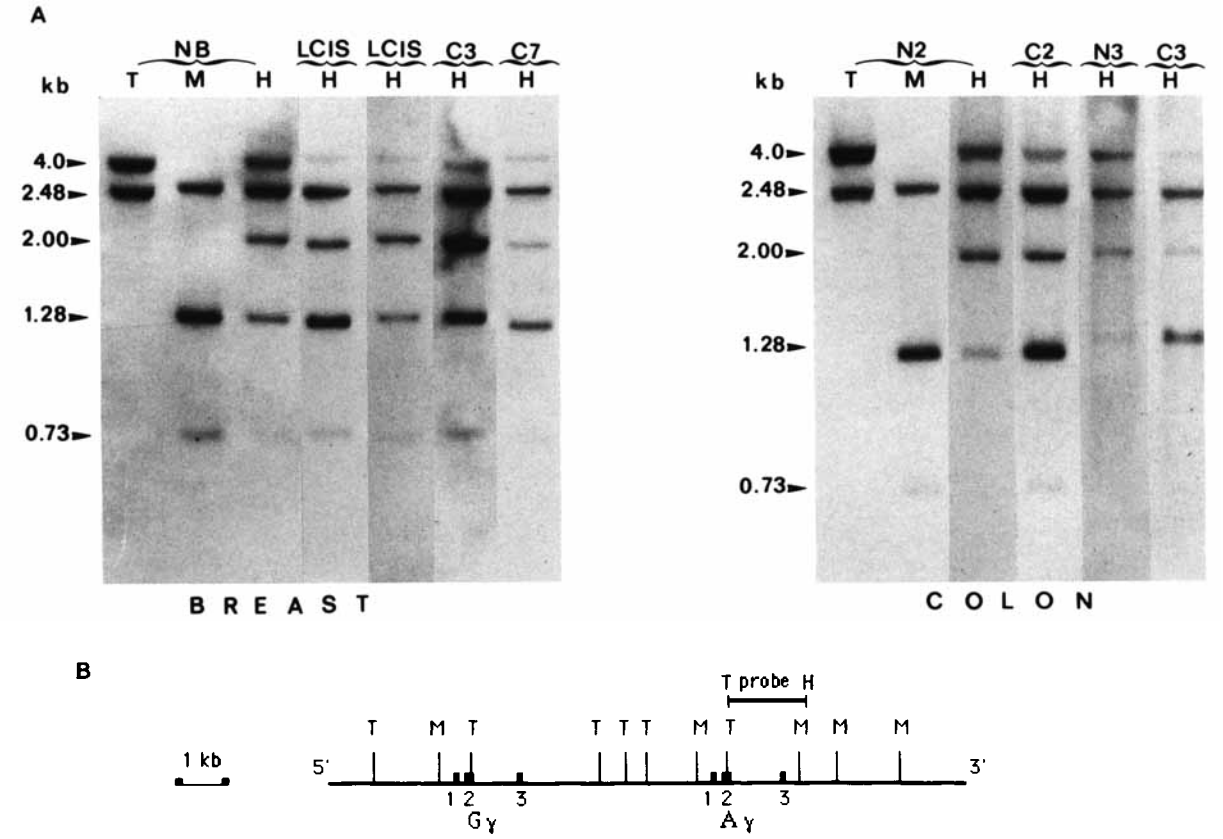

Fig. 1. DNA methylation patterns in part of the A-γ globin gene. DNAs extracted from normal breast: NB, normal colon mucosa: N, and from breast and colon carcinomas: C were cleaved with *Taq*I, T, *Taq*I-*Msp*I, M, or TaqI-HpaII, H. Southern blot experiments were performed as described in Materials and Methods. DNA methylation patterns shown are representative of those observed in normal and tumoral tissues studied. **A:** In pathological samples (lanes LCIS and C) *Hpa*II digestion yield increased intensity of the *Taq*I-*Msp*I 1.28 kb band and the appearance of a *Msp*I-*Msp*I 0.73 kb band when compared to normal tissues (lanes NB, N2, and N3). The intensity of the *Taq*I-*Taq*I 2.48 kb genomic fragment of the 3′ region of the G-γ globin gene indicates the loading in each lane since this fragment does not contain CCGG sites (see restriction map). **B:** Restriction map of the fetal globin gene. The black boxes indicate exons. T, *Taq*I; M, *Msp*I; H, *Hind*III.

of genomic DNA generates, when probed with this fragment, two bands of 4.0 (A-γ) and 2.48 kb (G-γ). Addition of *Msp*I cuts the 4.0 kb fragment and generates two DNA fragments at 1.28 kb and 0.73 kb (Fig. 1A, lanes M) consistent with the presence of two CCGG sites (Fig. 1B) at position 8866 and 9593 [Shen et al., 1981].

In most DNA extracted from breast (8/10) and colon (9/10) adenocarcinoma, *Hpa*II digestion revealed that these two sites were hypomethylated in a consistent part of the tumoral cellular populations (Fig. 1A, lanes: breast C3, C7 and colon C2, C3), when compared to normal colon mucosa (Fig. 1A, lanes N2 and N3) and to the normal breast specimen studied (Fig. 1A, lane NB). In addition, this hypomethylation was also found in the two lobular carcinoma in situ (LCIS) samples analyzed, which is an early stage of breast carcinogenis (Fig. 1A, lanes LCIS), suggesting, as was described in colon carcinoma [Goelz et al., 1985], that modification of DNA methylation patterns of γ globin gene is an early event in breast tumorigenesis.

Ha-ras Gene, 11p15.5

DNA methylation patterns at CCGG sites were visualized using a 6.6 kb probe (probe 1, Fig. 2C), which corresponds to the entire Ha-ras gene, from the 5′ promoter to the 3′ VTR (variable tandem repeats) region [Shih and Weinber, 1982]. As previously described [Krontiris et al., 1985, Pierotti et al., 1986] a multiallele polymorphism was observed after *Taq*I or *Taq*I-*Msp*I/*Hpa*II digestion in both normal and tumoral tissue (Fig. 2A). *Hpa*II digestion gave complex patterns because of the partial cleavage of most of the *Taq*I fragments which produced novel bands with different intensity. However, for each *Taq*I-*Hpa*II pattern no difference was observed between normal and tumoral tissue (Fig. 2A,

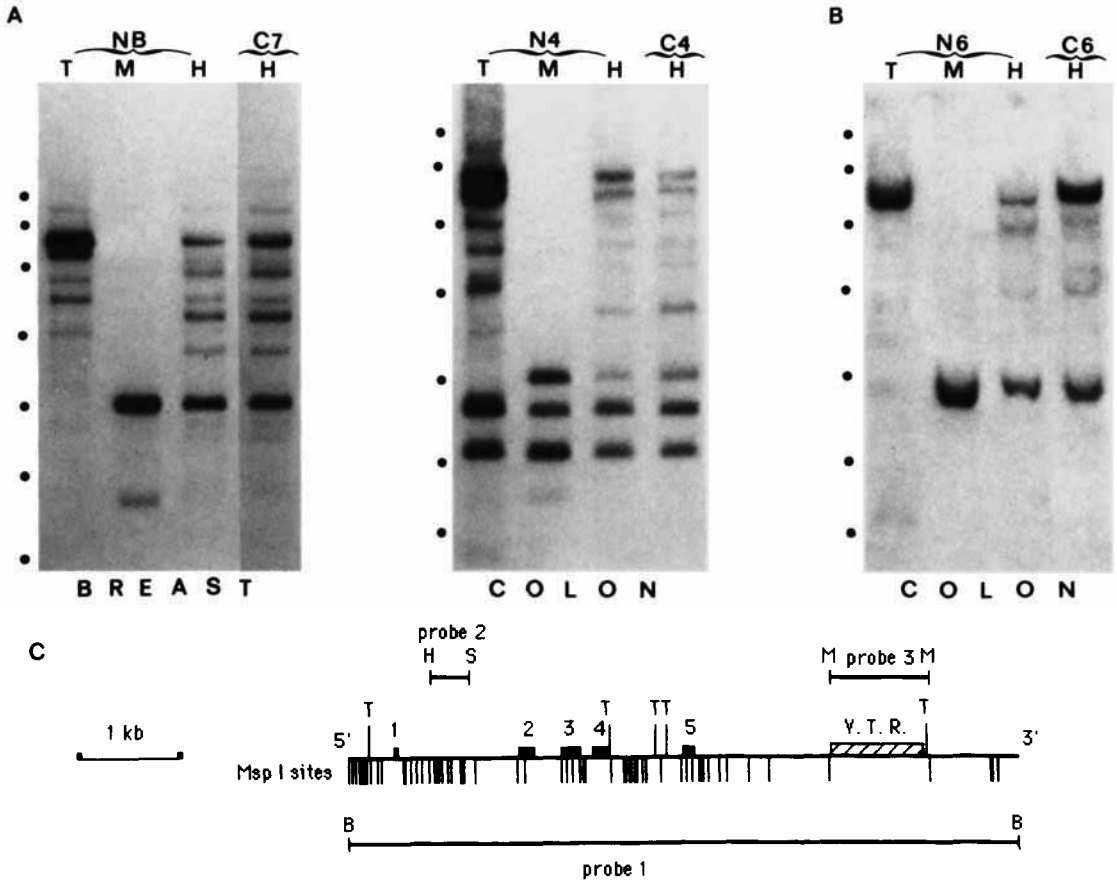

Fig. 2. DNA methylation patterns of Ha-ras gene. Experimental design was similar to the one described in the legend to Figure 1, and • represent some of the marker fragments of sizes 3.5, 2.7, 1.9, 1.4, 1.0, 0.68, and 0.49 kb, from top to bottom. **A:** Probe 1. When compared to normal breast (lane NB) and to normal colon tissues (lane N4) no significant methylation changes of Ha-ras gene are observed in tumoral tissues (lanes C). **B:** Probe 3. Colon C6 exhibits extensive methylation of the 3′ region, including the variable tandem repeats region (V. T. R.), of the gene. **C:** Restriction map of the Ha-ras gene. The black boxes indicate exons. Hatched bar, variable tandem repeats region. T, *Taq*I; M, *Msp*I; B, *BamH*I; H, *Hinf*I; S, *Sac*I.

lanes: breast NB/C7 and colon N4/C4). In one colonic adenocarcinoma an extensive hypermethylation of the CCGG sites of 3′ region including the VTR region (identified with the probe 3, Fig. 2C) of this gene was observed (Fig. 2B, lanes N6/C6). Hybridization with a 260 bp cloned genomic DNA fragment (probe 2, Fig. 2C), which allowed us to focus on 5′ region of the gene, showed that the CpG island located within this region [Shih and Weinber, 1982] was unmethylated in all the normal and tumors samples studied (data not shown).

Parathyroid Hormone Gene (PTH), 11p15.2/1

The methylation status of the CCGG site (localized by a restriction map of the probe and Southern blot experiments, Fig. 3A,B) of PTH gene was analyzed using a cloned genomic DNA probe [Vasicek et al., 1983]. *Taq*I digestion generated, as expected [Meyers et al., 1984], a constant band of 4.1 kb which produced two bands of 1.95 kb and 2.15 kb when cleaved with *Msp*I (Fig. 3A, lanes T/M). In DNA extracted from adenocarcinomas, *Hpa*II digestion of *Taq*I DNA fragments indicated that this site is hypomethylated in five of ten colon adenocarcinomas (Fig. 3A, lanes H) and one of ten breast adenocarcinomas.

Calcitonin Gene (CALCA), 11p 15.2/1

The cloned cDNA fragment (Fig. 4B) used as a probe revealed *Taq*I polymorphic fragments of 8.0 and 6.5 kb corresponding to the calcitonin gene (CALCA) and two constant bands of 3.0 and 2.3 kb corresponding to CALCP and CALCB genes respectively [Craig et al., 1982; Hoppener et al., 1988]. In almost all normal and tumoral tissues, CCGG sites visualized with this probe were unmethylated (Fig. 4A). However, in one colonic adenocarcinoma, a selective hypermeth-

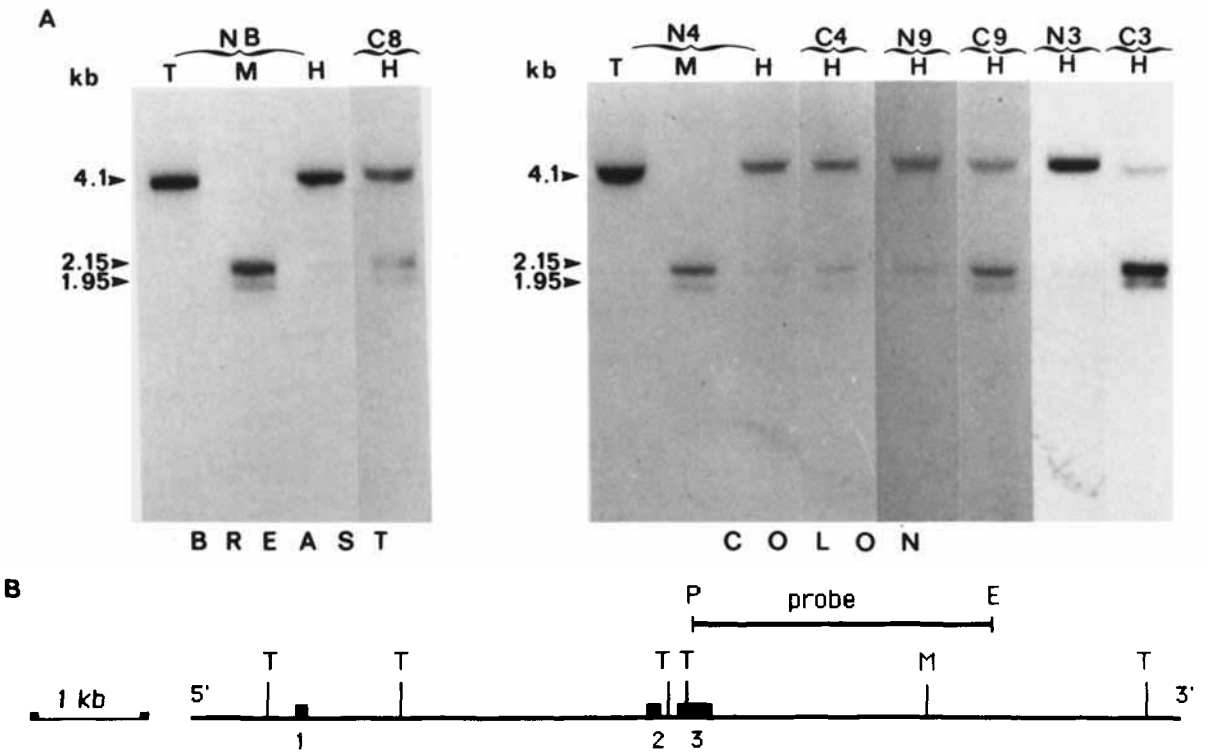

Fig. 3. DNA methylation patterns of the 3′ region of the parathyroid hormone gene. Experimental design was similar to the one described in the legend to Figure 1. **A:** In breast carcinoma C8 and colon carcinomas C9 and C3, *Hpa*II digestion yield increased intensity of the 2.15 and 1.95 kb band and a parallel decrease of the 4.1 kb band when compared to normal breast (lane NB) and normal colon mucosa (lanes N9 and N3). Colon C4 retains unchanged DNA methylation status. **B:** Restriction map of the parathyroid hormone gene. The black boxes indicate exons. T, *Taq*I; M, *Msp*I; E, *Eco*RI; P, *Pst*I.

ylation of the CALCA gene was observed, meanwhile the CALB and CALP genes also located in the same area (11p 15.2/1) retained a normal DNA methylation pattern (Fig. 4A, lane C3).

Catalase Gene, 11p13

DNA methylation patterns of catalase gene were analyzed using two cloned cDNA fragments (probe 1 contains exons 1 and 2, probe 2 contains exons 3 to 13, Fig. 5D) and a cloned genomic DNA fragment of the intron I (probe 4, Fig. 5D).

The *Taq*I 2.7 kb DNA fragment, detected by probe 1, was cleaved to 2.0 kb band by *Hpa*II (Fig. 5B, lanes: breast C4 and colon C3, C6) in tumors samples only, 4/10 in colon and 1/10 in breast. This region, which contains exon 2 of the catalase gene, was therefore hypomethylated in a consistent part of the tumoral cellular population.

The *Taq*I 4.5 kb DNA fragment also detected by probe 1 was cleaved by *Msp*I and by *Hpa*II to small DNA fragments ($\leq$250 bp), which are usually no longer detected under our experimental contitions (Fig. 5B, lanes M and H). Hybridization with probe 3 (Fig. 5D) indicate (data not shown) that this fragment contains the exon I and multiple CCGG sites located at 5′ region of the exon I [Quan et al., 1986].

In addition, hybridization with probe 2, despite numerous CCGG sites detected in *Taq*I DNA fragments (Fig. 5A, lanes M), failed to detect differences between normal and tumoral tissues in *Taq*I-*Hpa*II DNA digestion (Fig. 5A, lanes H). Furthermore, hybridization with a cloned fragment of the intron I (probe 4, Fig. 5D) also failed to detect differences between DNAs extracted from normal and tumoral colonic tissue (only one colonic tumor exhibited some hypomethylated sites, Fig. 5C, lanes N6/C6). Thus, the hypomethylation of the CCGG sites within the catalase gene in tumoral tissues seems to be restricted to the exon 2 region.

D11S347, 17p 13.3

To examine in normal and tumoral tissues the methylation status of the CpG island located at the 17p13.3 locus [Makos et al., 1992], DNAs

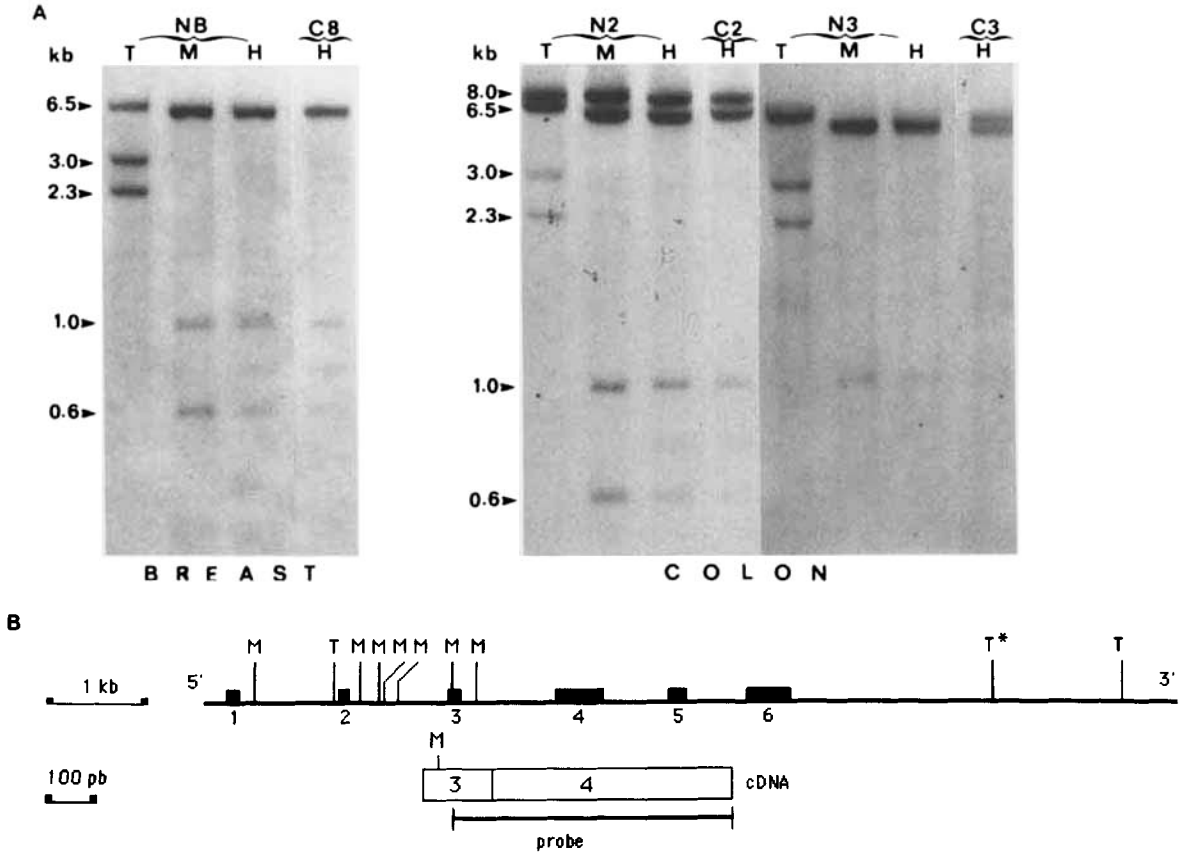

Fig. 4. DNA methylation patterns in part of the calcitonin (CALCA) gene. Experimental design was similar to the one described in the legend to Figure 1. **A:** Colon samples 2 exhibit the 8.0/6.5 kb CALCA polymorphic bands. The CALCP (3.0 kb band in lanes T), and CALCB (2.3 kb band in lanes T) genes, produce when cleaved with *Msp*I, the 1.0 and 0.6 kb bands (lanes M). **B:** Restriction map of the calcitonin gene. The boxes indicate exons. T, *Taq*I; T*, *Taq*I polymorphic site absent in the *Taq*I-*Taq*I 8 kb allele, M, *Msp*I.

were digested with *Not*I (*Not*I is blocked by the methylation of CpG at overlapping sites, [McClelland and Nelson, 1992]) and probed with a cloned genomic DNA (Fig. 6B). *Not*I sites were always unmethylated in normal tissues, as indicated by the constant presence of the normal polymorphic bands of 4/4.5 kb and the absence of bands superior to 4.5 kb in size (Fig. 6A, lanes NB, N7 and N9). However, in colon tumors methylated *Not*I sites were always detected, as shown by bands ranging from 5.5 to 7.5 kb in size (Fig. 6A). Of the nine breast tumors tested, four samples exhibited hypermethylated *Not*I sites (Fig. 6A,). Since in *Taq*I digestion CCGG sites located in the 5′ flanking region of the cluster of the *Not*I sites (Fig. 6B) were also visualized with this probe, we took this opportunity to compare these two regions. The polymorphic *Taq*I-DNA fragments ranging from 2.0 to 2.8 kb were cleaved to the same extent by *Msp*I and *Hpa*II in normal tissues (Fig. 6C, lanes NB and N4), whereas methylated CCGG sites were detected in one out of ten colon adenocarcinomas and three out of ten breast adenocarcinomas (Fig. 6C). However hypermethylation of the CpG frequently detected in *Not*I sites (Table I) does not seem to be correlated with that noted on the 5′ flanking sequences of this region (Table I).

Loss of Heterozygoty in Colon Adenocarcinomas

In colon adenocarcinomas, in good agreement with previous works [Fearon and Volgelstein, 1990], a high frequency (four out of five informative cases) of LOH at 17p13.3 was observed and only few LOH were noted in the 11p region (Ha-ras: one out seven informative cases, PTH: 0/6, CALCA 1/7 and catalase: 1/3, see Table I)

DISCUSSION

Analysis of the methylation patterns at CCGG sites in colon and breast carcinomas, at various loci on the short arm of chromosome 11 and at the 17p13.3 locus, show some variability be-

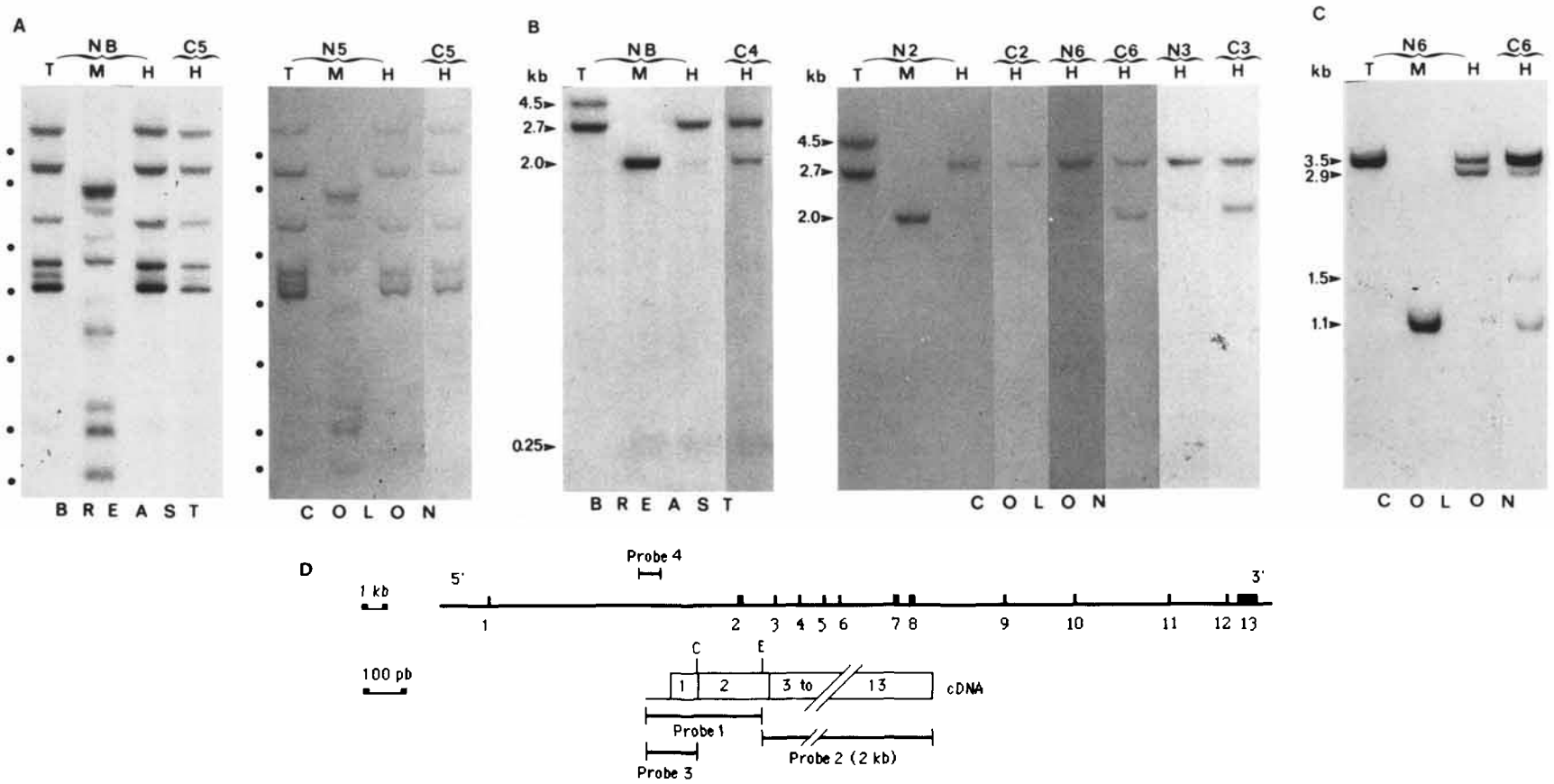

Fig. 5. DNA methylation patterns of the catalase gene. Experimental design was similar to the one described in the legend to Figure 2. **A:** Probe 2. DNA methylation pattern of catalase gene observed in normal tissues and tumoral tissues (lanes: breast NB/C5 and colon N5/C5). **B:** Probe 1. Lanes: breast C4 and colon C6 and C3 show representative patterns of DNA hypomethylation observed in tumoral tissues. Colon carcinoma C2 possess unchanged DNA methylation status. **C:** Probe 4. Hypomethylation of the intron 1 observed in colon carcinoma C6. **D:** Shematic of the catalase gene. The boxes indicate exons. T, *Taq*I; M, *Msp*I; E, *Eco*RI; C, *Cfo*I.

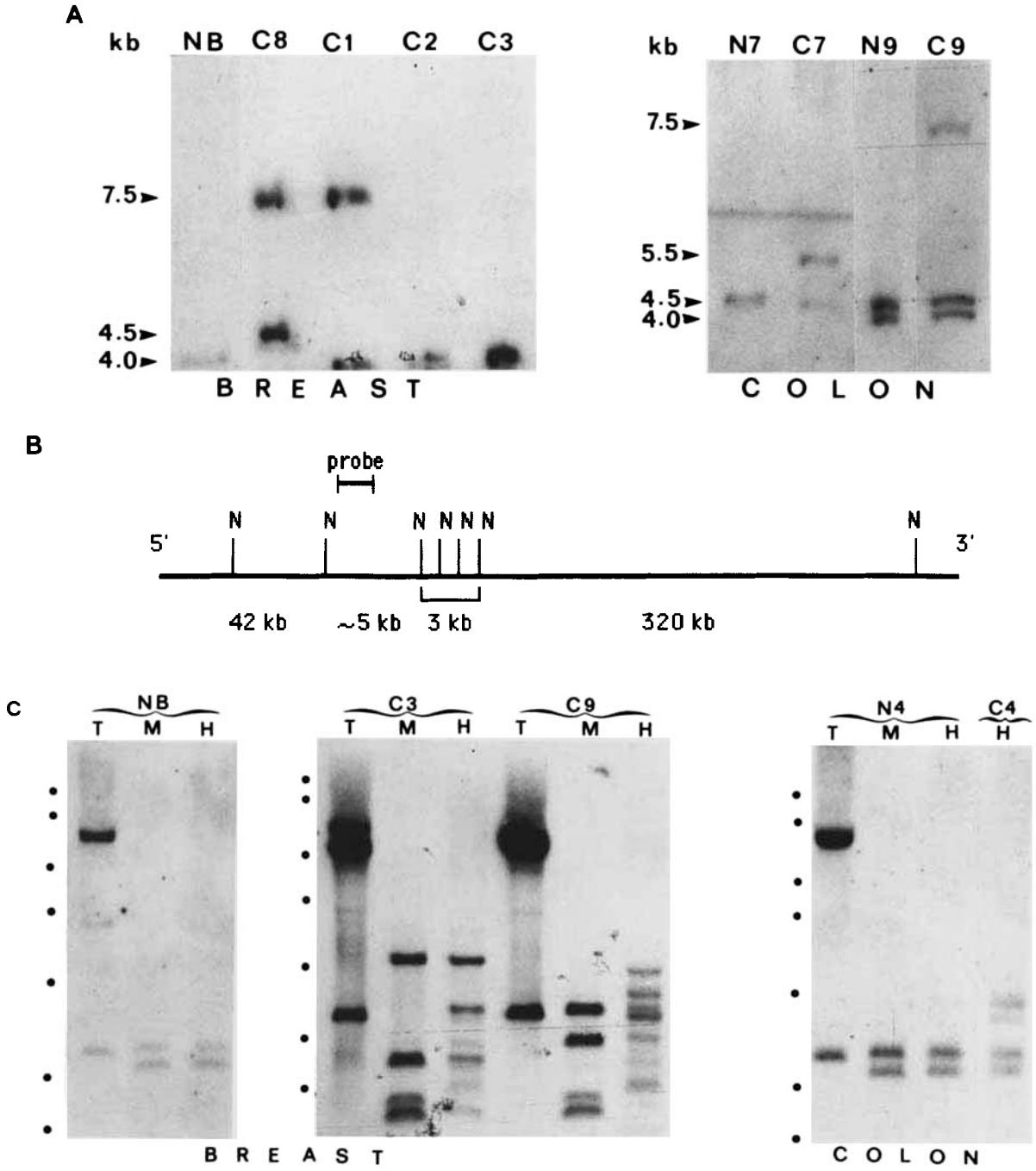

Fig. 6. DNA methylation patterns of the genomic fragment located at D17S5, using *Not*I or *Taq*I-*Hpa*II digestion. Southern blot experiments were performed as described in Materials and Methods. **A:** *Not*I digestion. DNA hypermethylations are observed in some breast carcinomas (lanes C8 and C1) and in colon carcinomas (lanes C7 and C9). Breast carcinoma samples C2 and C3 retain unchanged DNA methylation status. **B:** Schematic of the position of the *Not*I sites, with estimated sizes of *Not*I digestion fragments, around the recognition site for pYNZ22.1 [Makos et al., 1993b]. N, *Not*I. **C:** *Taq*I-*Hpa*II digestion. Experimental design was similar to the one described in the legend to Figure 2. The CCGG sites in normal tissues are not methylated as shown by identical patterns of *Msp*I and *Hpa*II digestion (NB and N4). Hypermethylation is observed in some breast carcinomas (lanes C3 and C9) and colon carcinoma (lane C4).

tween tumor samples. However, colon tissues, which allow a comparison between identically paired samples of human solid tumors and adjacent normal tissues, indicate that these differences are not related to interindividual variability. In good agreement with these findings, it had been reported for other genes or DNA segments that patterns of DNA methylation at the nucleotide or kilobase pair levels are not subject to interindividual variations [Kochanek et al., 1990, Behn-Krappa et al., 1991]. The modifications of methylation patterns observed in these tumors samples are therefore not due to interindividual variability but associated with tumor development.

DNA methylation patterns of the five DNA segments on the chromosome 11p15 and 11p13 analyzed do not exhibit tissue specificity since identical *Hpa*II patterns were observed in both normal breast tissue and normal colon mucosa. However, in the colon adenocarcinomas analyzed, several genes (γ-globin, PTH and catalase, see Table I) on the short arm of chromosome 11 are frequently hypomethylated, whereas in breast two of these loci retain in practically all the tumors a normal DNA methylation pattern (Table I). Although these data were obtained from a limited number of samples they suggest that in these two epithelial adenocarcinomas the modifications of the DNA methylation occur predominantly at specific loci according to the tumor type.

Furthermore, in the samples analyzed, the hypomethylation observed at some loci does not extend to all the 11p chromosome since some genes within this area are not affected (Table I) and for one gene studied (catalase) only one region of this gene is affected by the DNA methylation changes. This sequence specificity of the modification of the DNA methylation patterns was also observed at the 17p13.3 locus since the hypermethylation of a CpG island at this locus is not correlated with the hypermethylation detected in the 5′ flanking sequences (Table I). This concept is also supported by the observation that, in colon carcinomas, the 5′ region of the calcitonin exhibits at a high frequency (9/13) new methylation patterns (hypomethylation or hypermethylation) [Silverman et al., 1989], whereas in the samples analyzed in this study *Hpa*II digestion failed to detect modification of the CCGG sites within the calcitonin gene.

Alterations of DNA methylation have been evaluated by the use of restriction enzymes specific for CCGG sites and therefore the modifications of other CpG are not quantitated in this study. However, previous works, using genomic sequencing method, have suggested that the measurement of DNA methylation in the CCGG sites seems to be a good indicator of the overall levels of DNA methylation at CG sequences [Saluz et al., 1986, Kochanek et al., 1990]. With this restriction, in the samples analyzed alterations of the DNA methylation patterns do not seem to occur at random throughout the genome. In addition, it had been shown in cell lines [Antequera et al., 1990] and during differentiation [Saluz et al., 1986] that DNA methylation might be regulated by a combination of de novo methylation and demethylation. It will be therefore interesting to know if a modification of this equilibrium might be involved in the alterations of DNA methylation observed in tumor cells.

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