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HYPOMETHYLATION AND EXPRESSION OF PEPSINOGEN A GENES IN THE FUNDIC MUCOSA OF HUMAN STOMACH

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SUMMARY: We have examined the correlation between the extents of methylation and expression of pepsinogen A genes in normal human tissues. Expression of pepsinogen A mRNA was detected only in the fundic mucosa of the stomach and both CCGG and GCGC sites in the genes region were less methylated in the fundic mucosa than in other non-expressing tissues. Thus, there was an inverse correlation between the extents of methylation and expression of pepsinogen A genes and the role of DNA methylation in the regulation of pepsinogen A genes expression during normal differentiation was suggested.

INTRODUCTION: Pepsinogen, the precursor of pepsin is synthesized in the stomach mucosa and secreted into gastric lumen. Previous studies have shown that the human pepsinogens are separable into two enzymologically and immunologically distinct groups, namely pepsinogen A (PGA) and pepsinogen C (PGC) (1,2). PGA and PGC are derived from different parts of gastroduodenal mucosa: the former only from the peptic cells in the fundic mucosa and the latter

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from these cells, the pyloric glands and Brunner,'s glands (2). One of the central problem for pepsinogen is how it is selectively synthesized in the stomach mucosa. The results of recent studies strongly suggest that DNA methylation is involved in the regulation of gene expression during normal differentiation and an inverse correlation between the extents of methylation and expression has been found for several normal genes and integrated viral genes (3-5). However, it is becoming clear that not all genes are regulated by DNA methylation (6). In order to know whether DNA methylation is involved in pepsinogen gene regulation, we have analyzed the correlation between the extents of methylation and expression of PGA genes using methylation-sensitive restriction enzymes, MspI/HpaII and HhaI, and PGA cDNA as a probe.

MATERIALS AND METHODS

HUMAN SAMPLES: Human tissues were removed from surgical patients according to the regulation of Tokyo University Hospital. Normal part of the resected human samples were carefully dissected, immediately frozen and stored in liquid nitrogen until use. The dissected normal tissues were comfirmed by the pathological examination and pepsinogen A production in each tissue was analyzed by immunohistochemistry using ABC method (7) with anti-pepsinogen A antibody as described (8).

Isolation of DNA and RNA: High molecular weight DNA from various human tissues was prepared by the method of Gross-Bellard (9) and the total RNA was isolated by the guanidium/cesium chloride method (10).

Analysis of DNA and RNA: 10μg of DNA was digested to completion by restriction enzymes (5-10 U/μg DNA) by the manufacturer's recommended condition. Electrophoresis, transfer to nitrocellulose filter and detection of DNA were performed by the method of Southern (11). 10 μg of RNA was denatured (12) and subjected to electrophoresis on agarose-formalin gel by the method of Goldberg (13). Then the RNA was transferred to nitrocellulose filter, baked and hybridized as described (14). PGA cDNA insert in recombinant DNA clone, PTM003g-35 (15), was excised by EcoRl and BglII digestion, labeled by nick-translation (16) and used for the hybridization probe. This insert of about 1.2 kb contains roughly all the coding region and also the 3'-non-coding region (34 bp).

RESULTS: Northern blot analysis of total RNA from normal human tissues is shown in Fig. 1. PGA mRNA of about 1.8 kb in size was detected only in the stomach mucosa and not detected in other non-stomach tissues, namely, duodenum, large bowel, spleen, liver and lung. In the stomach mucosa, the expression of PGA mRNA was

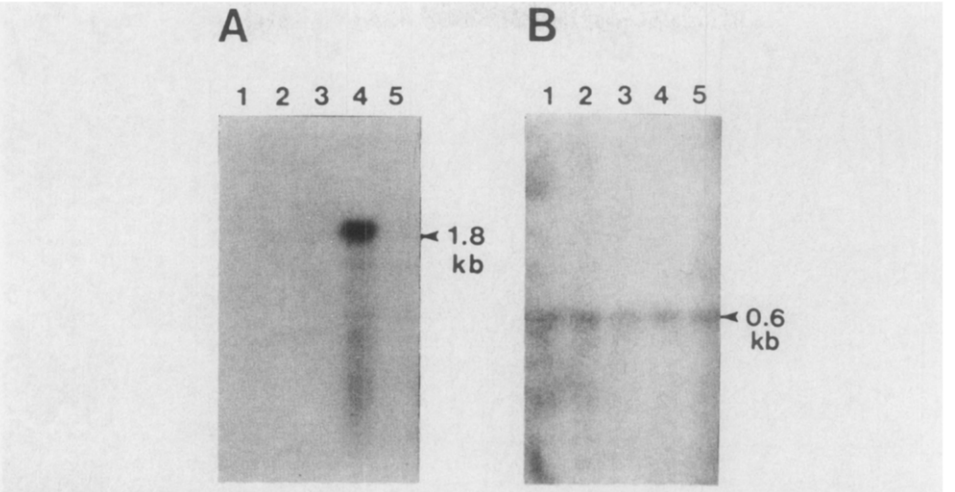

Fig. 1. Northern blot hybridization of total RNA from normal human tissues with pepsinogen A (A) and β_2-microglobulin cDNA probe (B). The same filter was hybridized successively with these two probes. The RNA samples (10 µg/lane) analyzed were as follows. Lanes: 1, large bowel; 2, duodenum; 3, pyloric mucosa; 4, fundic mucosa; 5, liver.

limited only to the fundic mucosa and not observed in the other part of the stomach mucosa, the pyloric mucosa (Fig. 1a). The lack of hybridizable signals was not caused by poor transfer or RNA degradation as shown by probing the same filter with ^{32}P-labeled β_2-microglobulin cDNA (17). Almost comparable signals for the β_2-microglobulin transcript were observed for all RNA samples analysed (Fig. 1b). Immunohistochemistry of these tissue samples also revealed the existence of PGA producing cells only in the fundic mucosa of the stomach (Fig. 2) and comfirmed the previous observation (2). These results suggest that PGA synthesis is controlled primarily by the process that regulates the level of its mRNA expression and there is little or no PGA mRNA in tissues that do not synthesize PGA.

We next investigated the methylation status of PGA genes. For methylation analysis, the methylation-sensitive restriction endonucleases, HpaII and HhaI were used. Both of these enzymes contain the doublet 5'-CG-3' within their recognition sequences (HpaII, CCGG; HhaI, GCGC) and do not function if the cytosine is methy-

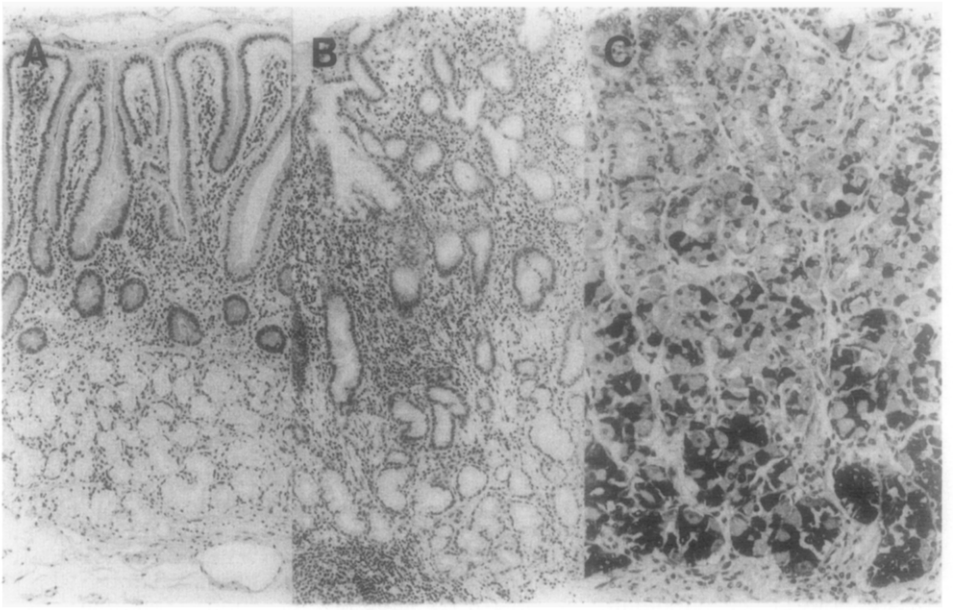

Fig. 2. Immunostaining of gastroduodenal mucosa with anti-pepsinogen A antibody by the ABC method. A; duodenal mucosa, x100, B;pyloric mucosa, x100, C; fundic mucosa, x200. Cells staining strongly for pepsinogen A are seen only in the fundic mucosa.

lated (18). The enzyme MspI provides a control for HpaII since it cleaves CCGG regardless of the methylation state of the internal cytosine (18). No such methylation-insensitive isoschizomer of HhaI has been found. When genomic DNAs from several tissues were digested with MspI and analyzed by Southern blotting using PGA cDNA as probe, an identical pattern was observed (Fig. 3a, Lane 1). Similarly, when several methylation-insensitive restriction enzymes (EcoRI, BamHI, HindIII, XhoI and Bgl II) were used for the digestion of these DNA samples, the hybridization patterns were also identical and comfirmed previous observation that there are at least three PGA genes (15,19). In addition, no polymorphism was found in Japanese population, when nearly a hundred subjects were analyzed (unpublished data). It appears, therefore, that no gross deletions, insertions or rearrangements have occurred in or around the PGA genes during normal differentiation. However, the hybridization patterns of HpaII digested DNAs were different from those of MspI digests in all the tissues analyzed, indicating the methy-

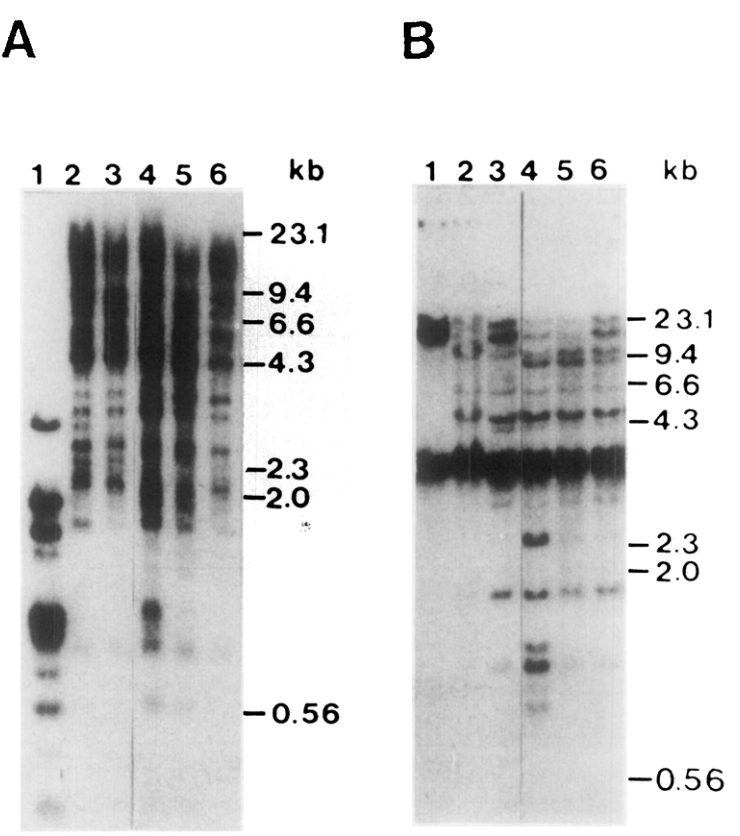

Fig. 3. Southern blot hybridization of high molecular weight DNA from normal human tissues (Lanes: 1 and 2, liver; 3, large bowel; 4, fundic mucosa; 5, pyloric mucosa; 6, duodenal mucosa). 10μg of DNA was digested with restriction enzymes and hybridized to ^{32}P-labeled pepsinogen A cDNA. Restriction enzymes used were MspI (A, lane 1), HpaII (A, lanes 2-6), EcoRI (B, lane 1) and EcoRI plus HhaI (B, lanes 2-6).

lation of CCGG sites in PGA genes region. These patterns were tissue specific and DNA from the fundic mucosa was less methylated than those from other non-expressing tissues as shown by the appearance of lower molecular weight bands (Fig. 3a). Likewise, the same analysis of EcoRI-HhaI digested DNA samples revealed that GCGC sites in PGA genes region were also less methylated in the expressing tissue than in non-expressing tissues (Fig. 3b). From the results of the hybridization analysis using various sized PGA cDNA as probe, these hypomethylated sites were situated both in the 5' and 3' halves of PGA genes region (not shown). When the same filters were hybridized with BglI-BglII genomic fragment of insulin gene (20), the gene region on the same chromosome as PGA

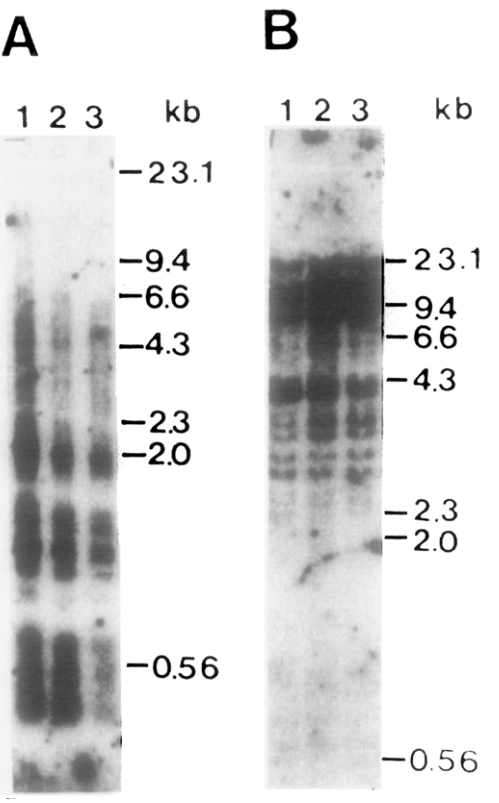

Fig. 4. Southern blot hybridization of high molecular weight DNA from normal tissues of a subject with Bgll-BglII genomic fragment of insulin gene. The same filter as used in Fig. 3 was hybridized successively with the probe. Lanes 1-3 correspond with lanes 4-6 of Fig. 3. Restriction enzymes used were HpaII (A) and EcoRl-Hhal (B) as in Fig. 3.

genes, polymorphic hybridization patterns were observed among DNAs from different subjects as described (21). However, the DNAs from the same subject revealed an identical pattern suggesting no difference in the methylation state of the insulin gene region among tissues which do and do not synthesize PGA (Fig. 4). Thus, these results indicate that PgA genes are selectively hypomethylated less in the expressing tissue, the fundic mucosa, than in other non-expressing tissues.

DISCUSSION: The results of the present study indicated that there was an inverse correlation between the expression and methylation of PGA genes and strongly suggest that DNA methylation is involved in the regulation of PGA genes expression during normal differen-

tiation. PGA genes were hypomethylated in the expressing tissue, the stomach. Since this hypomethylated pattern and the expression of PGA mRNA were found only in the fundic mucosa of the stomach, of which morphological analysis revealed the existence of PGA producing cells, PGA genes are probably hypomethylated in the PGA producing cells. Both the 5' and 3' halves of PGA genes were hypomethylated in the expressing tissue. However, at present it is difficult to correlate the hypomethylation of specific sites and expression of PGA genes, because there are at least three PGA genes and fine structures of these genes are not determined (15, 19). It is also difficult to determine the precise role of DNA methylation in the regulation of PGA genes expression by analyzing the human tissue samples. In rats, both CCGG and GCGC sites in and around the pepsinogen gene are hypomethylated when expressed and during the stomach development the demethylation changes of these sites appear to follow the onset of transcription, suggesting that they are not playing a causal role in gene activation (Ichinose M. et al. to be published). Further analysis utilizing other system such as DNA transfection with in-vitro methylation (22,23) will probably give further information as to how much of the PGA genes control is due to the modulation of DNA methylation and also as to the functional significance of the each methylated sites.

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