Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10744–10748, November 1993
Medical Sciences

Biliary glycoprotein, a potential human cell adhesion molecule, is down-regulated in colorectal carcinomas

(carcinoembryonic antigen/nonspecific crossreacting antigen/mRNA expression/tumor suppressor gene)

MICHAEL NEUMAIER*, STEFAN PAULULAT*, ANDREW CHAN*, PETER MATTHAES†, AND CHRISTOPH WAGENER*‡

*Abteilung für Klinische Chemie, Medizinische Klinik, Universitätskrankenhaus Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany; and †Chirurgische Abteilung, Israelitisches Krankenhaus, Orchideenstieg 14, D-22297 Hamburg, Germany

Communicated by Hans J. Müller-Eberhard, August 5, 1993 (received for review June 2, 1993)

ABSTRACT **Biliary glycoprotein (BGP) is the human homologue of a cell adhesion molecule (CAM) of the rat designated Cell-CAM. The BGP gene is a member of the carcinoembryonic antigen gene family, which belongs to the immunoglobulin superfamily. BGP is expressed in cells of epithelial and myeloid origin. In granulocytes, BGP is a main antigen of the CD66 cluster of differentiation antigens that mediate the binding to endothelial E-selectin. Since BGP is a major human CAM, the expression of BGP was studied in 21 colorectal carcinoma tissue specimens and in the respective adjacent normal mucosae. As an internal control for epithelial mRNA, the expression of cytokeratin 18 was evaluated in parallel. In addition, the expression of carcinoembryonic antigen and nonspecific crossreacting antigen, which are highly homologous to BGP, was investigated. Two BGP mRNAs of 3.9 and 1.5 kilobases were detected in the normal colonic mucosa samples. The median of the tumor-to-normal ratios of mRNA expression was 0.2 for both BGP mRNAs. In contrast, the median was 1.2 for cytokeratin, 1.0 for carcinoembryonic antigen, and 1.4 for nonspecific crossreacting antigen. Relative to cytokeratin 18 expression, the expression of BGP was reduced to ≤0.1 in half of the tumors and to ≤0.4 in >80% of the tumors. These findings indicate that the loss or reduced expression of the adhesion molecule BGP is a major event in colorectal carcinogenesis.**

Cell adhesion molecules (CAMs) play a key role in the generation and maintenance of tissue architecture (1). In comparison with normal tissues, malignant tumors are characterized by an altered phenotype and reduced intercellular binding forces. The loss of cell–cell binding, which closely correlates with differentiation and invasive potential of malignant tumors, is accompanied by a concomitant loss or altered expression of CAMs (2). For example, in poorly differentiated prostatic carcinomas and squamous cell carcinomas of the head and neck, the expression of E-cadherin is reduced in comparison with well-differentiated tumors (3, 4).

Biliary glycoprotein (BGP) is the human homologue of a CAM of the rat designated Cell-CAM (5–7). The designation "BGP" refers to a glycoprotein first described in human bile (8, 9). Based on internal amino acid sequences of this glycoprotein, we previously identified a BGP cDNA clone in a normal human colon library (5). The corresponding gene is a member of the carcinoembryonic antigen (CEA) family, which belongs to the immunoglobulin superfamily. BGP is expressed in cells of epithelial and myeloid origin (for review see ref. 10). After transfection into fibroblasts, the encoded protein mediates homophilic adhesion (11). In contrast to other CAMs of the CEA family or immunoglobulin family such as the neural cell adhesion molecule (N-CAM), and similar to cadherins, cell adhesion mediated by BGP and its murine homologue is Ca^{2+} and temperature dependent (11, 12). Thus, BGP combines structural features of the immunoglobulin superfamily with functional properties of the cadherin family of CAMs. Recently, we showed that BGP is the major antigen of the CD66 cluster of granulocyte differentiation antigens (13). Antigens of the CD66 cluster mediate binding of granulocytes to endothelial E-selectin (14).

In colonic carcinoma cell lines, several transcripts of the BGP gene have been identified (15). Since we cloned the BGP gene from a normal colon cDNA library, the BGP gene should be expressed in normal colon as well. Therefore, we asked the question if the expression of this adhesion molecule differs between normal colonic mucosa and colonic carcinoma tissue. The expression of BGP was compared with the expression of CEA itself and of a major CEA-related glycoprotein, the so-called "nonspecific crossreacting antigen" (NCA) (for review, see ref. 10). We provide evidence that, in contrast to mRNAs coding for CEA and NCA, the expression of two BGP specific mRNAs is down-regulated in >80% of colorectal carcinomas when compared to the corresponding normal mucosa.

MATERIALS AND METHODS

Tissue Samples. Tissue samples from colorectal adenocarcinomas (n = 21) and the corresponding normal mucosae (n = 20) were studied. Tumor samples were obtained from surgically removed colorectal carcinomas. From each individual patient, normal mucosa was also prepared from the resection borders. For RNA preparation, normal and malignant tissues were immediately frozen in liquid nitrogen until processed.

Probes. For Northern blots, the following probes were used: (*i*) A 327-bp PCR fragment (nucleotide positions 1030–1356) (15) containing all coding sequences of the A2 domain of the BGP gene. This fragment was also expressed as β-galactosidase fusion protein for the generation of BGP A2 domain-specific antisera (13). (*ii*) A restriction fragment of 396 bp (nucleotide positions 1677–2072) from the 3′ untranslated region (3′ UTR) of BGP A (5, 15) obtained from a BGP expression vector (kind gift by R. Paxton, Beckman Research Institute of the City of Hope, Duarte, CA). (*iii*) An amplified 131-bp PCR fragment (nucleotide positions 2154–2284) from the 3′ UTR of CEA (16). (*iv*) An amplified 289-bp PCR fragment (nucleotide positions 1383–1671) from the 3′ UTR of NCA (17). (*v*) A 1500-bp *Eco*RI restriction fragment of the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BGP, biliary glycoprotein; CAM, cell adhesion molecule; CEA, carcinoembryonic antigen; NCA, nonspecific crossreacting antigen; 3′ UTR, 3′ untranslated region.

‡To whom reprint requests should be addressed.

human cytokeratin 18 cDNA (kind gift from R. Leube; ref. 18).

Northern Blots. Total RNA was prepared according to Meese and Blin (19) and further purified by subsequent ethanol precipitations. Prior to Northern blot analysis, quantitation of RNA was done spectrophotometrically at 260 nm. Up to 50 μg of total RNA of each sample was analyzed by hybridization after agarose gel electrophoresis (20) and capillary transfer to GeneScreen*Plus* membranes (NEN/DuPont, Bad Homburg, Germany). Hybridizations were carried out overnight in a hybridization oven (GFL, Branschweig, Germany) using either standard hybridization solution as suggested by the manufacturer of the membrane (containing formamide, dextran sulfate, Denhardt's reagent, and denatured salmon sperm DNA) or, alternatively, rapid hybridization solution (Amersham). The blots were stripped for rehybridization by boiling in 0.1% SDS for 10 min. Labeling of probes was done by random priming (21) using the Megaprime DNA labeling system (Amersham) to specific activities of ≈ 4–9×10^8 dpm/μg. Approximately 10^6 dpm of the ^{32}P-radiolabeled probes per ml was used for hybridization at 42°C overnight, after which the membranes were washed at room temperature twice in 2× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), for 5 min each, followed by two stringent washes at 60°C in 2× SSC/0.1% SDS for 30 min, followed by a wash at room temperature in 0.1× SSC/0.1% SDS for 30 min. The filters were then exposed to x-ray film at −80°C with two intensifying screens for 24–48 hr.

Quantification of mRNA Expression. Autoradiograms were scanned with an AGFA Arcus scanner and subsequently analyzed using the densitometry software IMAGE 1.42 (public domain software of National Technical Information Service). Expression levels of BGP, NCA, CEA, and cytokeratin 18 were calculated from the integer over the mRNA bands in autoradiographs.

Statistical Methods. As nonparametric tests, the Wilcoxon signed rank test and the χ^2 test were used.

RESULTS

Selection of Probes. In general, cDNAs belonging to the CEA gene family exhibit high homologies between their coding sequences (≈90% on the nucleotide level). However, they differ in their 3′ UTRs. For this reason, probes corresponding to the 3′ UTR were used to study the expression of BGP, NCA, and CEA (3′ UTR probes). In contrast to CEA and NCA, some of the BGP-specific cDNAs contain a domain (A2 domain) that exhibits a significantly lower homology to the corresponding A domains of CEA and other members of the CEA family. A probe corresponding to this region allows the selective detection of BGP-specific mRNAs containing the A2 domain (A2 probe). Probes corresponding to the 3′ UTRs of the BGP, CEA, and NCA mRNAs as well as a probe corresponding to the A2 domain of BGP were used for Northern blots.

Northern Blots. Twenty-one tissue samples from adenocarcinomas and the corresponding normal mucosae were studied. Representative Northern blots with total RNA from normal and corresponding tumor tissues are shown in Fig. 1. Ethidium bromide stains of RNA were performed prior to blotting to allow a rough estimate of the amount of RNA loaded onto the gel and as a control of RNA integrity. RNA

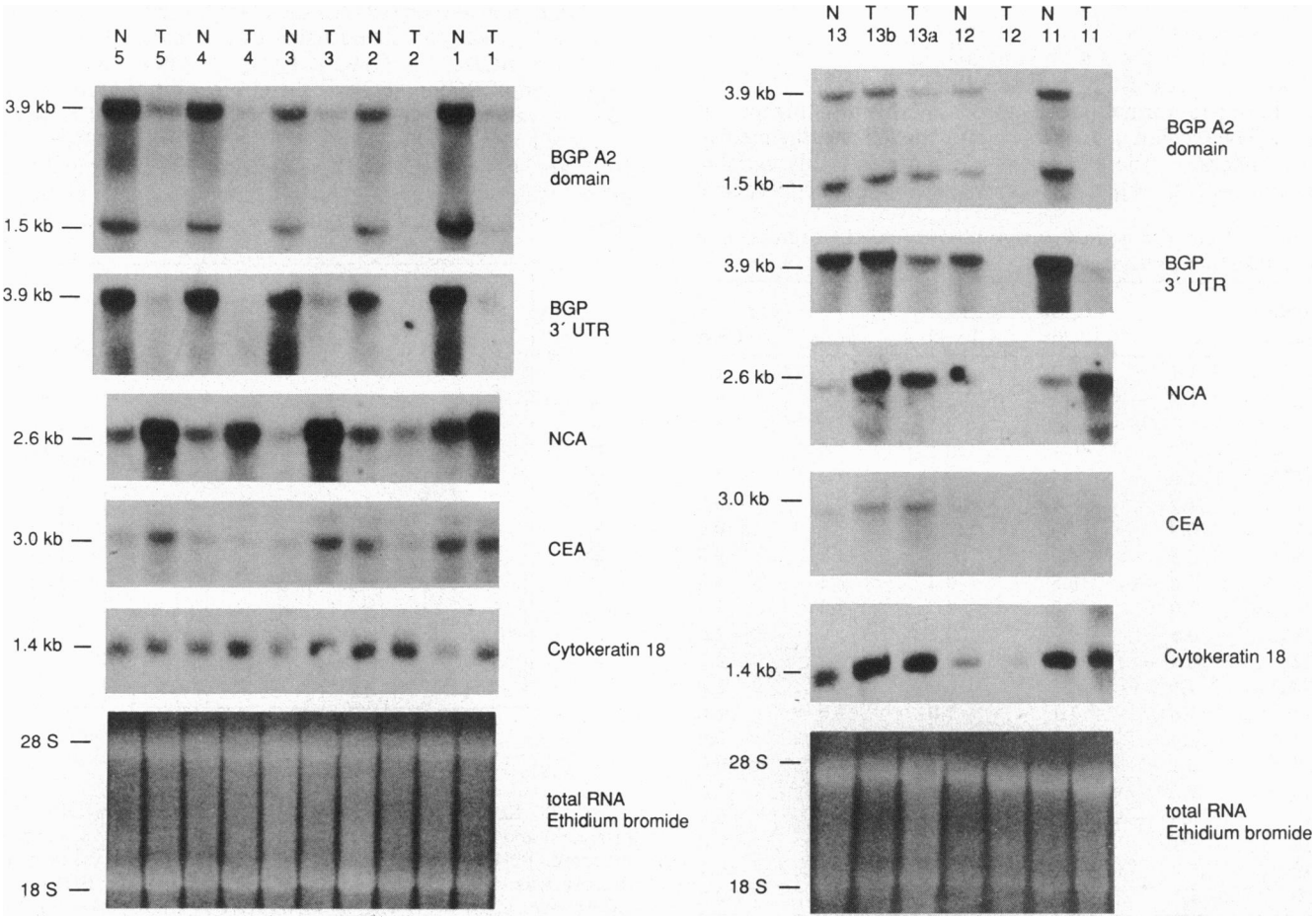

FIG. 1. Northern blots of mRNAs coding for BGP, NCA, CEA, and cytokeratin 18. Probes are indicated on the right of each panel. N, normal mucosa; T, tumor tissue.

from normal and tumor tissues revealed the expected sizes of 28 and 18 S. A single RNA species of 3.9 kb hybridized with the BGP 3′ UTR probe. A RNA of identical size was detected using the BGP A2 probe. In most tumor tissues, the expression of this RNA species was either reduced or absent. With the A2 probe, but not with the BGP 3′ UTR probe, a second band of 1.5 kb was detected. The expression of this band closely parallels the expression of the 3.9-kb band. When the expression of the BGP-specific mRNAs is compared with the cytokeratin 18 message, it is apparent that the expression of cytokeratin 18 mRNA does not parallel the expression of BGP-specific mRNAs. This finding excludes the possibility that the lower amounts of BGP-specific mRNA simply reflect a lower fraction of epithelial mRNA in the tumor tissue samples. The expression of CEA and NCA mRNAs was studied using a probe specific for the respective 3′ UTRs. It is interesting to note that, in a number of tumors, the expression of NCA exhibits an inverse correlation to the expression of BGP (see samples 1, 3, 4, and 5, Fig. 1 *Left*, and samples 11 and 13, Fig. 1 *Right*). The expression of CEA shows no consistent pattern.

Quantitative Evaluation of Northern Blots. To quantify mRNA expression, the Northern blots were evaluated by densitometry and ratios between the values obtained for tumors and corresponding normal tissues were calculated. Table 1 gives the results for cytokeratin 18, BGP (A2 probe), NCA, and CEA for all samples. In Fig. 2, a graphical presentation of the tumor-to-normal ratios is given for the BGP mRNAs and the cytokeratin 18 mRNA. The medians of the tumor-to-normal ratios were 0.2 for both BGP-specific mRNAs and 1.2 for the cytokeratin 18 mRNA. From Table 1 and Fig. 2 it follows that, for cytokeratin, tumor-to-normal ratios were always >0.7. In contrast, the ratio was ≤0.5 in 17/21 (81%) for the 3.9-kb band and in 19/21 (90%) for the 1.5-kb band of the BGP-specific mRNAs. In contrast to BGP, the tumor-to-normal ratios were significantly higher for NCA. The median of 1.4 was slightly higher than the median for cytokeratin. The expression of CEA was highly variable (median = 1.0). When the distributions of tumor-to-normal ratios are compared, differences on a highly significant level are obtained for cytokeratin 18 vs. BGP ($P < 0.001$ for both mRNAs). In contrast, no significant differences were calculated for cytokeratin 18 vs. NCA, cytokeratin 18 vs. CEA, and NCA vs. CEA (Wilcoxon signed rank test).

Table 1. Tumor-to-normal mucosa ratios of mRNA expression for cytokeratin 18 (Cyt), BGP, NCA, and CEA

Tumor no.	Cyt	BGP 3.9 kb	BGP 1.5 kb	NCA	CEA
1	1.7	0.2	0.3	1.3	1.0
2	1.0	0.2	0.2	0.7	0.3
3	1.2	0.3	0.2	2.9	5.1
4	1.2	0.1	<0.1	1.5	0.8
5	1.0	0.4	0.3	2.3	2.7
6	1.1	0.0	0.0	0.0	0.0
7	0.9	0.5	0.3	1.1	0.1
8	2.3	0.9	0.2	4.7	4.5
9	3.8	0.5	0.2	2.2	4.3
10	1.0	0.8	0.5	2.3	3.5
11	0.8	0.2	0.2	1.8	2.0
12	0.7	0.0	0.0	0.3	0.0
13a	1.5	0.6	0.7	2.2	2.9
13b	1.6	1.1	1.0	2.6	2.6
14	1.3	0.5	0.4	0.8	0.8
15	6.1	<0.1	<0.1	1.4	0.0
16	1.2	<0.1	0.2	1.1	1.5
17	0.9	<0.1	0.1	0.7	0.4
18	1.1	<0.1	0.0	1.3	0.8
19	1.4	0.0	0.0	1.7	<0.1
20	1.5	0.3	0.1	1.3	2.9

BGP mRNAs were hybridized to the A2 probe. Tumors 13a and 13b were two separate tumors from one patient. For these two tumors, a single normal mucosa sample was used as reference.

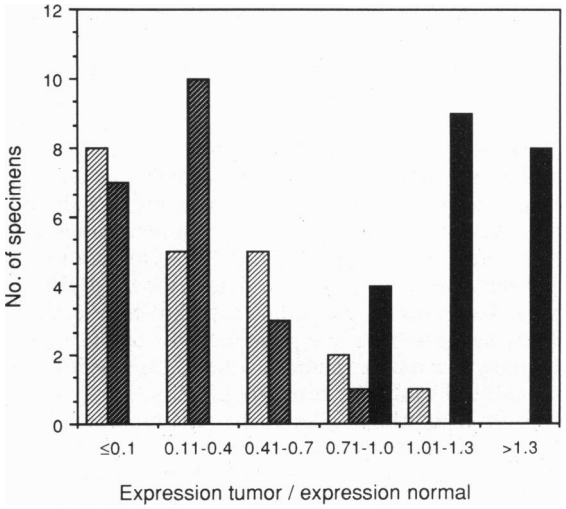

FIG. 2. Tumor-to-normal mucosa ratios of mRNA expression for two different mRNAs of 3.9 and 1.5 kb detected by the BGP A2 probe and of a mRNA of 1.4 kb detected by the cytokeratin 18 probe. ▨, The 3.9-kb band detected by the BGP A2 probe; ▩, the 1.5-kb band detected by the BGP A2 probe; ■, cytokeratin 18 probe.

Since the expression of cytokeratin 18 mRNA reflects the proportion of epithelial tissue, the relative expression (RE) of BGP in tumor tissues (TU) as compared to adjacent normal mucosa (N) was calculated using the formula $RE_{BGP\text{-}TU} = BGP_{TU} \times BGP_N^{-1} \times Cyt_N \times Cyt_{TU}^{-1}$, where BGP is the expression of BGP and Cyt is the expression of cytokeratin 18. The values for the expression of NCA and CEA were corrected accordingly. The results obtained for BGP and NCA are shown in Fig. 3. BGP expression (3.9-kb band, A2 probe) was ≤0.5 of normal in 18/21 tumors (86%); in 12/21 tumors (57%), the expression was ≤0.2 of normal. NCA

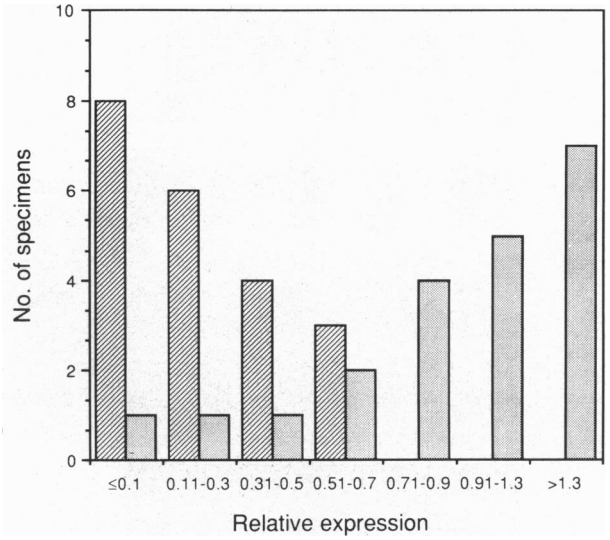

FIG. 3. Relative expression (RE) of BGP (3.9-kb mRNA, A2 probe, ▨) and NCA (□) in colorectal carcinoma tissues vs. normal mucosae. Relative expression of BGP in tumor tissue vs. normal mucosa was calculated using the formula $RE_{BGP\text{-}TU} = BGP_{TU} \times BGP_N^{-1} \times Cyt_N \times Cyt_{TU}^{-1}$, where Cyt is the cytokeratin 18 expression in normal mucosa (N) and tumor tissue (TU), respectively, and BGP is the expression of BGP in normal and malignant tissue, respectively.

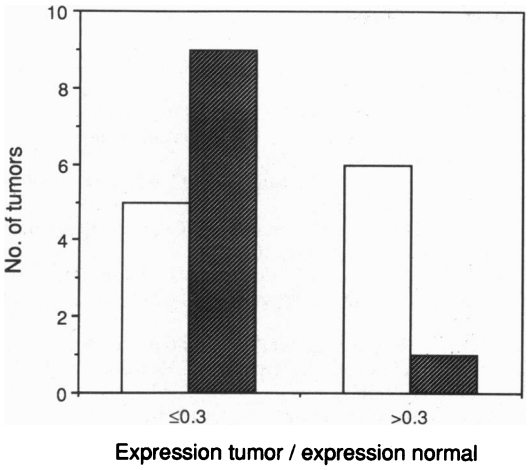

FIG. 4. Tumor-to-normal mucosa ratios of BGP expression (3.9-kb mRNA, A2 probe) in relation to lymph node involvement. □, N_0; ▨, N_1 and N_2.

expression was above 0.5 in 86%. When the normalized expression of NCA is related to that of BGP, in all but 1 tumor BGP expression was <50% of NCA expression. For CEA, the relative expression varied in a wide range (0–4.2).

BGP Expression and Lymph Node Involvement. Reduced levels of BGP were accompanied by a higher percentage of lymph node involvement. Lymph node metastases were present in 9/14 tumors (64%) with BGP mRNA tumor-to-normal ratio of ≤0.3 compared to only 1/7 (14%) with BGP ratios of >0.3 (3.9-kb mRNA, A2 probe) ($P < 0.01$, χ^2 test) (Fig. 4).

DISCUSSION

BGP is a major human adhesion molecule. Since cell adhesion is altered in malignant tissues, the expression of BGP-specific mRNAs was investigated in colorectal carcinoma tissue specimens and in corresponding normal mucosa samples. For comparison, the expression of two additional members of the CEA family—e.g., CEA and NCA—was studied. Two BGP-specific mRNAs were down-regulated in >80% of colorectal carcinomas in comparison with the corresponding normal mucosa, whereas the expression of the NCA mRNAs was up-regulated in a number of tumors. The expression of CEA was highly variable.

Because CAMs guide tissue architecture, one must assume a close interrelationship between gene expression and histology (1). For this reason we decided to avoid the use of cell lines and instead studied the expression of BGP-specific mRNA(s) in tissue samples of colonic carcinomas and adjacent normal colonic mucosae obtained during surgery. In several colonic cancer cell lines, the expression of two major BGP transcripts of 3.9 and 2.2 kb has been described (15). In normal colonic mucosa and in a fraction of the carcinoma specimens, we found two transcripts of 3.9 and 1.5 kb using the A2 domain probe. The size of the 3.9-kb mRNA is consistent with the transcript expressed in the cell lines. However, the second transcript of 1.5 kb has not been found in colonic cancer cell lines. Although not formally proven, the presence of the A2 domain and the absence of the 3′ UTR is reminiscent of BGP clone W211 described by Kuroki *et al.* (22).

A major difficulty with tissue specimens is the variable proportion of epithelial and stromal tissue elements. To compensate for different proportions of epithelial tissue, the expression of a mRNA coding for cytokeratin 18 was used as an internal control. The expression of cytokeratin 18 is restricted to simple epithelia where 100% cells of either normal or malignant tissues express this polypeptide in their cytoplasm (23). BGP is expressed in cells of myeloid origin in addition to epithelial tissues. For several reasons, a significant interference of mRNA from myeloid cells can be excluded. (*i*) One would expect granulocytes to be present in tumor tissue rather than in normal mucosa. For this reason, the amounts of BGP-specific mRNAs should be higher in tumor tissue. (*ii*) Since the NCA gene is similarly expressed in myeloid cells, the expression of mRNAs coding for BGP and NCA should show a positive correlation. (*iii*) Granulocytes contain low amounts of mRNA only.

BGP is the human homologue of Cell-CAM, for which a cell adhesion function has been established by the use of antibodies blocking the aggregation of hepatocytes *in vitro* (24). In line with this argument are transfection studies that show that human and murine BGPs are homophilic CAMs. In contrast to other CAMs of the immunoglobulin superfamily such as N-CAM, BGP shares functional properties with cadherins insofar as cell adhesion depends on Ca^{2+} and temperature (11, 12). In contrast, CEA and NCA mediate homophilic and heterophilic cell adhesion in a Ca^{2+}- and temperature-independent fashion after transfection of their cDNAs into fibroblasts (10), indicating a mechanism different from BGP. Moreover, the lack of transmembrane and cyto-

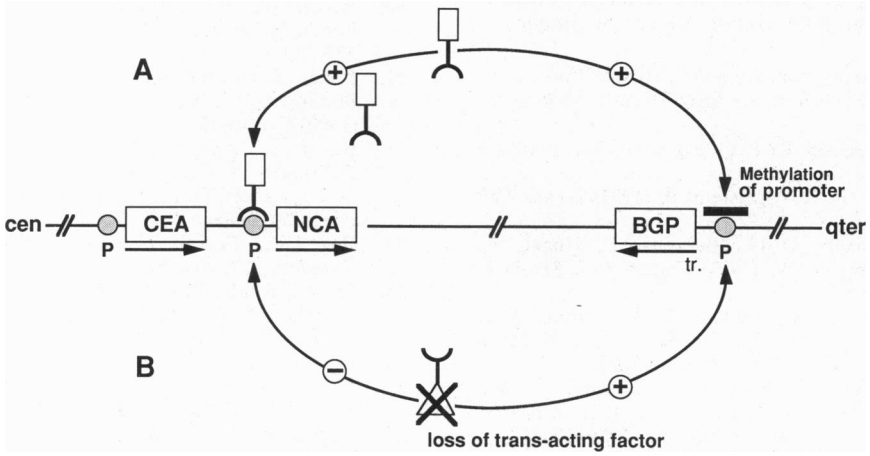

FIG. 5. Schematic model of linked gene expression for BGP and NCA. Representation of the chromosomal localization of CEA, NCA, and BGP as modified from ref. 10. cen, Centromer; qter, telomer. The direction of transcription (tr.) is indicated by horizontal arrows. Gene regulatory effects are indicated by (+) or (−). (*A*) Potential transcription factor, rectangular symbol; CEA and NCA promoters: P, shaded circle; the horizontal bar above the BGP promoter indicates binding inhibition by methylation. (*B*) Loss of a potential transacting factor is indicated by a crossed-out triangular symbol.

plasmic domains in CEA and NCA and their strictly luminal localization argue against an intercellular adhesion function in normal colon epithelium *in vivo* (25, 26). Since BGP-specific antibodies only became available very recently (13, 27), the cellular localization of BGP is not determined so far. If BGP functions as CAM in normal colonic mucosa, one would expect that the reduction of BGP expression in tumors should be accompanied by a more invasive growth and an increased percentage of lymph node metastases. Indeed, a higher proportion of lymph node involvement was found in tumors with BGP mRNA tumor-to-normal ratios of ≤ 0.3. The fact that in about one-third of tumors with a relative BGP expression of ≤ 0.3 lymph node metastases were not detected may be due to the redundancy of CAMs. The loss of additional CAMs may be necessary for cancer cells to become overtly metastatic. One candidate is the putative CAM DCC (deleted in colorectal carcinomas) (28). Furthermore, colorectal cancer cells may have to adopt new adhesion molecules for the establishment of metastases. As has been shown recently, a splice variant of CD44, which conferred the metastatic phenotype to a pancreatic carcinoma cell line of the rat, is expressed in a high percentage of human colorectal carcinomas. It has been proposed that the CD44 variant mediates the adherence of carcinoma cells to cellular elements in lymph nodes (29, 30).

The frequency of the down-regulation of BGP is comparable with the frequency of major genetic alteration in colorectal cancer such as mutations of the p53 (31, 32), *RAS* (33), and *APC* (34) genes. At present, one can only speculate on the molecular mechanism underlying the down-regulation of BGP and the concomitant up-regulation of NCA expression. Since the NCA gene is located in close proximity to the BGP gene on the long arm of chromosome 19 (10, 35), it is improbable that major deletions are the reason for the loss or reduction of BGP expression. Furthermore, gross alterations of chromosome 19 are not frequently encountered in colorectal carcinomas (36). At this point, we propose two alternative mechanisms that could account for the observed inverse relation of BGP and NCA expression in colorectal cancer (Fig. 5). During malignant transformation, the expression of BGP may be lost due to methylation of the BGP promoter. For a potential common transcription factor, this may exert a positive dose effect on NCA expression. Alternatively, BGP may be lost because of the down-regulation of a BGP-promoting trans-acting factor that acts as a repressor for NCA under normal conditions. Studies on the regulation of BGP expression may help to explain the basis of altered morphology and invasive potential of colorectal cancer.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Wa 473/5-1) and from the Fritz Thyssen Stiftung.

1. Edelman, G. M. & Crossin, K. L. (1991) *Annu. Rev. Biochem.* **60,** 155–190.
2. Hedrick, L., Cho, K. R. & Vogelstein, B. (1993) *Trends Cell. Biol.* **3,** 36–39.
3. Schipper, J. H., Frixen, U. H., Behrens, J., Unger, A., Jahnke, K. & Birchmeier, W. (1991) *Cancer Res.* **51,** 6328–6337.
4. Umbas, R., Schalken, J. A., Aalders, T. W., Carter, B. S., Karthaus, H. F., Schaafsma, H. E., Debruyne, F. M. & Isaacs, W. B. (1992) *Cancer Res.* **52,** 5104–5109.
5. Hinoda, Y., Neumaier, M., Hefta, S. A., Drzeniek, Z., Wagener, C., Shively, L., Hefta, L. J., Shively, J. E. & Paxton, R. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6959–6963.
6. Aurivillius, M., Hansen, O. C., Lazrek, M. B., Bock, E. & Öbrink, B. (1990) *FEBS Lett.* **264,** 267–269.
7. Lin, S. H. & Guidotti, G. (1989) *J. Biol. Chem.* **264,** 14408–14414.
8. Svenberg, T., Hammarström, S. & Hedin, A. (1979) *Mol. Immunol.* **16,** 245–252.
9. Svenberg, T. (1976) *Int. J. Cancer* **17,** 588–596.
10. Thompson, J. A., Grunert, F. & Zimmermann, W. (1991) *J. Clin. Lab. Anal.* **5,** 344–366.
11. Rojas, M., Fuks, A. & Stanners, C. P. (1990) *Cell Growth Differ.* **1,** 527–533.
12. Turbide, C., Rojas, M., Stanners, C. P. & Beauchemin, N. (1991) *J. Biol. Chem.* **266,** 309–315.
13. Stoffel, T., Neumaier, M., Gaida, F.-J., Fenger, U., Drzeniek, Z., Haubeck, H.-D. & Wagener, C. (1993) *J. Immunol.* **150,** 4978–4984.
14. Kuijpers, T. W., Hoogerwerf, M., van der Laan, L. J., Nagel, G., van der Schoot, C. E., Grunert, F. & Roos, D. (1992) *J. Cell Biol.* **118,** 457–466.
15. Barnett, T. R., Kretschmer, A., Austen, D. A., Goebel, S. J., Hart, J. T., Elting, J. J. & Kamarck, M. E. (1989) *J. Cell Biol.* **108,** 267–276.
16. Oikawa, S., Nakazato, H. & Kosaki, G. (1987) *Biochem. Biophys. Res. Commun.* **142,** 511–518.
17. Neumaier, M., Zimmermann, W., Shively, L., Hinoda, Y., Riggs, A. D. & Shively, J. E. (1988) *J. Biol. Chem.* **263,** 3202–3207.
18. Leube, R. E., Bosch, F. X., Romano, V., Zimbelmann, R., Höfler, H. & Franke, W. W. (1986) *Differentiation* **33,** 69–85.
19. Meese, E. & Blin, N. (1987) *Gene Anal. Tech.* **4,** 45–49.
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
21. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
22. Kuroki, M., Arakawa, F., Matsuo, Y., Oikawa, S., Nakazato, H. & Matsuoka, Y. (1991) *Biochem. Biophys. Res. Commun.* **176,** 578–585.
23. Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. & Krepler, R. (1982) *Cell* **31,** 11–24.
24. Ocklind, C. & Öbrink, B. (1982) *J. Biol. Chem.* **257,** 6788–6795.
25. Hammarström, S., Khan, W. N., Teglund, S., Hammarström, M.-L., Ramos, T., Baranov, V., Yeung, M. & Frängsmyr, L. (1993) in *Structure of Antigens*, ed. Van Regenmortel, M. H. V. (CRC, Boca Raton, FL), Vol. 2, pp. 341–376.
26. Fantini, J., Rognoni, J. B., Culouscou, J. M., Pommier, G., Marvaldi, J. & Tirard, A. (1989) *J. Cell. Physiol.* **141,** 126–134.
27. Drzeniek, Z., Lamerz, R., Fenger, U., Wagener, C. & Haubeck, H. D. (1991) *Cancer Lett.* **56,** 173–179.
28. Fearon, E. R., Cho, K. R., Nigro, J. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Preisinger, A. C., Thomas, G., Kinzler, K. W. & Vogelstein, B. (1990) *Science* **247,** 49–56.
29. Arch, R., Wirth, K., Hofmann, M., Ponta, H., Matzku, S., Herrlich, P. & Zöller, M. (1992) *Science* **257,** 682–685.
30. Heider, K. H., Hofmann, M., Hors, E., Vandenberg, F., Ponta, H., Herrlich, P. & Pals, S. T. (1993) *J. Cell Biol.* **120,** 227–233.
31. Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K., Hamilton, S. & Vogelstein, B. (1990) *Cancer Res.* **50,** 7717–7722.
32. Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., vanTuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R. & Vogelstein, B. (1989) *Science* **244,** 217–221.
33. Bos, J. L., Fearon, E. R. & Hamilton, S. R. (1987) *Nature (London)* **327,** 293–297.
34. Powell, S. M., Zilz, N., Beazer Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., Vogelstein, B. & Kinzler, K. W. (1992) *Nature (London)* **359,** 235–237.
35. Brandriff, B. F., Gordon, L. A., Tynan, K. T., Olsen, A. S., Mohrenweiser, H. W., Fertitta, A., Carrano, A. V. & Trask, B. J. (1992) *Genomics* **12,** 773–779.
36. Vogelstein, B., Fearon, E. R., Kern, S. E., Hamilton, S. R., Preisinger, A. C., Nakamura, Y. & White, R. (1989) *Science* **244,** 207–211.