

Small Integrin Binding Ligand *N*-Linked Glycoprotein Gene Family Expression in Different Cancers

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ABSTRACT

Purpose: Members of the small integrin binding ligand *N*-linked glycoprotein (SIBLING) gene family have the capacity to bind and modulate the activity of matrix metalloproteinases (MMPs). The expression levels of five SIBLING gene family members [bone sialoprotein (BSP), osteopontin (OPN), dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), and dentin sialophosphoprotein (DSPP)] and certain MMPs were determined using a commercial cancer array.

Experimental Design: Cancer profiling arrays containing normalized cDNA from both tumor and corresponding normal tissues from 241 individual patients were used to screen for SIBLING and MMP expression in nine distinct cancer types.

Results: Significantly elevated expression levels were observed for BSP in cancer of the breast, colon, stomach, rectum, thyroid, and kidney; OPN in cancer of the breast, uterus, colon, ovary, lung, rectum, and thyroid; DMP1 in cancer of the breast, uterus, colon, and lung; and dentin sialophosphoprotein in breast and lung cancer. The degree of correlation between a SIBLING and its partner MMP was found to be significant within a given cancer type (e.g., BSP and MMP-2 in colon cancer, OPN and MMP-3 in ovarian cancer; DMP1 and MMP-9 in lung cancer). The expression levels of SIBLINGs were distinct within subtypes of cancer (e.g., breast ductal tumors compared with lobular tumors). In general, SIBLING expression increased with cancer stage for breast, colon, lung, and rectal cancer.

Conclusions: These results suggest SIBLINGs as potential markers of early disease progression in a number of

different cancer types, some of which currently lack vigorous clinical markers.

INTRODUCTION

The small integrin-binding ligand *N*-linked glycoprotein (SIBLING) gene family is clustered on human chromosome 4, and its members include bone sialoprotein (BSP), osteopontin (OPN), dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), and dentin sialophosphoprotein (DSPP; ref. 1). SIBLINGs are normally thought to be restricted in expression to mineralizing tissue such as bones and teeth (1). Retrospective studies using pathological specimens have shown that OPN expression occurs in cancer of the breast, colon, stomach, ovary, lung, thyroid, kidney, prostate, and pancreas (2, 3). The expression of other SIBLING members in cancer has not been extensively studied. BSP expression was reported in breast (4, 5), prostate (6), lung (7), and thyroid cancer (8). DMP1 has been shown to be strongly up-regulated in lung cancer (9). Elevated levels of MEPE mRNA expression by tumors from patients with hypophosphatemia and osteomalacia have been reported (10). The neoplastic expression pattern of DSPP has not been defined.

Matrix metalloproteinases (MMPs) are critical for development, wound healing, and the progression of cancer. We have recently shown that BSP, OPN, and DMP1 specifically bind to pro-MMP-2, pro-MMP-3, and pro-MMP-9, respectively, thereby activating the latent proteolytic activity (11). Furthermore, it was shown that active MMPs inhibited by either tissue inhibitors of MMPs or low molecular weight synthetic inhibitors were reactivated by their corresponding SIBLING. The current study was undertaken to determine the mRNA expression patterns of SIBLINGs in nine different types of cancer. An additional goal was to determine whether SIBLINGs exhibited expression levels that correlated with their MMP partners as well as various measures of tumor progression.

MATERIALS AND METHODS

Cancer Array Analysis. A cancer profiling array (product 7841-1; Clontech, Palo Alto, CA) containing normalized cDNA from tumor and corresponding normal tissues from 241 individual patients was used to screen for SIBLING and MMP expression (12). Several cancer profiling arrays were hybridized in ExpressHyb hybridization solution (Clontech) with ³²P-labeled cDNA probes as per the manufacturer's instructions. Briefly, 1 to 2 × 10⁷ cpm of random-prime labeled cDNA was made single stranded by heating to 95°C for 5 minutes and allowed to hybridize with the prepared membrane overnight at 65°C. Membranes were washed in a series of high stringency washes as recommended by the manufacturer. The washed membranes were quantified by exposure to PhosphorImager screens for 1 to 24 hours, and the exposed screen was analyzed on a PhosphorImager (Amersham Biosciences, Piscataway, NJ)

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using the manufacturer's ImageQuant program. All polymerase chain reaction (PCR) products were subcloned into a shuttle plasmid, cloned, and sequenced, and the inserts were gel-purified before ^{32}P labeling by random priming. Unincorporated label was removed before hybridization.

SIBLING Probes. The labeled DNA used for probing was obtained as follows. Human BSP and OPN were cDNA inserts released from OP-10 and B6-5g plasmids, respectively (13, 14). Human DMP1 insert was the ~ 1.4 -kb coding region of exon 6 (15) amplified from human genomic DNA subcloned into pBluescript at the *EcoRI* and *BamHI* sites using oligonucleotides ATTATAGAATTCAAATGAAGACCCAGTGACAG (forward) and TAATTAGGATCCAATAGCCGCTTG-GCAGTC (reverse). The MEPE probe was a 1.45-kb, exon 5, cDNA insert corresponding to the last exon of human MEPE, which constitutes 95% of the mature protein as defined by Rowe *et al.* (10). The exon was amplified by PCR from human genomic DNA using a 5' oligonucleotide with a *NdeI* restriction site engineered in AGTACCCATATGAAAGACAATATTGG-TTTTCACCAT and a 3' oligonucleotide with a *BamHI* site (CTGATGGGATCCCTAGTCACCATCGCTCTCAC). The PCR product was subcloned into pBluescript and sequenced, and the ~ 1.5 -kb insert was released with *NdeI* plus *BamHI* and labeled. The DSPP probe corresponding to the last exon was similarly amplified using a 5' oligonucleotide with a *HindIII* restriction site engineered in CTGTTGGTACCGATATC-GAAATCAAGGGTCCCAGCAG and a 3' oligonucleotide with a *KpnI* restriction site (GTGCAAAGCTTCTAATCATCACTGGTTGAGTGG), subcloned, and sequenced, and the released ~ 2.6 -kb insert was labeled.

Matrix Metalloproteinase Probes. Specific probes of ~ 300 bp each for human MMP-2, MMP-3, and MMP-9 were made by PCR using human genomic DNA as template and the following oligonucleotides: MMP-2, ATTAGGATCCGGTCA-CAGCTACTTCTTCAAG (forward with *BamHI* site added for subcloning) and ATATGGATCCGCCTGGGAGGAGTACAG (reverse with *BamHI* site); MMP-3, ATATGGATCCAGCTG-GCTTAATTGTTGAAAG (forward with *BamHI*) and TAA-TGGATCCAAGTACAAATCGTCTTTATTA (reverse with *BamHI*); and MMP-9, AATTGAATTCAGAGAAAGCCTA-TTCTGCCAG (forward with *EcoRI*) and TAATGAATTCG-GTTAGAGAATCCAAGTTTATTAG (reverse with *EcoRI*). In each case, the PCR products were subcloned into pBluescript and verified by sequencing, and the ~ 0.3 -kb inserts were released and labeled. Membranes were used up to three times, each time removing the previous probe according to the manufacturer's instructions. The stripped membranes were reimaged by PhosphorImager to verify the removal of the previous probe.

Statistical Analysis. Clinical data linked to samples spotted on the cancer profiling array were accessed through the manufacturer's World Wide Web-based database.³ Comparisons between normal and tumor tissue (derived from the same subject) were performed using a paired *t* test. The coordinated expression of SIBLINGs with MMP binding partners in tumors was tested by regression analysis. Significant differences in

tumor subtype expression of SIBLINGs was tested by Student's *t* test. The association of SIBLING expression levels with tumor stage was investigated using a conservative statistical approach. The nonparametric Spearman rank order correlation was used to examine the correlation of tumor stage and SIBLING expression. The analysis was performed on untransformed data, and the adjusted Spearman correlation coefficient (r_s) is reported. All statistical calculations were carried out using StatView software (SAS Institute, Inc., Cary, NC).

RESULTS

SIBLINGs Are Elevated in Multiple Cancer Types.

Because BSP and OPN protein expression have been found to be greatly increased in many separate, often immunohistochemistry-based studies of different neoplasms, the expression levels of five SIBLING gene family members were determined using a commercial cancer array. The array included normalized cDNA from tumor and corresponding normal tissues from 241 individual patients, as well as certain internal controls (Fig. 1). Because the sample sizes were too small for some tumor types on the array, the tissues reported for this study include only breast, uterus, colon, stomach, ovary, lung, kidney, rectum, and thyroid. In each array experiment, the patient's normal and tumor cDNA was separately hybridized with ^{32}P -labeled probes for BSP, OPN, DMP1, MEPE, and DSPP, and the array was digitized by PhosphorImager. Whereas BSP, DMP1, and DSPP exhibited minimal normal tissue expression, significant OPN expression by normal tissues was observed. In fact, the highest levels of expression of OPN were seen in normal kidney. Because MEPE expression was minimal in both normal and tumor tissue, its expression was not analyzed further (data not shown). The amount of hybridized probe was quantified, and the average expression values of BSP, OPN, DMP1, and DSPP in normal and tumor tissue were compared (Fig. 2). The expression levels of BSP were significantly elevated (from 2- to 6-fold) in cancer of the breast, colon, rectum, thyroid, and kidney. OPN expression was significantly elevated (2- to 4-fold) in cancer of the breast, uterus, colon, ovary, lung, rectum, and thyroid. DMP1 exhibited significant (1.7- to 3-fold) elevated expression in cancer of the breast, uterus, colon, and lung, whereas DSPP exhibited significant (2-fold) increase in cancer of the breast and lung. Elevated SIBLING family expression was greatest in breast cancer, in which expression of four different family members was increased. Colon, lung, and thyroid cancer had significantly elevated expression of three different SIBLING family members. Of the nine different types of tumors quantified, each one had a significantly high expression of at least one SIBLING.

Matrix Metalloproteinases Are Elevated in Multiple Cancer Types.

We have recently shown that three members of the SIBLING family can specifically bind and modulate the activity of three different MMPs (11). The SIBLINGs BSP, OPN, and DMP1 were found to bind to and modulate the activity of MMP-2, MMP-3, and MMP-9, respectively. Corresponding MMP partners for DSPP and MEPE, if any, have yet to be identified. Because MMPs have been postulated to play major roles in tumor cell progression and metastasis (16), the expression levels of SIBLING-matched MMPs were screened in

³ <http://bioinfo.clonetechn.com/dparray/array-list-action.do>.

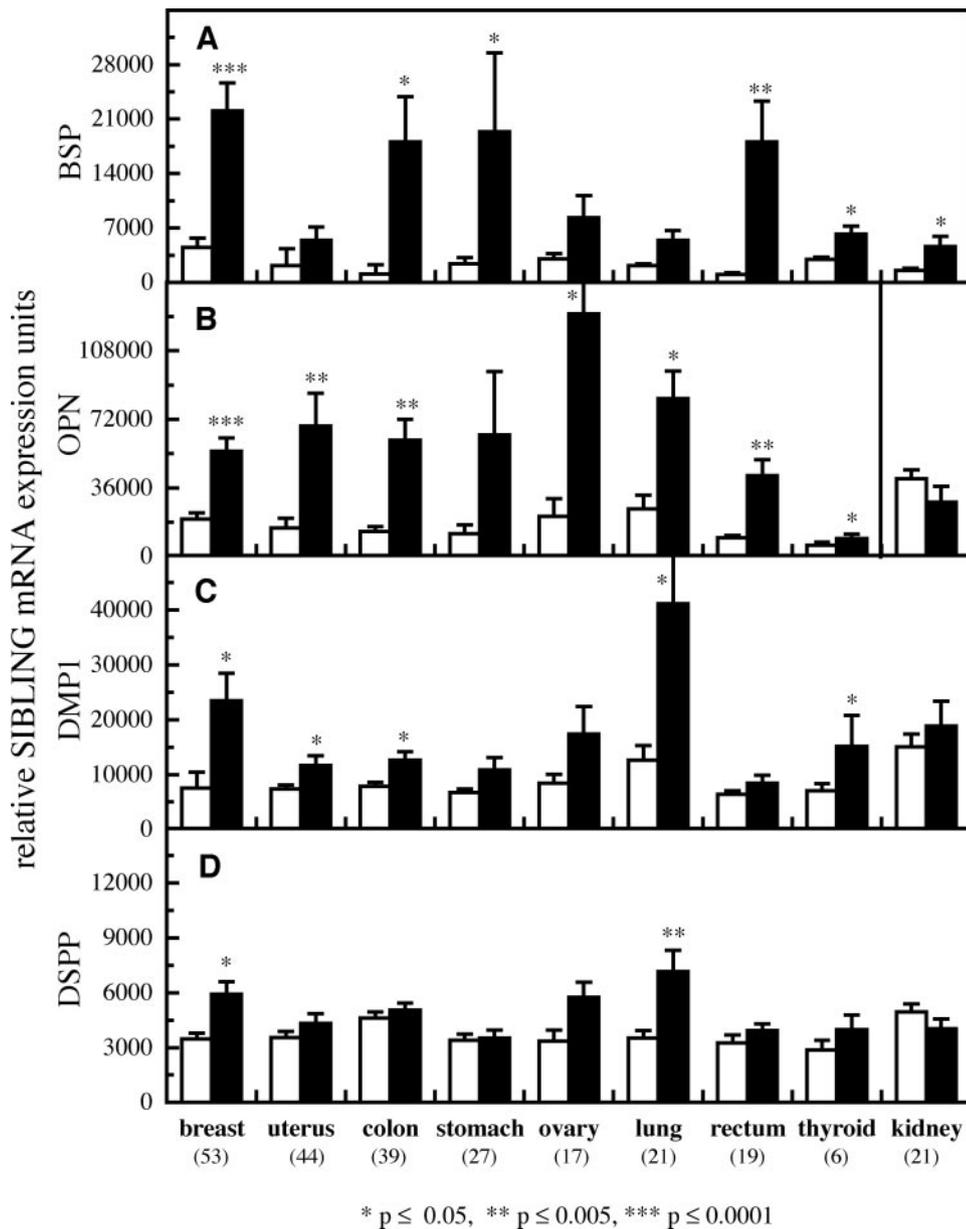


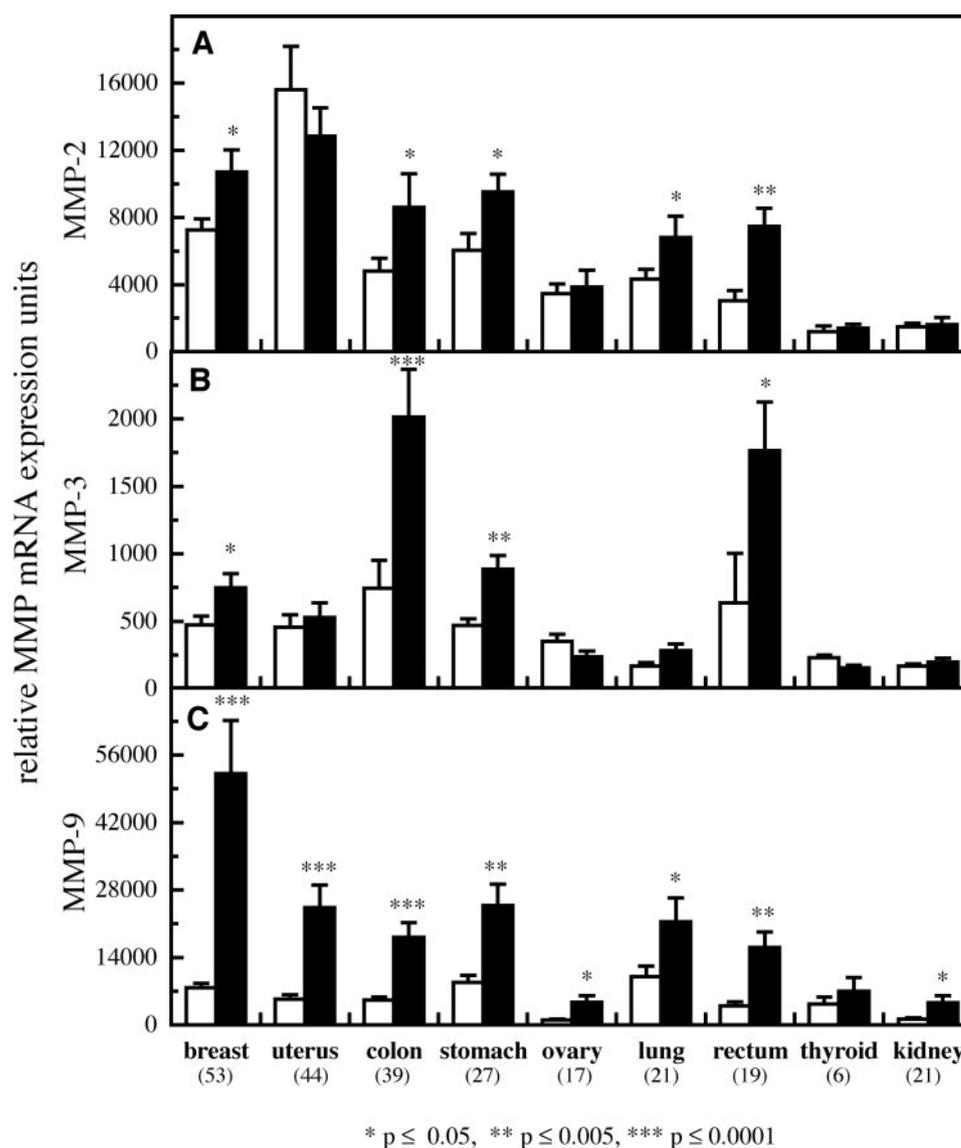
Fig. 2 SIBLING mRNAs are induced in multiple cancer types. Digitized exposures from Fig. 1 were quantified using ImageQuant software, and the mean values of relative expression of BSP (A), OPN (B), DMP1 (C), and DSPP (D) in normal tissue (□) and tumor tissue (■) were determined for each of nine different cancer types. Asterisks denote the statistical significance as determined by paired *t* tests. *, $P \leq 0.05$; **, $P \leq 0.005$; ***, $P \leq 0.0001$. Error bars represent the SE, and numbers in parentheses represent the number of subjects. OPN expression in both normal and tumor tissue from kidney is shown at one-tenth the actual mean values.

expression in breast cancer tissue were segregated by the pathological classification, and the average values of each group were compared (Fig. 5A). SIBLING mRNA levels were significantly higher in the ductal cancer groups, whereas the levels in the lobular group were intermediate between normal and ductal levels.

A similar analysis was carried out on uterine cancer samples, where there were sufficient numbers to permit segregation into clinically defined subtypes of adenocarcinoma, squamous cell, and benign tumors (Fig. 5B). OPN expression was significantly different between the two subtypes of malignant uterine tumors ($P \leq 0.005$) and between malignant and benign tumors ($P \leq 0.05$). The adenocarcinoma subtype expressed higher levels than the squamous cell subtype.

SIBLING Expression and Tumor Stage. Defined cancer stages represent how large the tumor is and how far it may have spread. The association of SIBLING expression levels with tumor progression was investigated by identifying tumor types with sufficient clinical detail to stratify into different tumor stages. Tumors from colon, rectal, and lung cancer were grouped by stage, and the distribution of SIBLINGs was compared (Fig. 6). In general, cancer stages mark tumors that were either localized and had a relatively small size (stage I), localized and larger in size (stage II), metastasized to lymph nodes (stage III), or metastasized to distant sites (stage IV). Colon cancer tumors exhibited mean values of BSP, OPN, DMP1, and DSPP that increased between stage I and stage III. Colon tumors with distant metastases exhibited SIBLING values with a sim-

Fig. 3 MMP mRNAs are induced in multiple cancer types. Cancer profiling arrays were hybridized with cDNA probes for different MMPs, the amount of hybridized probe was quantified using ImageQuant software, and the mean values of expression of MMP-2 (A), MMP-3 (B), and MMP-9 (C) in normal tissue (□) and tumor tissue (■) were determined for each of nine different cancer types. Asterisks denote the statistical significance as determined by paired *t* tests. *, $P \leq 0.05$; **, $P \leq 0.005$; ***, $P \leq 0.0001$. Error bars represent the SE, and numbers in parentheses represent the number of subjects.



ilar or lower pattern of distribution than that of stage III tumors. Rectal cancer tumors showed increasing BSP, OPN, and DMP1 levels from stage I to stage IV, whereas DSPP values were unchanged across different stages. In lung cancer, BSP, OPN, and DSPP levels increased with increasing stage. When the association of SIBLING expression and tumor stage in colon cancer was analyzed by Spearman rank order correlation analysis, only BSP was significantly correlated (Table 1). In rectal tumors, BSP, OPN, and DMP1 levels correlated with stage, whereas for lung cancer, BSP, OPN, and DSPP levels correlated with stage.

Breast cancer tumors were stratified into tumor-node-metastasis (TNM) stages, which reflect tumor size (T), lymph node involvement (N), and metastatic state (M). Enough breast tumor samples were analyzed to enable the analysis of SIBLING expression and tumor progression. Tumors were grouped by TNM stage, and the stages were ordered in sequence of

increasing progression. The sequence of tumors ranged from those with no nodal involvement or metastasis state (N_0M_0) that increased in size as well as N_1M_0 tumors that increased in size. For BSP, OPN, DMP1, and DSPP, significant differences were observed in the expression pattern as a function of tumor progression (Fig. 7; Table 1). Spearman rank order correlation analysis of SIBLING values and TNM stage yielded significant correlation for all four SIBLINGS.

DISCUSSION

Microarray technology has been typically used to screen the simultaneous expression of many genes using an array spotted with thousands of genes and measuring hybridization of target cDNA generated from a given tissue or cell type. In contrast, the cancer profiling array used in the current study was developed to enable the quantification of expression of a single

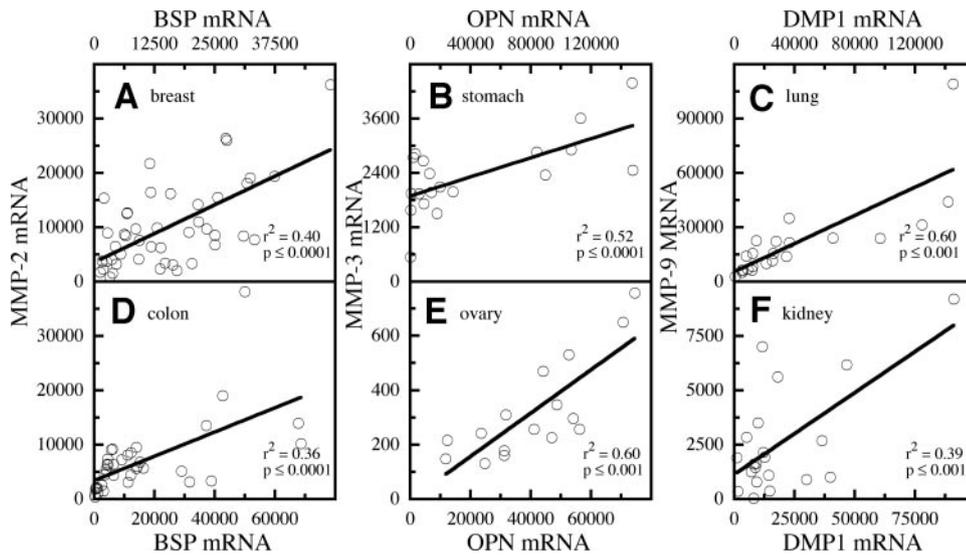


Fig. 4 Paired SIBLING and MMP expression is correlated in specific cancers. The expression levels of SIBLINGs and their respective binding partner MMPs were analyzed by regression analysis. BSP and MMP-2 levels in breast (A) and colon cancer (D), OPN and MMP-3 levels in stomach (B) and ovarian cancer (E), as well as DMP1 and MMP-9 levels in lung (C) and rectal cancer (F) were paired by subject and analyzed by regression analysis.

gene across multiple tissue types and tumor stages. The cancer profiling array contained multiple cDNA pairs from normal and tumor tissues including breast, uterus, colon, stomach, ovary, lung, kidney, rectum, thyroid, prostate, small intestine, pancreas, and cervix. Complementary DNA was generated by an efficient cDNA amplification technique that is based on the switching

mechanism at the 5' end of mRNA templates (17). This methodology has been shown to yield a high representation of mRNA transcripts, avoidance of biased amplification, linearity of signal, and recapitulation of the complexity of the original mRNA (12). Because the expression of individual housekeeping genes varies between normal and tumor tissue (18–20), the

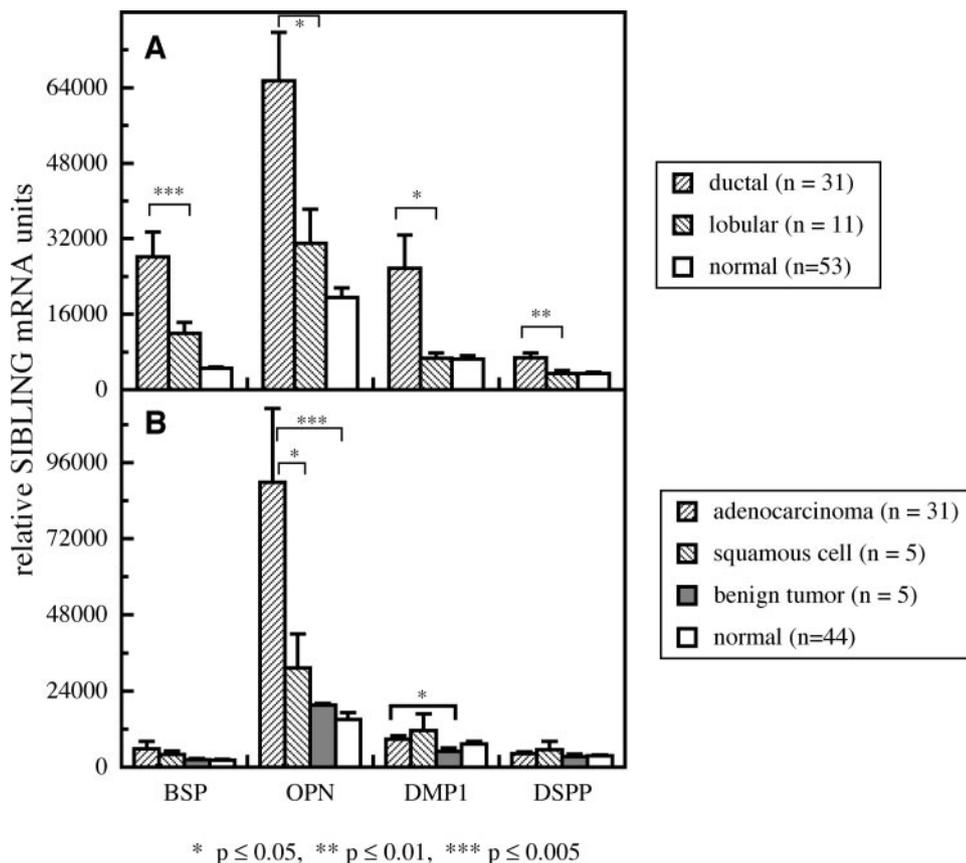
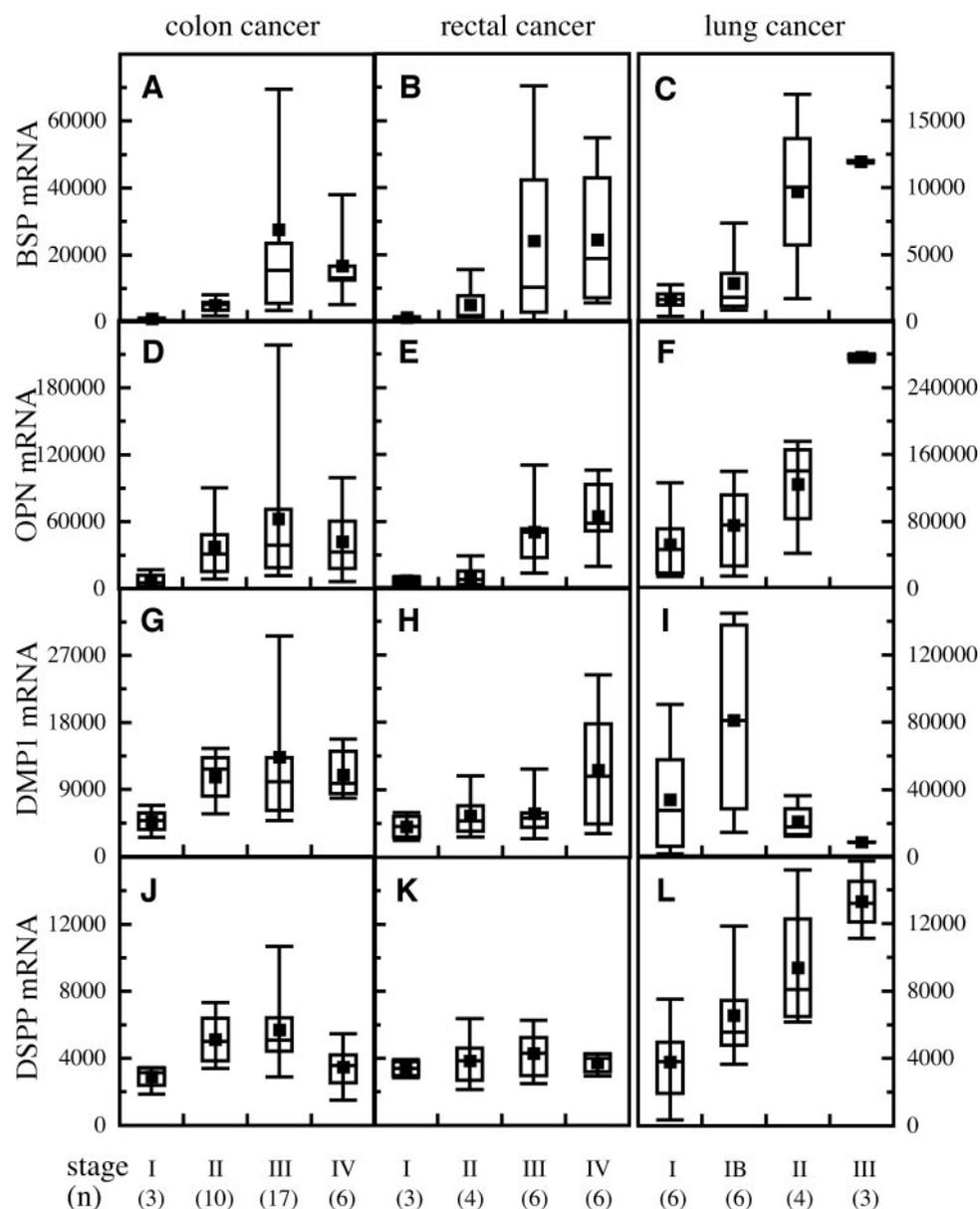


Fig. 5 SIBLING expression distinguishes cancer subtypes for breast and uterine tumors. The expression values of BSP, OPN, DMP1, and DSPP by breast cancer tumors were stratified by pathological classification (ductal versus lobular), and the average values were compared (A). Similarly, the expression of SIBLINGs by uterine tumors stratified into groups defined as adenocarcinoma, squamous cell, or benign tumor were averaged and compared (B). Asterisks denote the statistical significance as determined by *t* test. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$. Error bars represent the SE.

Fig. 6 Comparison of SIBLING mRNA levels with tumor stage in colon, rectal, and lung cancer. The expression values of BSP (A–C), OPN (D–F), DMP1 (G–I), and DSPP (J–L) by colon cancer tumors (A, D, G, and J), rectal cancer tumors (B, E, H, and K), and lung cancer tumors (C, F, I, and L) were stratified by pathological classification (stage), and the average values were compared. *Top line, bottom line, and line through the middle* correspond to 75th percentile, 25th percentile, and 50th percentile (median), respectively. *Error bar whiskers* represent the 10th and 90th percentile, whereas ■ indicates the arithmetic mean. Rectal and colon cancer stages were as follows: I, tumor invaded submucosa; II, tumor invaded through muscularis propria; III, invasive tumor with metastasis in one to three pericolic or perirectal lymph nodes; and IV, invasive tumor with metastasis in pericolic or perirectal lymph nodes and distant metastasis. Lung cancer stages were as follows: I, tumor < 3 cm in greatest dimension; IB, tumor > 3 cm in greatest dimension, involved main bronchus, associated with atelectasis or obstructive pneumonitis; II, metastasis to ipsilateral peribronchial and/or ipsilateral lymph nodes; and III, metastasis to ipsilateral mediastinal, and/or subcarinal lymph nodes. The number of subjects (*n*) for each group is shown at the *bottom*.



equal loading of cDNA onto the array membrane was carried out by normalizing to the average expression of three housekeeping genes: ubiquitin, β -actin, and M_r 23,000 highly basic protein (12, 21). The array has recently been used to profile a number of genes that exhibited either up- or down-regulation in cancer including gelsolin and glutathione peroxidase (12), netrin 1 (22), thiamin transporter THTR2 (23), PAGE 4 (24), and XAGE-1 (25). Strong correlation between tumor tissue expression by the current cDNA microarray and by *in situ* hybridization (24, 25) as well as reverse transcription-PCR and immunohistochemical staining (26, 27) has been observed.

The microarray design pairing normalized cDNA from an individual subject's tumor and normal tissue enabled differences in expression to be analyzed by paired *t* test, which provided a greater power to detect significant differences. Another method

of evaluating the significance of biomarker elevation is to compare target tissue measures to a cut point of the mean of normal levels plus twice the SD ($m + 2$ SD). A value of $>m + 2$ SD translates to a $<5\%$ probability that the elevation is due to chance (95% of normal values will lie within the $m + 2$ SD range). The overall significance of the microarray results was assessed by comparing concordance between these two methods of analysis, as well as comparison with the published results of other studies (Table 2). Elevated BSP expression was identified in two tissues (breast and thyroid), in agreement with previous studies. The current results for BSP did not replicate previous reports on elevated expression in cancer of the uterus or lung. Novel expression was identified in four different cancer types (colon, stomach, rectum, and kidney). Elevated OPN expression was observed in the current study in four different cancer types

Table 1 SIBLING expression and tumor staging

Spearman rank order correlation	BSP	OPN	DMP1	DSPP
Colon cancer*				
Spearman coefficient (r_s)	0.61	0.29	0.26	0.20
P	<0.001	>0.05	>0.05	>0.05
Rectal cancer*				
Spearman coefficient (r_s)	0.61	0.72	0.49	0.28
P	<0.005	<0.001	<0.05	>0.05
Lung cancer*				
Spearman coefficient (r_s)	0.70	0.70	-0.18	0.77
P	<0.001	<0.001	>0.05	<0.0005
Breast cancer†				
Spearman coefficient (r_s)	0.62	0.38	0.37	0.47
P	<0.0005	<0.05	<0.05	<0.005

* Spearman rank order correlation between mean SIBLING values and tumor stage. The Spearman coefficient value (r_s) is an adjusted value (corrected for ties). Tumor stages for colon, rectal, and lung cancer were defined as stated in the Fig. 5 legend.

† Correlation between mean SIBLING values and breast tumor progression. Spearman rank order correlation was performed on breast tumor SIBLING expression levels grouped by TNM stage and ordered across increasing progression ($T_1N_0M_0$, $T_2N_0M_0$, $T_3N_0M_0$, $T_1N_1M_0$, $T_2N_1M_0$, $T_3N_1M_0$). Breast tumor T stages were defined as stated in the Fig. 6 legend.

(breast, colon, ovary, and lung) in agreement with other published studies. For cancer of the stomach, thyroid, and kidney, the OPN expression levels and published literature were not in concordance. Novel expression of OPN in cancer of the uterus

and rectum was identified. Elevated DMP1 expression was confirmed in lung cancer and newly identified in breast cancer. DMP1 levels in cancer of the uterus and colon, although significantly elevated by paired t test, did not satisfy the $>m + 2$

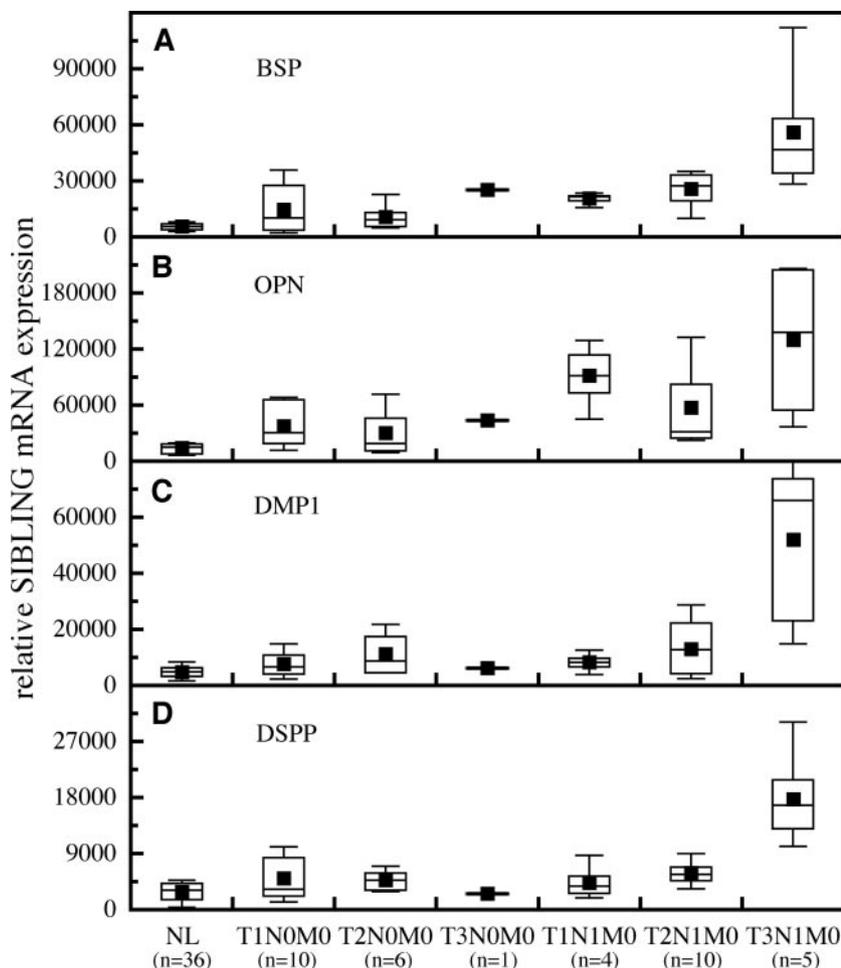


Fig. 7 Comparison of SIBLING mRNA levels and tumor stage in breast cancer. The expression values of BSP (A), OPN (B), DMP1 (C), and DSPP (D) by breast cancer tumors were stratified by increasing TNM stage, and the values were compared. Top line, bottom line, and line through the middle correspond to 75th percentile, 25th percentile, and 50th percentile (median), respectively. Error bar whiskers represent the 10th and 90th percentile, whereas ■ indicates the arithmetic mean. Breast cancer TNM staging was as follows: T_1 , tumor ≤ 2 cm in greatest dimension; T_2 , 2 cm < tumor < 5 cm; T_3 , tumor > 5 cm; N_0 , no regional lymph node metastasis; N_1 , metastasis to movable ipsilateral axillary lymph node(s); N_2 , metastasis to movable ipsilateral axillary lymph node(s) fixed to one another or to other structure; and M_0 , no distant metastasis; and M_1 , distant metastasis. The number of subjects (n) for each group is shown at the bottom. The normal group consisted of the 36 normal breast tissue samples corresponding to the 36 paired tumor tissues with well-defined TNM stage.

Table 2 SIBLING expression in different cancer types

	Breast cancer	Uterine cancer	Colon cancer	Stomach cancer	Ovarian cancer	Lung cancer	Rectal cancer	Thyroid cancer	Kidney cancer
BSP									
<i>t</i> test*	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes
> <i>m</i> + 2 SD†	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Other studies (ref. no.)‡	4 and 28	29				7		8	
OPN									
<i>t</i> test*	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No
> <i>m</i> + 2 SD†	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Other studies (ref. no.)‡	30 and 31		32 and 33	34 and 35	36 and 37	38 and 39		40	2
DMP1									
<i>t</i> test*	Yes	Yes	Yes	No	No	Yes	No	No	No
> <i>m</i> + 2 SD†	Yes	No	No	No	No	Yes	No	No	No
Other studies (ref. no.)‡						9			
DSPP									
<i>t</i> test*	Yes	No	No	No	No	Yes	No	No	No
> <i>m</i> + 2 SD†	No	No	No	No	No	Yes	No	No	No
Other studies (ref. no.)‡									

* Significant elevation defined by a paired *t* test pairing individual subject's normal and tumor tissue expression levels.

† Significant elevation defined by a mean cancer tissue level of expression >*m* + 2SD.

‡ Published studies finding increased expression of SIBLINGs in a given tumor type.

SD criteria. DSPP expression was elevated significantly by both criteria in lung cancer, but only by paired *t* test in breast cancer. Cancers for which the two analysis methods were not in accordance are obvious targets for further, more extensive studies.

The observed increase in MMP-2 expression observed in tumor samples is consistent with previous studies of breast (41, 42), colon (43–47), stomach (48, 49), lung (50–53), rectal (43, 54), and kidney cancer (55–57). Whereas a strong association of increased MMP-3 has been found in breast cancer (41, 58–61), the increased expression levels observed in other tumor types are not as well supported by published literature. Altered MMP-3 levels have been observed in colon (62–64), stomach (65–67), and rectal (68) cancer, although in some cases, the increases were relatively small. In addition, studies have indicated that the MMP-3 source was not necessarily tumor cell but stromal cell or another infiltrating cell type, distinct from the tumor. The observed increases in MMP-9 expression are consistent with published studies of breast (41, 69), uterine (70, 71), colon (46, 53, 72), stomach (73–75), ovarian (76, 77), lung (50, 78), rectal (43, 79), and kidney cancer (56, 80).

A correlation of SIBLING message expression levels with MMP message levels of their partners (BSP with MMP-2, OPN with MMP-3, and DMP1 with MMP-9) was observed. That, in association with the recently described ability of these SIBLINGs to bind to and modulate the activity of specific MMPs, suggests that the same factors that activate SIBLING genes in tumor progression may be the same ones that can activate the corresponding MMP genes. It is also possible that the expression of one SIBLING member in a tumor may induce the production of its corresponding MMP partner, or *vice versa*. Interestingly, SIBLING production by tumors could facilitate angiogenesis because both BSP and OPN have been shown to possess angiogenesis activity *in vivo* (81, 82).

SIBLING expression was different between different subtypes of cancer. Whereas the historical basis for the distinction between the main two types of breast cancer (the belief that ductal carcinomas arose from ducts and lobular carcinomas

from lobules) is subject to debate (both can arise from the terminal duct lobular unit), there is evidence that the two classes as used clinically refer to disease entities that differ in tumor size, shape, dissemination, and proliferation rates (83). The most common hallmark associated with the lobular classification is multifocality. Lobular tumors tend to be more slowly proliferating than ductal tumors. They also tend to frequently exhibit hormone receptor positivity and show distinct chromosomal changes (84, 85). The more rapidly progressing ductal tumors had an associated higher level of SIBLING expression. OPN was recently identified by microarray analysis as a discriminating marker between ductal and lobular cancer (86). In our current study, OPN, as well as BSP, DMP1, and DSPP were significantly different between lobular and ductal tumors. Similarly, the association of higher OPN expression with adenocarcinomas as opposed to squamous cell carcinomas in uterine cancer may be associated with different size, shape, and progression rates.

SIBLING expression correlated with tumor stages associated with changing size and lymph node involvement. These observations are consistent with SIBLING expression coupled with MMP activity modulation having an effect on early tumor progression. These results suggest SIBLINGs as potential markers of early disease progression in a number of different cancers. Future studies of SIBLING expression and serum levels will address the degree to which these tumor biomarkers can be correlated with disease progression.

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