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Breast-specific expression of MGB1/mammaglobin: an examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers

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Previously, we used the reverse transcription-polymerase chain reaction (RT-PCR) to show that mammaglobin (MGB1) can serve as a differential marker of breast cancer metastasis from primary lung cancer. However, mRNA-based methods are not appropriate for use in clinical practices. In this study, we examined MGB1 protein expression in 480 tumors from various organs using immunohistochemical detection and a tissue microarray technique. Breast cancers expressing MGB1 were also analyzed clinicopathologically to determine whether these cancers constitute a characteristic subset. Immunohistochemically, MGB1 was expressed specifically in breast cancers. Of the other cancers examined, including 29 of the head and neck, eight of the thyroid, 106 of the lung, 35 of the gastrointestinal tract, three of the pancreas, 14 of the uterine cervix and 13 of the ovary, none were positive for MGB1 except a proportion of salivary gland tumors (6/11, 55%) and endometrial cancers (3/23, 13%). Among the 238 breast cancers, MGB1 was expressed in 114 (48%), most of which were classified histologically as invasive duct or lobular carcinomas. Clinicopathologically, MGB1 expression was associated with positive expression of estrogen receptors and negative expression of CK5, but not with pathological stage, HER2 gene amplification or p53 immunoreactivity. Kaplan–Meier analysis revealed prolonged disease-free survival in patients with MGB1-positive breast cancers (log rank test, $P=0.016$), but the Cox proportional hazard model failed to confirm that MGB1 was an independent prognostic factor (hazard ratio 1.77, $P=0.1755$). In terms of practical diagnosis, MGB1 immunohistochemistry can serve as a differential marker of breast cancer metastasis from primary lung cancer for two reasons. Firstly, HER2-positive breast cancer frequently lacks estrogen receptor expression, but MGB1 is expressed in about half of this subtype. Secondly, as primary lung adenocarcinomas may express estrogen receptors, MGB1 expression provides further discrimination of the origin of breast cancers.
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The lung is a major target of hematogeneous metastases from a variety of cancers. Thus, a diagnostic procedure for differentiating between primary and metastatic cancers is always required in clinical practice. Accordingly, the differential diagnosis determines the treatment of choice. For example, in the case of a small solitary lung tumor without any lymph node swelling, the patient may be treated with chemotherapy or may undergo partial resection of the lung when the lung tumor is diagnosed as a metastatic breast cancer. On the other hand, standard lobectomy may be the treatment of choice when the diagnosis is of a primary non-small-cell lung cancer.

The mammaglobin gene encodes a 10 kDa molecule,[1–3] which is related to a family of secretory proteins that includes rat prostatic steroid-binding protein subunit C3, human Clara cell 10 kDa protein, and rabbit uteroglobin. Mammaglobin (MGB1) and

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mammaglobin-2 are highly homologous[4] and are expressed specifically in breast tissue.[1–3] Some studies have shown that MGB1 and mammaglobin-2 are useful mRNA markers for detecting lymph node metastasis of breast cancer.[5,6] Using the SAGE database of the NCBI, we independently found that this molecule is a differential marker between primary lung cancer and metastatic cancer from the breast.[7] The RNA-based assay for MGB1 adequately distinguished breast cancer metastasis from primary lung cancer. Recently, an anti-MGB1 antibody, which can be applied to formalin-fixed, paraffin-embedded sections, was made commercially available. Therefore, to evaluate whether conventional immunohistochemical analysis of this molecule on paraffin sections could be used as a marker of breast cancer, we studied primary breast cancers and a wide range of cancers from various organs.

Materials and methods

Patients

We constructed tissue microarray sections of 455 tumors from various tissues (Table 1), a part of which had been used in the other studies reported previously.[8–10] This series included 238 primary breast cancer cases recorded between February 2002 and November 2003 at the Department of Pathology and Molecular Diagnostics, Aichi Cancer Center, Nagoya, Japan. Of these breast cancer patients, 22 were excluded from the prognostic analysis because of noncurative operations (17 patients) or insufficient clinical information (five patients). Pathological staging was determined according to the sixth edition of the AJCC Cancer Staging Manual.[11] In addition to these tumors, 14 endometrial tumors and 11 salivary gland tumors were examined using immunohistochemistry.

Table 1 MGB1 expression in tumors from various organs

Organ	*Histology*	*MGB1*		
		n	*Positive*	*Percentage*
Head and neck	SQ	20	0	0
Thyroid	Papillary carcinoma	8	0	0
Breast	AD	238	114	48
Non-small-cell lung cancer	AD,79; SQ,17; others 10	106	0	0
Esophagus	SQ,8; AD,1	9	0	0
Stomach	AD	19	0	0
Colon	AD	16	0	0
Pancreas	AD	3	0	0
Uterine cervix	SQ,10; AD,4	14	0	0
Endometrium	AD	9	1	11
Ovary	AD,10, adenoma,3	13	0	0
Total		455	115	25

SQ, squamous cell carcinoma; AD, adenocarcinoma.

Breast Cancer Classification

The breast cancers were classified histologically according to the WHO classification scheme.[12] We also applied molecular classification according to Nielsen *et al.*[13] Briefly, the molecular subtypes were defined as follows: (1) when the HER2 gene was amplified, the tumor was considered a HER2-amplified subtype; (2) when the tumor was negative for HER2 gene amplification but positive for ER or PR expression, it was classified as a luminal cell-like subtype; (3) when the tumor was negative for HER2, ER and PR but positive for at least one basal marker (CK5 or EGFR), it was classified as a basal cell-like subtype; (4) when ER, PR, HER2, CK5 and EGFR were all negative, the tumor was classified as a negative subtype.

Immunohistochemistry

Immunohistochemical examination of tissue microarrays and whole sections was conducted according to the standard avidin–biotin–peroxidase complex method. The following antibodies were used: MGB1 (304-1A5, DAKO, Copenhagen, Denmark), ER (6F11, Novocastra, Newcastle, UK), PR (PR88, Biogenex, San Ramon, CA, USA), CK5 (XM26, Novocastra), EGFR (EGFR.25, Novocastra), p53 (DO7, DAKO).

Each dot of the tissue microarray that stained for MGB1 and CK5 was evaluated using the following criteria because the positive reaction was so distinct. A signal of more than moderate intensity was considered positive. The reaction was defined as negative when $<5\%$ of tumor cells were positive, as focal positive when 5–90% of tumor cells were positive, as diffuse positive when more than 90% of tumor cells were positive. EGFR and p53 were evaluated semi-quantitatively because the intensity of these reactions varied from weak to strong and the proportion of cells that displayed a positive reaction varied. Staining intensity was scored from 0 to 3 (0 = none, 1 = weak, 2 = moderate, 3 = strong) and the proportion of positive tumor cells was scored from 0 to 4 (0 = none, 1 = 1–25%, 2 = 25–50%, 3 = 50–75%, 4 = more than 75%). The staining score of each dot was defined as the product of the intensity score and the proportion score. Samples were considered EGFR-positive when the mean EGFR score was 10 or more and p53-positive when the p53 score was 6 or more. ER and PR were evaluated according to Harvey *et al.*[14] HER2 gene amplification was determined by *in situ* hybridization assays according to the manufacturer's instructions (PathVision, Vysis Inc., Downers Grove, IL, USA).

Statistical Analysis

Pearson's χ^2 test and Fisher's exact test for independence were used to compare incidences of MGB1 expression and frequencies of clinicopathological

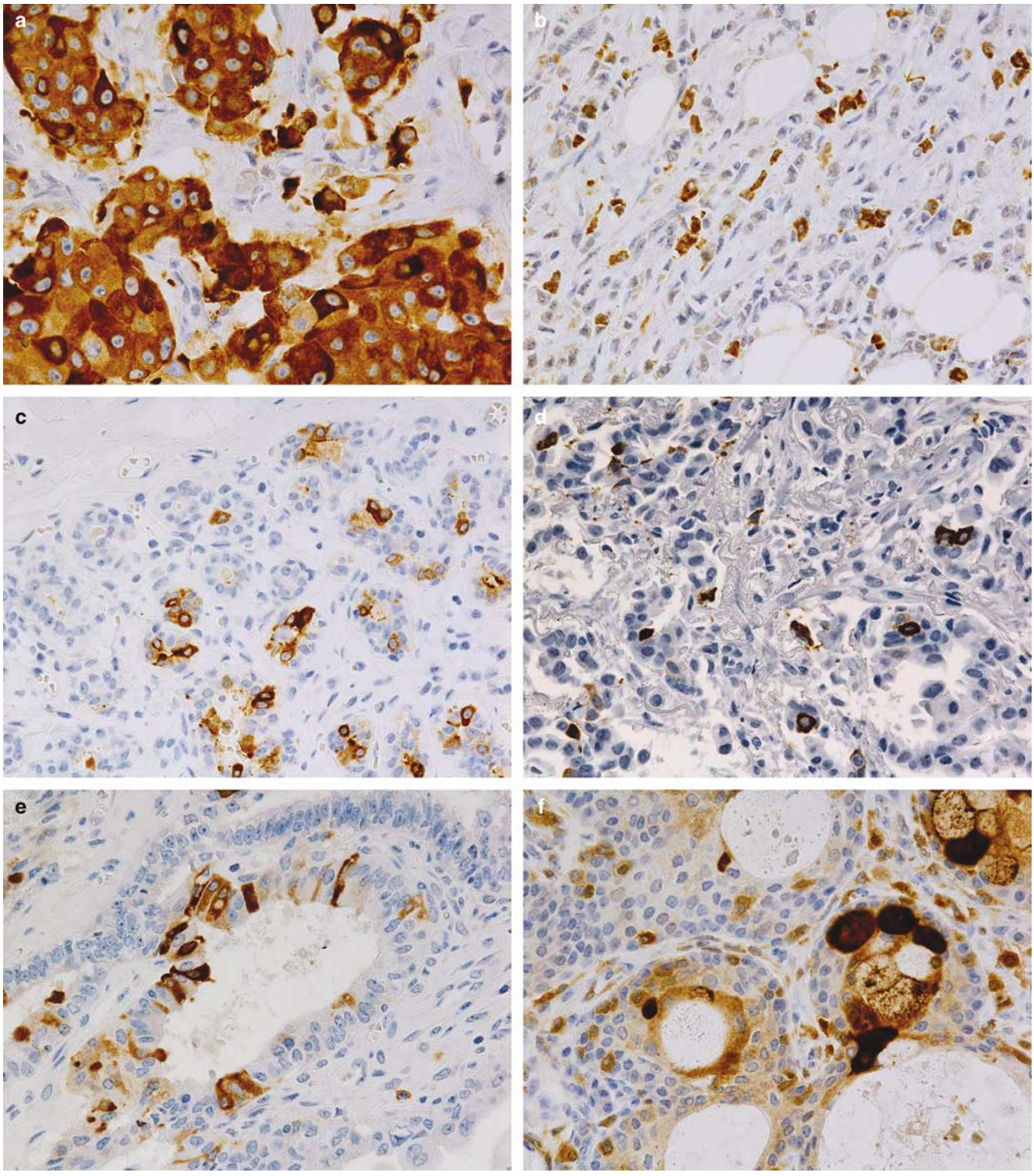

Figure 1 MBG1 expression in cancers and the normal breast. Diffuse intense staining (**a**) was seen in 20% of MGB1-positive cancers, and the remainder showed a scattered staining pattern (**b**) similar to that of normal breast tissue (**c**). The distinct positive staining pattern in sharp contrast to the negative background was obtained from a needle biopsy specimen of a breast cancer that metastasized to the lung (**d**). This staining property facilitates easy interpretation of MBG1 expression. In addition to breast cancers, three of 24 endometrial cancers were positive for MGB1 (**e**). MGB1 expression was also detected in six of 11 salivary gland tumors. A positive mucoepidermoid carcinoma is shown (**f**).

variables. Survival analysis, including Kaplan–Meier survival analysis and the Cox proportional hazard model, were executed using SYSTAT Software (SYSTAT Software Inc., Richmond, CA, USA). A *P*-value of less than 0.05 was considered statistically significant.

Results

MGB1 Expression Specific for Breast Cancers

Among the 455 tumors from various organs, MGB1 expression was detected only in breast cancers, except for a tumor of the endometrium (Table 1). Representative staining of a breast cancer sample for MGB1 is shown in Figure 1. The positive reaction of breast cancers was confirmed with an additional 20 tumors using regular whole tissue sections. The result was consistent with mRNA expression patterns described previously.[7] As endometrial and salivary gland cancers were reported to express MGB1 mRNA,[7,15] 25 additional endometrial and salivary gland tumors were examined. MGB1 was positive in two of 14 endometrial tumors (14%) and in six of 11 salivary gland tumors (55%; three of four pleomorphic adenomas, one of four adenoid cystic carcinomas, one of one mucoepidermoid carcinoma and one of two salivary duct carcinomas). In the two endometrial cancers, expression was focal positive and of weak to moderate intensity (Figure 1). In contrast, all salivary gland tumors excepting a salivary duct carcinoma had intense but focal positive reactions.

MGB1 Expression and Breast Cancer Subtype

As summarized in Table 2, this series contained 11 *in situ* duct carcinomas and 227 invasive carcinomas. Although MGB1 expression was less frequent in the *in situ* duct carcinomas (36%), about half the invasive breast cancers were positive for MGB1. Invasive lobular carcinomas had a high frequency of positive MGB1 reactions, whereas special types of breast cancer such as medullary carcinoma were mostly negative for MGB1. Among the 114 MGB1-positive tumors, staining was diffuse and intense in 23 (20%), whereas the remainder (91 tumors, 80%) exhibited a scattered positive reaction with moderate to strong intensity. The absence of weak intensity and ambiguous background staining made positive reactions very distinct.

We used a molecular classification schema for breast cancers as reported[13,16–19] to class those that were positive for MGB1. Basal cell-like subtypes were significantly less frequent among breast cancers that expressed MGB1 (χ^2 test, $P=0.01$). It is of note that about half the HER2 subtype cancers (26 of 49, 53%) were also positive for MGB1, similar to that of cancers of the luminal cell-like subtype.

Clinicopathological Features of MGB1-Positive Breast Cancer

The clinicopathological features of MGB1-positive and MGB1-negative breast cancers were evaluated (Table 3). MGB1 expression was not associated with disease progression, extension of primary tumors (pTs), nodal metastasis (pN) or histological grade of the tumor, whereas hormone-receptors and MGB1 were frequently positive simultaneously. CK5, a marker of basal cell-like subtypes, was inversely associated with MGB1 expression.

Table 2 MGB1 expression according to breast cancer subtype

	n	*Positive (%)*		*Distribution*	
				Diffuse	*Scattered*
Histological classification					
In situ duct carcinoma	11	4	36	2	2
Invasive carcinoma	227	110	48	21	89
Ductal, NOS	214	105	49	20	85
Lobular	5	4	80	1	3
Mucinous	4	1	25	0	1
Medullary	1	0	0	0	0
Metaplastic	2	0	0	0	0
Small cell	1	0	0	0	0
Molecular classification					
Luminal cell-like subtype	156	81	52	17	64
HER2 subtype	49	26	53	6	20
Basal cell-like subtype	25	5	20	0	5
Negative subtype	8	2	25	0	2

Figure 2 shows the relationship between patient outcome and MGB1 expression. Patients with MGB1-positive tumors survived for a significantly longer disease-free time than those with MGB1-negative tumors ($P=0.02$, log rank test). However, multivariate analysis revealed that pathological stage, ER expression, negative HER2 gene amplification and median age, but not MGB1, significantly and independently affected disease-free survival (Table 4).

Application of MGB1 Immunohistochemistry for Differential Diagnosis between Primary Lung Cancer and Metastatic Cancer from the Breast

Previously, we used the reverse transcriptase-PCR to show that MGB1 can serve as a differential marker of breast cancer metastasis from primary lung cancer. As RNA-based assays are not appropriate for routine clinical use, we used MGB1 immunohistochemistry for differential diagnosis of seven fine-needle biopsies and three surgically resected tissues of metastatic cancers of the breast, which had been confirmed positive by the mRNA-based assay. MGB1 was positive in six of the 10 cancers (Figure 1). Although not all of the metastatic cancers were positive, it is of note that unequivocal positive staining of even part of the tumor can easily result in an interpretation of positive MGB1 expression. The staining pattern in breast cancers contrasted with the completely negative reaction in 106 primary lung cancers.

Discussion

Immunohistological analysis of paraffin sections is useful for confirmation of differential diagnosis

Table 3 Clinicopathological features of breast cancers according to MGB1 expression

	n	*Positive*	*(%)*	P
Age distribution				0.37
>median/≤median	114/124	51/63	45/51	
Pathological stage				0.37
0/I/II/III/IV	11/45/103/60/8	4/25/54/24/3	36/56/52/40/38	
pT				0.25
is/1/2/3/4	11/81/110/16/13	4/43/54/4/5	36/53/49/25/38	
pN				0.08
0/1/2/3	103/67/28/30	52/35/7/15	50/52/25/50	
Histological grade				0.83
Grade 1/2/3	38/126/74	19/58/37	50/46/50	
ER status				0.01
Negative/positive	62/176	21/93	34/53	
PR status				0.05
Negative/positive	110/128	45/69	41/54	
HER2 gene amplification				0.43
Negative/positive	189/49	88/26	47/53	
CK5				0.03
Negative/positive	209/29	106/8	51/28	
p53				0.47
Negative/positive	174/64	86/28	49/44	

Table 4 Multivariate analysis of disease-free survival rate

Favorable	*Unfavorable*	*Hazard ratio*	*95% confidence interval*		
			Lower	*Upper*	P
Pathological stage I or II	Pathological stage III	8.065	3.623	17.857	<0.001
Negative for p53	Positive for p53	1.890	0.801	3.484	0.15
Negative for CK5	Positive for CK5	1.154	0.287	4.646	0.84
Less than or equal to median age	More than median age	2.746	1.239	6.083	0.013
Histological grade 1 or 2	Histological grade 3	1.411	0.491	4.056	0.52
Adjuvant chemotherapy	No adjuvant chemotherapy	2.421	0.257	22.727	0.44
No HER2 gene amplification	HER2 gene amplification	6.317	1.514	26.359	0.014
Positive for estrogen receptor	Negative for estrogen receptor	10.205	2.175	47.874	0.003
Positive for MGB1	Negative for MGB1	1.77	0.775	4.044	0.18

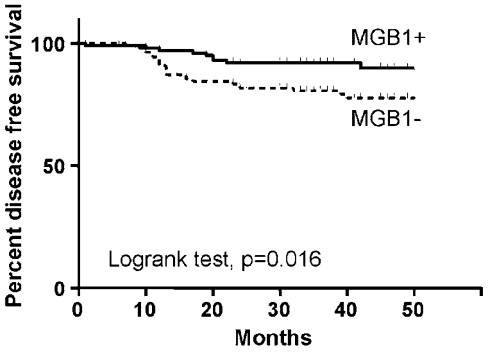

Figure 2 Kaplan–Meier curve for disease-free survival. Patients with MGB1-positive breast cancer had longer disease-free survival than MGB1-negative breast cancer patients.

between primary and metastatic cancers. There are several immunohistochemical markers for differential diagnosis. For example, differential expression of CK7 and CK20,[20] specific expression of TTF-1 in lung and thyroid,[21] and a panel of antibodies for the diagnosis of mesothelioma[22] are commonly used. Although we reported that mRNA expression of MGB1 could be used for differential diagnosis of breast cancer,[7] examination of mRNA expression using clinical material, especially biopsy specimens, is very difficult. Therefore, we examined MGB1 protein expression using an antibody that has recently become commercially available.

In this study, we confirmed that results obtained using MGB1 immunohistochemistry were similar to

those obtained using MGB1 mRNA analysis. With the exception of salivary gland tumors and rare endometrial cancers, MGB1 expression was specific for breast cancer. Notably, our study revealed that only 48% of breast cancers were immunohistochemically positive for MGB1, although mRNA expression was detected in most primary and metastatic breast cancers. The number of cases examined using RT-PCR was much less than that for immunohistochemistry. However, some of the mRNA-positive cancers were negative for MGB1 immunohistochemistry. Differences in sensitivity between the techniques might be the cause of this discrepancy. Alternatively, the staining pattern of MGB1 may be responsible. Of the 114 MGB1-positive breast cancers, 91 showed scattered reaction patterns and the remainder showed uniformly intensive reaction patterns. In contrast, because mRNA analysis does not take patterns of expression into account, a positive result would be recorded even when a very small proportion of the tumor cells expressed the molecule. Indeed, some lobules in normal breast tissue are positive and others are negative.

An MGB1-positive frequency of 50% in primary breast cancers may not be sufficiently high to use as a differential marker. However, the positive reaction is very distinct and is easily observed even with a small number of MGB1-expressing cells. Primary lung adenocarcinomas may be positive for ER,[23–25] but MGB1 expression was not detected in ER-positive lung adenocarcinomas. Therefore, expression of MGB1 in addition to positive ER highly indicates metastasis from breast cancer. ER expression is frequently absent from HER2-positive breast cancers, and thus there is no good marker for the subtype. In this event, MGB1 could be used because 50% of HER2-positive breast cancers are positive for MGB1. These findings indicate that MGB1 can serve as a useful marker for differential diagnosis between primary lung adenocarcinoma and metastatic cancer from the breast.

Kaplan–Meier survival analysis revealed a significant difference between MGB1-positive and MGB1-negative breast cancer. However, the Cox proportional hazard model suggested that the difference was affected by the close correlation of MGB1 with ER expression. Indeed, MGB1 has steroid-responsive elements in its promoter region,[1] and recent analysis based on mRNA expression also revealed an association of MGB1 with ER expression.[26,27]

In conclusion, MGB1 is a highly specific marker of breast cancers, and its expression was detected in about half the breast cancers examined. The expression of MGB1 was correlated with that of ER, but was unaffected by other clinicopathological features, including pathological stage, HER2 gene status and disease-free survival rate. In clinical practice, MGB1 could be used as a marker to distinguish breast cancer metastasis from primary lung adenocarcinoma.

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References

1 Watson MA, Darrow C, Zimonjic DB, *et al.* Structure and transcriptional regulation of the human mammaglobin gene, a breast cancer associated member of the uteroglobin gene family localized to chromosome 11q13. Oncogene 1998;16:817–824.
2 Watson MA, Fleming TP. Isolation of differentially expressed sequence tags from human breast cancer. Cancer Res 1994;54:4598–4602.
3 Watson MA, Fleming TP. Mammaglobin, a mammary-specific member of the uteroglobin gene family, is overexpressed in human breast cancer. Cancer Res 1996;56:860–865.
4 Becker RM, Darrow C, Zimonjic DB, *et al.* Identification of mammaglobin B, a novel member of the uteroglobin gene family. Genomics 1998;54:70–78.
5 Ouellette RJ, Richard D, Maicas E. RT-PCR for mammaglobin genes, MGB1 and MGB2, identifies breast cancer micrometastases in sentinel lymph nodes. Am J Clin Pathol 2004;121:637–643.
6 Ooka M, Sakita I, Fujiwara Y, *et al.* Selection of mRNA markers for detection of lymph node micrometastases in breast cancer patients. Oncol Rep 2000;7:561–566.
7 Koga T, Horio Y, Mitsudomi T, *et al.* Identification of MGB1 as a marker in the differential diagnosis of lung tumors in patients with a history of breast cancer by analysis of publicly available SAGE data. J Mol Diagn 2004;6:90–95.
8 Yatabe Y, Koga T, Mitsudomi T, *et al.* CK20 expression, CDX2 expression, K-ras mutation, and goblet cell morphology in a subset of lung adenocarcinomas. J Pathol 2004;203:645–652.
9 Yatabe Y. Role of expression of Thyroid transcription factor-1 in pulmonary adenocarcinoma. In: Hayat MA (ed). Immunohistochemistry and In Situ Hybridization of Human Carcinomas. Elsevier Science/Academic Press: New York, NY, USA, 2004, pp 169–179.
10 Yatabe Y, Mitsudomi T, Takahashi T. Maspin expression in normal lung and non-small-cell lung cancers: cellular property-associated expression under the control of promoter DNA methylation. Oncogene 2004;23:4041–4049.
11 Greene FL, Page DL, Fleming ID, *et al* (eds). AJCC Cancer Staging Manual, 6th edn. Springer-Verlag: New York, 2002.
12 Tavassoli FA, Devilee P, (eds). Pathology and Genetics of Tumors of the Breast and Female Genital Organs. IARC Press: Lyon.
13 Nielsen TO, Hsu FD, Jensen K, *et al.* Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004;10:5367–5374.
14 Harvey JM, Clark GM, Osborne CK, *et al.* Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999;17:1474–1481.

15 Grunewald K, Haun M, Fiegl M, *et al.* Mammaglobin expression in gynecologic malignancies and malignant effusions detected by nested reverse transcriptase-polymerase chain reaction. Lab Invest 2002;82: 1147–1153.
16 Rouzier R, Perou CM, Symmans WF, *et al.* Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005;11:5678–5685.
17 Perou CM, Sorlie T, Eisen MB, *et al.* Molecular portraits of human breast tumours. Nature 2000;406: 747–752.
18 Sotiriou C, Neo SY, McShane LM, *et al.* Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci USA 2003;100:10393–10398.
19 Wang ZC, Lin M, Wei LJ, *et al.* Loss of heterozygosity and its correlation with expression profiles in subclasses of invasive breast cancers. Cancer Res 2004;64:64–71.
20 Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol 2000;13:962–972.
21 Ordonez NG. Thyroid transcription factor-1 is a marker of lung and thyroid carcinomas. Adv Anat Pathol 2000;7:123–127.
22 Ordonez NG. Immunohistochemical diagnosis of epithelioid mesothelioma: an update. Arch Pathol Lab Med 2005;129:1407–1414.
23 Ollayos CW, Riordan GP, Rushin JM. Estrogen receptor detection in paraffin sections of adenocarcinoma of the colon, pancreas, and lung. Arch Pathol Lab Med 1994;118:630–632.
24 Lau SK, Chu PG, Weiss LM. Immunohistochemical expression of estrogen receptor in pulmonary adenocarcinoma. Appl Immunohistochem Mol Morphol 2006;14:83–87.
25 Wu CT, Chang YL, Shih JY, *et al.* The significance of estrogen receptor beta in 301 surgically treated non-small cell lung cancers. J Thorac Cardiovasc Surg 2005;130:979–986.
26 Guan XF, Hamedani MK, Adeyinka A, *et al.* Relationship between mammaglobin expression and estrogen receptor status in breast tumors. Endocrine 2003;21:245–250.
27 Span PN, Waanders E, Manders P, *et al.* Mammaglobin is associated with low-grade, steroid receptor-positive breast tumors from postmenopausal patients, and has independent prognostic value for relapse-free survival time. J Clin Oncol 2004;22:691–698.