Table 4. Molecular markers frequently used in breast cancer.

Molecular markers	Comments
Carcinoembryonic antigen (CEA)	Glycoprotein molecule with an oncofetal expression pattern
Cytokeratin 19	An epithelial marker for detection of cancer cells in mesenchymal organs by RT-PCR, as the type of cytoplasmic intermediate filaments primarily exist in epithelial cells and cancer cell derived from epithelia, but is normally not expressed in cells of non-epithelial origin
MUC1	Mucins are the major protein constituents of the intestinal mucous gel which coats and protects epithelial surface. Among several different mucins, MUC1, a membrane-bound form, is a core protein of polymorphic epithelial mucin, and it is uniformly and highly expressed in epithelial tumors including breast cancer, and rarely expressed in nonepithelial tissues
Maspin	Homologous to the serine superfamily of protease
Mammaglobin (MGB/MGB1)	A mammary specific member of the uteroglobin gene family, and encode a glycoproteins. The human MGB gene is localized on 11q12.3-13.1. In non-malignant tissues, expression is strictly limited to the mammary epithelium, and is highly expressed in primary breast cancer cells
Mammaglobin B (MGB2)	A recently cloned member of uteroglobin gene family, and highly homologous to mammaglobin

Table 5. Representative studies of micrometastases in lymph nodes.

Author, Year	Targets	Positive rates in breast cancer patients		Positive rates in LNs from controls	Remarks
		Histologically positive LNs	Histologically negative LNs		
Noguchi, 1994 [204]	MUC1	100% (9)	14.6% (41)	0% (10)	Sensitive
Schoenfeld, 1994 [205]	CK19	100% (18)	10% (39)	0% (11)	By ethidium brimide staining
	CK19	-	28% (39)	0% (11)	By Southern blotting and hybridization
Mori, 1995 [206]	CEA	100% (6)	0% (5)	0% (5)	Included various types of cancer
Hoon, 1996 [207]	βhCG	90% (10)	25% (8)	0% (8)	Sensitive and specific
Noguchi, 1996 [208]	CK19	90% (20*)	14 .2% (106*)	-	Useful to detect micrometastasis
Noguchi, 1996 [209]	CK19	100% (10)	9% (53)	0% (10)	Useful to detect micrometastasis
	MUC1	100% (10)	6% (53)	0% (10)	Useful to detect micrometastasis
Schoenfeld , 1996 [210]	CK19	-	20% (530)	0% (28)	Improve detection of micrometastasis
Lockett, 1997 [211]	Multimarker [1]	94% (16)	48% (29)	-	Useful
Bostick, 1998 [212]	CEA	70%(10)	42% (12)	100% (3)	No diagnostic value
	CK19	80% (10)	67% (12)	100% (3)	No diagnostic value
	CK20	20% (10)	8% (12)	0% (3)	Low sensitivity, high specificity
	GA733.2	70% (10)	92% (12)	100% (3)	No diagnostic value
	MUC1	70% (10)	83% (12)	100% (3)	No diagnostic value

Table 5. Continued

Gerhardt, 1998 [213]	HER2[2)]	83.3% (6)	-	-	
Lockett, 1998 [214]	Multimarker[3)]	-	40% (35)	-	Useful
Lockett, 1998 [215]	Multimarker[3)]	92% (24*)	40% (37*)	0% (9*)	Useful
Aihara, 1999 [216]	MGB2	100% (11)	31% (45)	0% (15)	Useful to detect micrometastasis
Merrie, 1999 [217]	CK8	81% (313)	-	96% (54)	No diagnostic value
	CK16	23% (313)	-	18% (146)	No diagnostic value
	CK19	71% (313)	-	67% (61)	No diagnostic value
	Maspin	55% (90)	-	48% (113)	No diagnostic value
	MUC1	18% (159)	-	49% (90)	No diagnostic value
	ST3	70% (342)	-	64% (64)	No diagnostic value
Watson, 1999 [218]	CK19	100% (11*)	100% (3*)	-	Less specific
	MGB1	91% (11*)	0% (3*)	-	Promising than CK19
Leygue, 1999 [219]	MGB1	100% (13)	0% (7)	-	Correlated with H &E staining
Masuda, 2000 [220]	CEA	-	31.0% (129*)	-	Correlated with prognosis
Kataoka, 2000 [221]	CEA	100% (17)	25% (48)	0% (30)	Improve detection of micrometastasis
	MGB1	70.6% (17)	20.8% (48)	0% (30)	Improve detection of micrometastasis
Ooka, 2000 [222]	CEA	35.7% (14)	1.2% (163)	0 (16)	Less sensitive
	CK20	-	-	0% (16)	Not suitable because of low expression in tumor
	MAGE1	-	-	0% (16)	Not suitable because of low expression in tumor

Table 5. Continued

Ooka, 2000 [222]	CEA	35.7% (14)	1.2% (163)	0 (16)	Less sensitive
	CK20	-	-	0% (16)	Not suitable because of low expression in tumor
	MAGE1	-	-	0% (16)	Not suitable because of low expression in tumor
	MAGE3	-	-	0% (16)	Not suitable because of low expression in tumor
	MGB1	100% (14)	30.1% (163)	0% (16)	Specific
	MGB2	100% (14)	17.8% (163)	0% (16)	Specific
	PSA	57.1% (14)	43.7% (163)	0% (16)	Less sensitive
Mitas, 2001 [223]	Multimarker[4)]	-	38% (21)		Applicable as a panel
Branagan, 2002 [224]	MGB1	17.2% (29)	8% (119)	-	
Mitas, 2002 [225]	CEA	-	4.5% (22)	-	
	PIP	-	27.3% (22)	-	
	PSE	82% (22)	14% (22)	2% (51)	Informative

Abbreviations: CEA, carcinoembryonic antigen; CK, cytokeratin; GA733.2, gastrointestinal tumor-associated antigen-733.2; βhCG, β human dhorionic gonadotropin; LN, lymph node; MGB1, mammaglobin; MGB2, mammaglobin B; PIP, prolactin inducible protein; PSA, prostate-specific antigen; PSE, prostate-specific Ets; ST3, stromelysin 3.

() Number of LNs.

1) Combination of 4 genes including CK19, c-myc, HER2, PIP.

2) Alternatively spliced HER2 was used as target.

3) Combination of CK19, c-myc, PIP.

4) Combination of MGB1, MGB2, CK19, MUC1, CEA, HER2, PIP.

* Number of patients.

Table 6. Representative studies of micrometastases in bone marrow.

Author, Year	Targets	Positive rates in breast cancer		Positive rates in controls	Remarks
		Primary, NMD	Metastatic disease		
Datta, 1994 [250]	CK19	-	75% (8)	6.7% (30)	Sensitive and specific
Gerhard, 1994 [251]	CEA	66.7% (6)*		0% (56)	
Brown, 1995 [252]	DF3	11.1% (9)	-	0% (4)	
	CK18	-	-	100% (4)	Not usuful
Fields, 1996 [253]	CK19	55% (33)	82% (50)	3.7% (214)	Sensitive and associated with prognosis
Krüger, 1996 [254]	CK19	35% (20)	75% (4)	0% (5)	Sensitive and specific
Luppi, 1996 [255]	Maspin	-	Pos**	0% (4)	Sensitive and specific
Moscinski, 1996 [256]	CK19	13.3% (30)	73% (30***)	3.8% (52)	Highly sensitive and specific
Schoenfeld, 1997 [257]	CK19	35% (65)	80% (5***)	-	Improve detection of micrometastasis
Wulf, 1997 [258]	PTHrP	26.5% (34)	-	12% (25)	Less specific
Zippelius, 1997 [259]	CEA	-	-	26.3%	
	CK18	-	-	71.4%	Not useful
	DPI I	-	-	100% (5)	Not useful
	EGP-40	-	-	100% (53)	Not useful
	HER2	-	-	71.4% (7)	Not useful
	HER3	-	-	85.7% (7)	Not useful
	PSA	0% (10)	-	0% (53)	
	PSM	-	-	44.4% (9)	Not useful
Gerhardt, 1998 [213]	HER2[11]	-	100% (6)	-	
Vannucchi, 1998 [260]	CK19	48% (33)	-	13.9% (43)	Correlated with positivity in PBST
Shammas, 1999 [261]	CK19	25% (12)	-	-	Sensitive, but overlapping

Table 6. Continued

Slade, 1999 [262]	CK19	83% (23)		-	60% (30)	Highly sensitive, more likely to be positive in BM
Zhong, 1999 [263]	CEA	27.6% (181)		-	0% (8)	Limitation due to heterogeneity
Zhong, 1999 [264]	GA733.2	100% (33)		-	100% (8)	Not specific
Zhong, 1999 [265]	CK19	40.9% (115)		-	0% (8)	
Berois, 2000 [266]	CEA	29% (42)		-	-	More sensitive in BM than in PB
	CK19	48% (42)		-	-	More sensitive in BM than in PB
Ikeda, 2000 [267]	CK19	34.2% (117)		-	-	Prognostic value
Shaw, 2000 [193]	CK19	56% (32)		-	-	
Zhong, 2000 [268]	CK19[2)]	69.2% (26)		-	0% (8)	Sensitive and specific
Ooka, 2001 [269]	MGB	29.7% (111)		-	0%	Correlated with prognosis
Shammas, 2001 [270]	CK19	20.6% (141)		-	4% (48)	Serial sampling may be useful
Silva, 2001 [271]	CK20		100% (1)*		100% (2)	Not reliable
Silva, 2002 [272]	MGB	40% (5)		86% (7)	0% (15)	Sensitive and specific
Stathopoulou, 2002 [273]	CK19	63% (27)		74% (46)	-	

Abbreviations: CEA, carcinoembryonic antigen; CK, cytokeratin; DF3, human breast carcinoma-assocaited antigen; DPI, desmoplakin I; EGP, epithelial glycoprotein; GA733.2, gastrointestinal tumor-associated antigen-733.2; MGB, mammaglobin; NMD, non metastatic disease; PBST, peripheral blood stem cell; PSA, prostate-specific antigen; PSM, prostate-specific membrane antigen; PTHrP, parathyroid hormone related protein.

() Number of patients.

1) Alternatively spliced HER2 was used as target.

2) Cells were separated using immunomagnetic beads.

* Breast cancer, NOS, ** Positive in 3 BM, NOS, *** Patients with bone marrow metastasis.

Table 7. Representative studies of circulating tumor cells in peripheral blood.

Author, Year	Targets	Sample source	Positive rates in breast cancer		Positive rates	Remarks
		Patients : Controls	Primary, NMD	Metastatic disease	in controls	
Datta, 1994 [250]	CK19	B, PBSC : B	0% (8)	21.1% (19)	2.5% (39)	Sensitive and specific
Hoon, 1996 [207]	βhCG	B : B	80% (10)**		0% (28)	
Krüger, 1996 [254]	CK19	PBSC : B	37.5% (16)	60% (10)	0% (10)	Sensitive and specific
Krüger, 1996 [289]	CK19	PBSC : -	40% (10*)	-	-	Mobilized by GCSF
Lehrer, 1996 [290]	PSA	B : B	23.1% (78)	-	0% (42)	Useful
Luppi, 1996 [255]	CK19	B : B	33.3% (9)	25.0% (20)	29.4% (17)	
	Maspin	B : B	0% (9)	20% (20)	0% (17)	Sensitive and specific
Mori, 1996 [291]	CEA	B : B	25% (8)	100% (1)	0% (22)	Useful
Mapara, 1997 [292]	EGFR	PBSC : B	-	58.8% (17*)	-	Better than CK19
Schoenfeld, 1997 [257]	CK19	B : B	25% (75)	80% (5)	-	Improve detection of micrometastasis
Traystman, 1997 [293]	CK19	B : B	25% (20)	63.6% (11)	0% (22)	Sensitive
Wulf, 1997 [258]	PTHrP	B : B	30% (30)	-	0% (30)	Very sensitive
Bostick, 1998 [212]	CEA	B : B	NA	NA	46% (13)	High false positive
	CK19	- : B	-	-	77% (13)	High false positive
	CK20	B : B	-	-	0% (13)	
	GA733.2	B : B	-	-	54% (13)	High false positive
	MUC1	- : B	-	-	46% (13)	High false positive
Leitzel, 1998 [294]	EGFR	B : B	0% (13)	22% (18)	0% (23)	Sensitive and specific
Luke, 1998 [295]	CK19	B-IM : B-IM	-	67% (15)	0% (39)	More sensitive than GCDFP
	GCDFP	B-IM : B-IM	-	27% (15)	13% (39)	Less sensitive than CK19
Vannucchi, 1998 [260]	CK19	PBSC : PBSC	57.5% (33)	-	3.3% (30)	Mobilized by GCSF

Table 7. Continued

Lambrechts, 1999 [296]	CK19	B : B	-	94.3% (35)	95% (20)	Not suitable
Shammas, 1999 [261]	CK19	B : -	-	25% (12)	-	Sensitive, but overlapping
Slade,1999 [262]	CK19	B : B	70% (23)	76% (37)	51% (45)	Highly sensitive
Soria, 1999 [297]	Telomerase	B-IM : B-IM	-	84% (25)	0% (9)	Useful
Watson, 1999 [218]	MGB	PBSC : -	-	60% (15)	-	
Zach, 1999 [298]	MGB	B : B	28% (18)	49% (43)	0% (27)	Useful
Zhong, 1999 [263]	CEA	- : B	-	-	0% (96)	
Zhong, 1999 [264]	GA733.2	- : B	-	-	40% (40)	Not specific
Zhong, 1999 [265]	CK19	PBSC : B	7.7% (13)	37.5% (16)	0% (96)	
Berois, 2000 [266]	CEA	B : B	3% (37)	-	0% (20)	More sensitive in BM than in PB
	CK19	B : B	35% (37)	-	0% (20)	More sensitive in BM than in PB
	MUC1	- : B	-	-	60% (20)	High false positive
de Cremoux, 2000 [299]	MUC1	B-IM : B-IM	43.3% (97)	52.2% (23)	11% (28)	Sensitive and specific
Grünewald, 2000 [300]	CK19	B : B	48% (133)	-	39% (31)	Not specific
	EGFR	B : B	10% (133)	-	25% (31)	
	MGB	B : B	8% (133)	-	0% (31)	Specific marker for spread of tumor
Ikeda, 2000 [267]	CK19	- : B	-	-	0% (15)	
Kahn, 2000 [301]	CK19	B : B	34.6% (81)	71.4% (28)	0 (45)	Correlated with prognosis
Sabbatini, 2000 [302]	Maspin	B : B	4.8% (21)	0% (8)	-	Mobilized by chemotherapy
Shaw, 2000 [193]	CK19	B : -	-	67% (18)	-	Sensitive
Smith, 2000 [303]	CK19	B : -	-	49.6% (145*)	-	Correlated with response
Zhong, 2000 [268]	CK19	- : B	-	-	0% (117)	
Aerts, 2001 [304]	CK19	B : B	31.6% (19)	71.4% (14)	8.7% (23)	
An, 2001 [305]	CEA	B : B	38% (32)	-	0% (11)	Correlated with prognosis

Table 7. Continued

Houghton, 2001 [306]	MGB	B : B	-	62.5% (32)	0% (11)	Microarray study
	Multimarker[1]	B-IM : B-IM	-	84.4% (32)	0% (11)	
Hu, 2001 [307]	βhCG	B : B	12.4% (72)**		0% (30)	Correlated with stage
	CK19	B : B	9.7% (72) **		10% (30)	
	CK20	B : B	2.8% (72) **		0% (30)	Not useful
Matin, 2001 [308]	Cluster[2]	B : B	77% (13)	-	19% (15)	
Ooka, 2001 [269]	MGB	- : B	-	-	0%	
Silva, 2001 [271]	CK20	B : B	100% (2)***		74% (31)	Not reliable
		PBSC : PBSC	100% (2)***		80% (5)	Not reliable
Shammas, 2001 [270]	CK19	B : B	1.3% (74)	-	4% (48)	Not useful
Silva, 2002 [272]	MGB	B : B	22% (65)	38% (13)	0% (47)	Sensitive and specific
Stathopoulou, 2002 [273]	CK19	B : B	29.7% (148)	52% (46)	3.7% (54)	Correlated with prognosis

Abbreviations: B, Blood; B-IM, immunomagnetic separation of cancer cells from blood; CEA, carcinoembryonic antigen; CK, cytokeratin; EGFR, epidermal growth factor receptor; GA733.2, gastrointestinal tumor-associated antigen-733.2; GCDFP, gross cystic disease fluid protein; βhCG, β human chorionic gonadotropin; LOH, loss of heterozygosity; MGB, mammaglobin; MI, microsatellite instability; NMD, non metastatic disease; PBSC, peripheral blood stem cell; PSA, prostate-specific antigen ; PTHrP, parathyroid hormone related protein.

() Number of patients.

1) Combination of MGB1, B305D, B311D, B533S, B726P, GABAπ.

2) A group of SRP19, CD44, TRP-2-8b, Maspin, HSIX1, Gro α, Myosin light chain, MDM2, ZZ38, β–tubulin, N33, Laminin α3.

* Number of patients or samples, ** Metastatic disease was included, *** Breast cancer, NOS.

Table 8. Representative studies of disseminated tumor cells in pleural effusion, cerebrospinal fluid.

Author, Year	Targets	Positive rates in breast cancer according to cytological diagnosis			Positive rates in control	Remarks
		Positive	Negative	Suspected		
Cerebrospinal fluid						
Datta, 1994 [250]	CK19	100% (1)	-	-	0% (2)	Detectable
Pleural effusion						
de Matos Granja, 2002 [353]						
	LOH	38% (24)*	0% (3)*	36% (11)*	-	Useful

Abbreviations: CK, cytokeratin; LOH, loss of heterozygosity.

() No of patients.

* Percentage of positive cases for at least one locus.

effusion of patients with breast cancer [250, 353-357], or axillary drainage fluid after surgery [358-361]. The representative studies at the dawn of a new golden age of molecular detection and characterization of micrometastasis, DTCs and CTCs in breast cancer are listed in table 5-8.

LN metastasis is usually determined by histologic analysis of one or a few hematoxylin and eosin sections from each LN, and axillary LN status is the most powerful predictor of patient outcome. However, patients without axillary LN metastasis are not completely devoid of risk for relapse. Therefore, considerable efforts have been made to find prognostic markers such as HER2, p53 and cathepsin D in breast cancer tissue, although their clinical significance is less conclusive because of limited and conflicting data. Another concern is false negative pathological findings. In this respect, a study of particular interest indicated that the serial sectioning technique revealed micrometastases in 9% of breast cancer patients who were diagnosed as node-negative by routine histological examination, and these patients had a poor disease-free and overall survival [362]. In agreement with this study, several studies have shown that the serial sectioning technique with or without IHC can detect micrometastases [363-366]. Thus, routine histological examination may underestimate the true incidence of metastasis. Unfortunately, the serial section technique is not practical as a routine method, as it is cumbersome and time-consuming. On the other hand, RT-PCR based methods are highly sensitive, require relatively less time to perform and are cost effective [214, 367]. So far, several studies have demonstrated that micrometastases could be detected at a significant frequency in histologically negative LNs, and it is interesting to note that micrometastases in LNs detected by RT-PCR for CEA were associated with reduction of both disease-free survival and overall survival [220].

Recent studies have shown that the sentinel lymph node (SLN), which is the first drainage LN from the site of the breast cancer, can be used to predict nodal status [368-370]. The fundamental concept underlying sentinel node mapping is that the lymphatic effluent of a tumor drains initially to a sentinel node (or to a few SLNs) before other nodes in the group receive tumoral drainage. SLN biopsy may allow patients with breast cancer to avoid the morbidity of formal axillary clearance [370, 371]. For intraoperative assessment, the recent European Working Group for Breast Screening Pathology guidelines advocate multilevel assessment of grossly or intraoperatively negative sentinel nodes with levels separated by a maximum of 1 mm [372]. In this respect, several reports have suggested that RT-PCR based methods may be useful for more accurate diagnosis of metastasis in SLN [221, 222, 224, 234, 237, 241, 244, 246-248, 373]. For perioperative

diagnosis, the rapid molecular test assay appears to be a favorable method for analysis [367, 374, 375].

Detection of BM micrometastases may have a role in monitoring treatment, predicting prognosis, and understanding tumor biology. At the time the primary breast cancer is diagnosed, metastatic bone lesions are usually beyond detection by conventional methods such as radiography, or skeletal scintigraphy. Furthermore, conventional histological methods could find cancer cells in only a few cases. However, the addition of immunohistological methods has led to improvements in the ability to detect breast cancer cells in BM aspirates or biopsy samples. Importantly, immunologic detection of epithelial cells in the BM correlated with a significantly shorter disease-free survival in breast cancer [177, 376-380]. Similarly, BM micrometastases detected by RT-PCR for mammaglobin (MGB) were correlated with early distant recurrence of breast cancer [269]. Moreover, one study using RT-PCR for cytokeratin (CK) 19 indicated that the presence of BM micrometastases was associated with a high risk of relapse in patients with proven metastatic breast cancer undergoing BM transplantation [253]. The detection of occult carcinoma in BM or PB stem cell (PBSC) collections may be of special concern in the setting of autologous BM transplantation for breast cancer patients, as high dose chemotherapy or granulocyte-colony stimulating factor may mobilize cancer cells into circulation [260, 381]. The possibility of contaminated PBSC collections was significantly higher in patients with CK19 positivity in BM at diagnosis, and there was a trend towards longer relapse free survival in patients transplanted with CK19-negative PBSC collections as compared with the others [260]. Similarly, conventional-dose chemotherapy caused cancer cell mobilization into the circulation, and the presence of circulating maspin positive cells was associated with disease progression [302].

On the other hand, obtaining serial blood samples is much easier and less invasive than the process involved in obtaining BM aspirates or biopsy samples. Currently available IHC methods are not sensitive enough to detect low numbers of CTCs in PB [257], but the introduction of RT-PCR techniques have opened up the way to a more sensitive and meaningful analysis of CTCs in PB [250, 291, 382]. Despite the relatively recent introduction of this methodology, several studies have demonstrated preliminary but promising data. For instance, in patients with gastrointestinal or breast cancer, the detection rates for CEA mRNA in PB increased with advancing stage of disease; moreover, in patients who underwent curative surgery, those with positive for CEA mRNA showed higher relapse rates than those who were negative [383]. Furthermore, another study found a significant association between positivity for CK19 mRNA and the presence

of distant metastasis [301]. In this study, increased intensity of CK19 RT-PCR signal, presumably indicating higher numbers of CTCs, was associated with reduced survival. In addition, the detection of CK19 mRNA positive cells in the PB of patients with stage I and II breast cancer before the initiation of any adjuvant treatment was an independent prognostic factor for disease relapse and death [273]. In patients with progressive, locally advanced, and metastatic breast cancer, circulating CK19 positive cells detected by quantitative RT-PCR reflected the changes in disease load [303]. In this study, the trend over a period of months was particularly important. A study using DF3 as molecular marker demonstrated that operative manipulation enhanced cancer cell dissemination [252], indicating that cancer surgery will result in increased shedding of cancer cells into the circulation. Moreover, when CTCs from PB were analyzed before and during therapy for EpCAM, MUC1 and HER2 transcripts, molecular profiling of CTCs could offer superior prognostic information and predictive judgement of therapeutical regimens in the study of Tewes et al [384]. Thus, molecular characterization of CTCs might contribute to improving targeted and more individualized cancer therapies. In this field, important progress has also arisen from the development of an automated enrichment and immunocytochemical detection system for CTCs [177, 385].

In general, however, the metastatic process is grossly inefficient, and the presence of cancer cells in the blood circulation does not necessarily indicate the subsequent appearance of systemic disease [176, 386]. Moreover, it is generally believed that the majority of cancer cells that shed into the blood circulation, are cleared and do not survive in the peripheral circulation, only a very few cancer cells succeeding in establishing secondary tumors [176, 387, 388]. Therefore, there seems to be dissociation between molecular positivity and true metastatic disease, and a more detailed molecular study might give information about the metastatic potential as well as the natural history of CTCs in PB.

3. Sensitivity, specificity, and problems in assay

Several factors may affect the sensitivity and specificity of PCR-based methods. For example, methods of an endpoint analysis to evaluate PCR and RT-PCR products may affect the sensitivity. To date, the majority of studies have used ethidium bromide (EtBr) staining as an endpoint analysis to evaluate these products. However, Southern blot analysis increases the sensitivity compared with EtBr staining [205, 212]. In addition, immunomagnetic bead technology may provide a sensitive and specific means for identifying very small numbers of breast cancer cells in PB [295, 299].

More importantly, as PCR-based methods are up to 100 times more sensitive than conventional methods in detecting micrometastases, this extreme sensitivity confers an inherent disadvantage of producing false positive results [389]. An important cause of false positive results is contamination by carry-over products from the previous PCR amplifications, or contamination by cancer cells or normal epithelial cells. When using epithelial markers, the first blood sample should be discarded to avoid epithelial contamination. For BM aspiration, the skin should be incised before the aspirates are taken. Moreover, the existence of a processed pseudogene or illegitimate transcription of CKs in hematopoietic cells may cause false positive results [259, 390]. Importantly, even a small number of copies of mRNA transcripts presents a problem, not only for CK but for any targeted gene that is expressed at low levels in noncancer cells. One approach to overcome this problem is to develop a quantitative method in which a competitive sequence is used in PCR titration assay, and to introduce an appropriate cut-off value [262, 267, 304]. When using epithelial markers for molecular detection, it must be remembered that the presence of amplified products of these markers is not specific for breast cancer, as other epithelial neoplasms may also produce a positive results. Even benign breast epithelial cells could be mobilized during breast surgery for benign disease [391]. In contrast, false negative results may occur because of the deficient expression of the marker gene in micrometastatic cancer cells. For example, when using CK19, RT-PCR may fail to detect cancer cells that do not express CK19 transcript but rather express one or more of the other CK genes such as CK7, CK8 or CK18 [392]. Therefore, breast cancer specific markers seem to be crucially important for detection of micrometastasis. Given the lack of such breast cancer specific markers, tissue specific markers, including maspin and MGB, seem to be the next choice.

Maspin is homologous with the serine superfamily of protease inhibitors. It is shown to be produced by normal mammary epithelial cells, but its expression is down regulated or absent in breast cancer cells and tissues, suggesting that maspin functions as a tumor suppressor [393]. Two studies have indicated that maspin is useful to detect cancer cells in PB or BM [255, 302]. However, the sensitivity and specificity of maspin has been questioned in other studies [217, 248]. MGB exhibits homology to several secretory epithelial proteins of the uteroglobin gene family, and the human MGB gene is located on 11q13, a region that is amplified in a subset of breast cancer [394]. In non-malignant tissues, expression of MGB is strictly limited to the mammary epithelium, and it is highly expressed in primary breast cancer cells [218, 394, 395]. Moreover, MGB B/MGB2 is a recently cloned member of the uteroglobin gene family that is homologous to the MGB gene [396].

To date, the physiological role of these protein in breast tissue is unknown, as is their involvement in breast tumorigenesis. However, based on their breast cancer association and somewhat unique breast-specific pattern of expression, these proteins appear to be excellent candidates for molecular markers of breast cancer [216, 221, 222, 248, 298, 300, 397].

An ideal molecular marker to detect micrometastasis should be specific to cancer cells and not to normal cells, or to the tissue from which the tumor cell originated. It should not be present in any other cells and tissues than tumor cells. Furthermore, it should be easily detectable with little variance. More importantly, it should be clinically significant. As mentioned above, no single marker evaluated can be used for every breast cancer patient, partly because of heterogeneity of gene expression in cancer cells. Therefore, multi-marker analysis seems to be promising for accurate examination of micrometastasis [214, 222, 223, 248, 266, 309]. Ultimately, microarray analysis will be more effective and may yield more useful data. A study of particular interest demonstrated that complementation of MGB with additional genes including B305D, B311D, B533S, B726P and GABAπ increased the detection rate [306]. In a similar study using high-sensitivity array analysis of 170 candidate marker genes, cluster analysis identified a group of 12 genes that were elevated in the blood of cancer patients [308]. These genes included SRP19, CD44, TRP-2-8b, maspin, HSIX1, Groα, myosin light chain, MDM2, β-tubulin, N33, and lamininα3. Mean expression levels of these genes were elevated in 77% of breast cancer patients, and cluster analysis could correctly classify healthy volunteers and patients.

Thus, molecular methods seem to be useful not only for accurate diagnosis but also for detection of micrometastasis in LN, BM, PB and other body fluids. Potential uses of molecular detection of micrometastasis include: aiding in the selection of patients for earlier treatment for minimal residual disease, improvement of preoperative staging, and monitoring the effectiveness of therapy. Moreover, DNA microarray analysis may allow patient-tailored therapy strategies. A study of particular interest has suggested that gene expression profile will outperform all currently used clinical parameters in predicting disease outcome of breast cancer [168]. These genes consisted of those regulating cell cycle, invasion, metastasis and angiogenesis. However, before these techniques enter routine use in cancer patients, it remains to be evaluated in prospective studies with a large number of patients whether PCR-based methods may provide any diagnostic and prognostic information. For this purpose, sample collection, processing, choice of primers, use of proper controls and meticulous contamination-minimizing techniques should be standardized.

Conclusion

Recent advances in molecular technology have created the opportunity to understand the biology of breast cancer and the heterogeneous nature of this disease. Ultimately, full awareness of the factors underlying individual differences in cancer etiology and drug response will allow the development of more specific diagnosis and treatment.

Acknowledgement

This study was supported by Japan's Ministry of Health, Labor and Welfare research grant, a study on the construction of algorithm of multimodality therapy with biomarkers for primary breast cancer by a formulation of decision making process, led by Masakazu Toi(H20-3JIGAN-IPPAN-007).

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