Metallothionein as a Prognostic Biomarker in Breast Cancer

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Breast cancer is the most common cancer in women, with a general upward trend in incidence. Basic and clinical breast cancer research has continued at a rapid pace, in the endeavor to understand the biology of the disease so as to improve management of patients. Besides traditional pathological indicators, expression of molecular markers in breast cancer has also been comprehensively investigated. This paper will focus on the prognostic utility of metallothioneins (MTs), a family of low molecular weight metal binding proteins encoded by at least 10 functional MT genes that are associated with cell proliferation in breast cancer. Evidence that MT is a potential prognostic biomarker for breast cancer is supported by many reports in the literature. Expression of the MT protein has been detected by immunohistochemistry in a significant portion of invasive ductal breast cancers. MT expression has also been well studied in association with traditional clinico-pathological parameters of breast cancers. Generally, higher MT expression in breast cancers is predictive of worse patient outcomes. The relationship of MT isoforms to histological grade, estrogen receptor (ER) status, and prognosis will also be discussed. Exp Biol Med 231:1516–1521, 2006

Key words: breast carcinoma; prognosis; metallothionein; metallothionein isoforms

The first occurrence of breast cancer, recorded in the Edwin Smith Papyrus, was found in the breast of an ancient Egyptian male at about 2500 B.C. (1). Today, breast cancer is the most common cancer in women, with an incidence that varies considerably around the world, but which shows a general upward trend (2, 3). Women in North American and European countries are reported to have the highest rates of breast cancer (4). The occurrence (from the years 1968–2002) of breast cancer in women in Singapore represents 28% of all female cancers in that country (3). The age standardized incidence rate for breast cancer is 54.9 per 100,000 women per year in Singapore. The incidence of breast cancer in the Chinese population in Singapore is higher than that of the Chinese population in Shanghai and Hong Kong but lower than that of Hawaii, showing geographical variation of the disease among women of the same ethnic group (3, 5).

In contrast to the recommendations of early physicians such as Hippocrates and Claudio Galen, who advocated nonintervention for patients afflicted with breast cancer, the approach has moved toward a more individualized clinical management of the disease. Basic and clinical studies in breast cancer have continued at a frantic pace in an attempt to understand its biology and, consequently, has led to progress in early diagnosis, optimization of treatment regimes, and development of novel drugs with improved safety and tolerability (6). In 2005 alone, there were 8277 published reports in the biomedical literature related to breast cancer (National Center for Biotechnology Information PubMed search; website: http://ncbi.nlm.nih.gov/query.fcgi?CMD=search&DB=pubmed (16 August 2006)).

Pathological Indices in Breast Cancer

The most common histological type of breast cancer is invasive ductal carcinoma, also known as infiltrative ductal carcinoma (Table 1). Histological grade has always been an essential component in the pathologic assessment of invasive breast carcinomas. The prognostic significance of histological grade has been shown by several investigators (12, 13). Most of the grading schemes are based on criteria such as the extent of tubule formation, nuclear pleomorphism, and mitotic rate, which were first laid down by Bloom and Richardson (14). Other histological features that have been studied for prognostic value include necrosis, lymphovascular invasion, and angiogenesis. Clinico-pathologic parameters such as tumor size and axillary lymph node status are known to correlate with patient survival (6, 15, 16). Regional metastasis to the axillary lymph node has also

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Table 1. Histological Types of Breast Cancer

Invasive ductal	Lobular	Mixed	Others[a]	Reference
53%	5%	28%	14%	7
66%	14%	—	20%	8
75%	10%	—	15%	9
70%	10%	2%	18%	10
49%	16%	14%	21 %	11
79.2%	4.5%	—	16.3%	3

[a] Others include medullary, tubular, mucinous, cribiform, and papillary histological types.

been regarded as a standard indication for adjuvant therapy (17). The hormonal status of breast cancer tissues has been utilized to identify breast cancer patients who are most likely to benefit from endocrine therapy in recurrent and metastatic breast cancers (18). Estrogen receptor (ER)-positive breast cancer patients are more likely to respond to hormonal treatment than ER-negative breast cancer patients.

Biological Markers of Breast Carcinoma

The expression of molecular markers in breast cancer has been investigated extensively with a view to providing early diagnosis and prediction of outcome. Mutations of the breast carcinoma susceptibility genes, BRCA1 (19) and BRCA2 (20) are known to be associated with an increased lifetime risk of developing breast cancer (21, 22). The necessity for close surveillance of carriers of BRCA1 and BRCA2 mutations has therefore been advocated.

Mutations of the p53 gene, the most frequently identified genetic alteration in breast cancer (23), have been associated with worse prognosis in breast cancer in many reports (24, 25). Studies have shown that mutant p53 is associated with resistance to endocrine therapy, chemotherapy, and radiotherapy. However, the potential therapeutic predictive value of p53 for breast cancer therapy is still controversial.

The c-erbB2 (HER-2/neu) oncogene, a member of the type I transmembrane tyrosine kinase family, has also been linked with poor prognosis in breast cancer patients, especially in patients with axillary lymph node involvement (26, 27). The development of antibodies that downregulate c-erbB2 expression was the result of laboratory findings that established the linkage of c-erbB2 overexpression to increased metastatic potential, angiogenesis, and drug resistance. Clinical experience with the anti–c-erbB2 antibody, Trastuzumab (Herceptin) in breast cancer has been promising, as manipulation of growth factor signaling appears to enhance cytotoxic drug sensitivity (28). In fact, anti–c-erbB2 passive immunotherapy has also become an accepted and conventional method of treatment for metastatic breast cancer (29).

Examples of other tumor markers of breast cancer that have been reported in the literature are provided in Table 2. Recently, there has been an interest in determining the prognostic value of cell proliferation markers in breast cancer. There is evidence that the nuclear protein Ki67 is an early predictor of the efficacy of therapeutic regimes and is prognostic of long-term outcome (40). However, because of inconsistent reports, its reliability in the prediction of disease outcome has been questioned (6). Cyclin E, a member of the cyclin family that regulates the G1/S cell cycle transition, was found to correlate with poor disease survival (41). Cyclin E has been touted as the "best indicator of outcome" in breast cancer patients, even surpassing that of lymph node status, which is generally accepted as the best prognostic factor available (42).

This paper will focus on metallothionein (MT), which is also associated with cell proliferation, as a potential prognostic biomarker of breast cancer.

Biology of MT

MTs were first identified as cadmium-binding proteins in equine kidneys almost half a century ago by Margoshes and Vallee (43). These small molecular weight proteins were subsequently purified and characterized by Kagi and Vallee (44). MTs are known to exhibit selective binding to heavy metals such as zinc, copper, and cadmium (45). Cysteine residues in MTs bind seven bivalent metal ions (46). Three-dimensional structures of rat MT-2 (47, 48) have verified the presence of an α domain and a β domain, which binds four metal ions and three metal ions, respectively. A molecular model of rat liver MT-2 (generated from the protein data bank, accession code 4MT2) using RasMol software (49) is shown in Figure 1.

MTs are known to participate in cell proliferation (50, 51), a process which is believed to be important in carcinogenesis (Fig. 2). Furthermore, MTs are reported to scavenge free radicals and confer protection against apoptosis (52, 53). MTs have also been linked with chemoresistance (54). In humans, MT proteins are encoded by a family of genes consisting of at least 10 functional MT isoforms, and subdivided into four groups, MT-1, MT-2, MT-3, and MT-4. Human MT isoforms have tissue-specific expression patterns (55).

The potential role of MT in carcinogenesis gained attention after the seminal paper by Nartey *et al.* (56) which, in 1987, first described the expression of MT in human thyroid neoplastic tissues.

Prognostic Utility of MT in Breast Cancer

Immunohistochemical staining with the commercially available anti-MT E9 antibody shows that MT is localized in myoepithelial cells in normal breast tissues (57, 58). In a significant proportion of invasive ductal breast cancers, both cytoplasmic and nuclear MT staining has been observed. Lobular cancers were observed to exhibit weak-to-no MT staining, whereas mucinous and intraductal papillary cancers were negatively stained (57, 59).

Several investigators found that MT immunopositivity

Table 2. Sampling of Tumor Markers Analyzed in Breast Cancer

Tumor marker	Reference
Serum CA 15–3	30
Serum CEA (carcinoembryonic antigen)	31
DNA ploidy	32
Cathepsin-D	33
Bcl-2	34
E-cadherin	35
Serum CYFRA 21–1	36
Heat shock protein 27 and pS2	37
Urokinase-type plasminogen activator	38
Glutathione-S-transferase pi	39

is not correlated with age, tumor size, or lymph node metastasis in breast cancer (57, 60–62). There was only one study, by Haerslev *et al.* (63), that showed a correlation between MT expression with tumor size and lymph node metastasis. Histological grade is generally found to be associated with MT positivity, with higher grade tumors displaying increased MT expression (57, 62, 64–66); however, this was not observed by Goulding *et al.* (61). Although association of MT overexpression with menopausal status was reported (63, 64), this finding was refuted by others (60, 61). MT-positive tumors have also been observed to be inversely correlated with progesterone receptor positivity (38, 63).

Generally, MT positivity is associated with poor prognosis and a more aggressive phenotype (57, 61, 65, 67). Fresno *et al.* (57) found that MT immunostaining is predictive of a worse prognosis in the subgroup of lymph node–negative and ER-negative patients. In a group of 116 female patients with breast cancer from Singapore, we observed that a high MT expression level is associated with increased risk of recurrence and a shorter disease-free survival period (Fig. 3). MT-positive tumors appear to develop metastasis more commonly than MT-negative ones (67). Vasquez-Ramirez *et al.* (68) reported that while MT positivity is associated with metastatic potential, there is no correlation with survival or recurrence. Haerslav *et al.* (64) and Ioachim *et al.* (38) used multivariate analysis to show that MT positivity is not correlated with survival. However, this finding might be confounded by the strong association of MT expression with factors that predict poor outcome (69). Interestingly, in a recent article, Surowiak *et al.* (70) demonstrated by multivariate analysis that elevated MT expression is associated with shorter overall survival.

With regard to the role of MT isoforms in breast cancer, expression of MT-2A mRNA (the most abundant MT isoform) was observed to be significantly higher in histological grade 3 tumors compared to grade 1 and 2 tumors, but not correlated with patient age and axillary lymph node status (62). The MT-1F isoform was also observed to be upregulated in tumors with higher histological grades (66). MT-1E mRNA was detected to be highly expressed in ER-negative human invasive ductal breast cancer (71). In their analysis of the ductal carcinoma *in situ* component of breast cancer, Sens *et al.* (72) observed

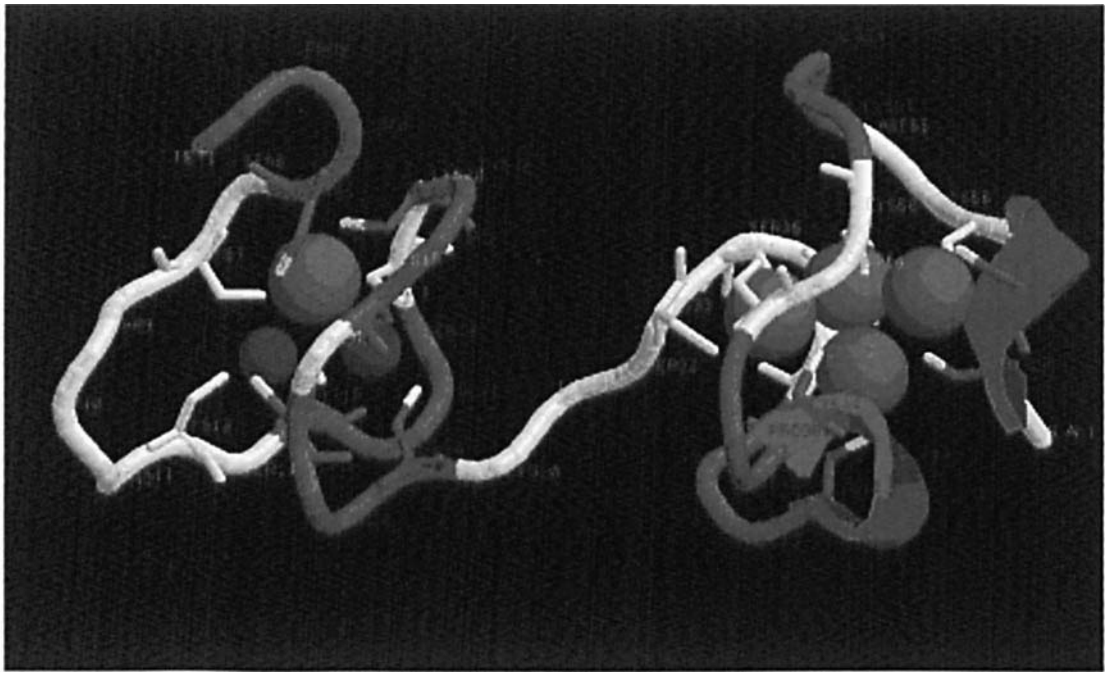

Figure 1. Three-dimensional structure of rat liver metallothionein. The molecular model was generated using RasMol software as previously described by Bay *et al.* (49) except that the model represented here is illustrated in greater detail. The protein backbone is denoted as a cartoon with heavy metal ions shown as spheres of arbitrary size. α-Helices and turns are darkly colored and loop regions are white cysteine side chains are shown as short sticks emanating from the protein backbone.

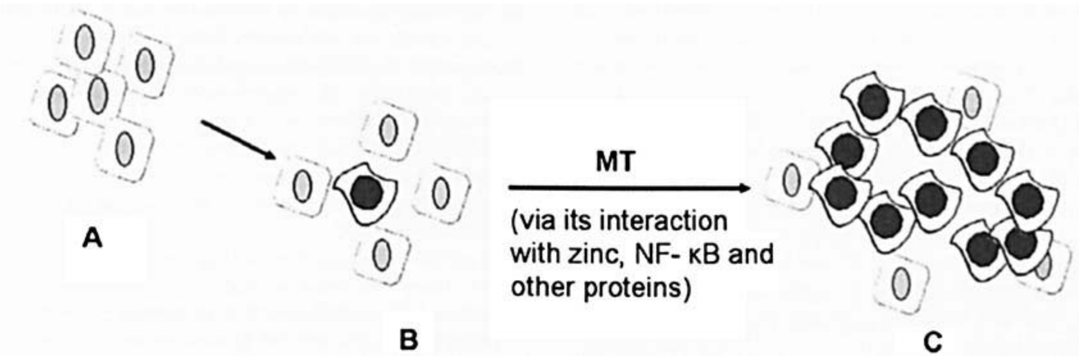

Figure 2. Schematic representation of cell proliferation in tumorigenesis. (A) Normal cells. (B) A normal cell has undergone genetic alteration (dark nucleus) and become malignant. (C) MT promotes proliferation of malignant cells (dark nuclei) and enhances tumor growth through its interaction with zinc, NF-κB, and other proteins.

significantly increased MT-3 staining in patients with bad outcomes when compared to those with good prognosis.

There have been mixed reports on the role of MT in mediating chemoresistance. MT was observed to have no relationship with chemotherapy or radiation therapy in breast cancer (63). However, elevated MT expression was recently reported to predict tamoxifen resistance in invasive ductal breast cancer (70). Indeed, the situation is rather complex because of the multifactorial nature of drug resistance (73).

Conclusion

MT is a promising prognostic biomarker in breast cancer. It may be appropriate to validate the usefulness of MT as a predictive marker in prospective patient studies and establish MT assays for use in clinical practice. In any case, knowledge gleaned from studying the role of MT isoforms in breast cancer may be useful in clarifying the clinical significance of this family of proteins (74) and developing novel strategies in the ongoing battle against breast cancer.

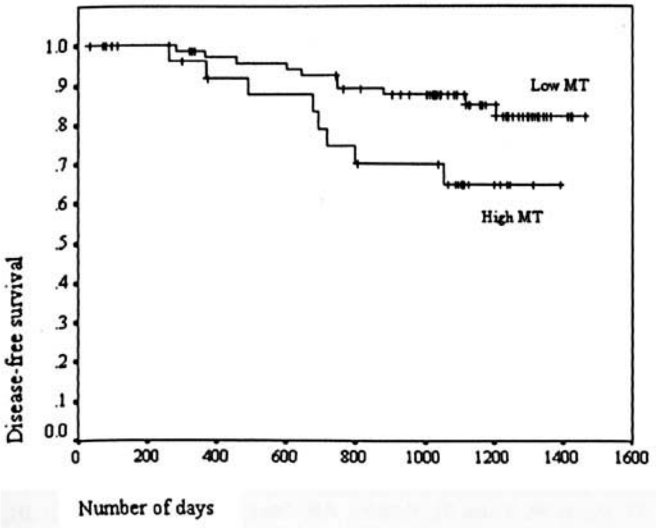

Figure 3. Patients with low MT expression have significantly longer disease-free survival than those with high MT expression ($P = 0.037$; log-rank test). The median follow up was 1117 days. Survival curves were plotted by the Kaplan-Meier method. This study was approved by the Singapore General Hospital Review Board.

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