Kaohsiung Journal of Medical Sciences (2011) 27, 167–172

available at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.kjms-online.com

ORIGINAL ARTICLE

# Expression of manganese superoxide dismutase in patients with breast cancer

## 乳癌病人乳房組織錳超氧化物歧化酶表現之研究

**Shih-Meng Tsai [a], Ming-Feng Hou [b,c], Szu-Hsien Wu [d,e], Bao-Wen Hu [f], Sheau-Fang Yang [g], Wan-Tzu Chen [g], Chee-Yin Chai [g], Hsu Ma [d,e], Li-Yu Tsai [f,*]**

蔡世盟 [a], 侯明鋒 [b,c], 吳思賢 [d,e], 胡寶文 [f], 楊曉芳 [g], 陳婉姿 [g], 蔡志仁 [g], 馬旭 [d,e], 蔡麗玉 [f,*]

[a] *Department of Public Health, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b] *Department of Surgery, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *Cancer Center, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan*
[d] *Division of Plastic Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan*
[e] *Department of Surgery, School of Medicine, National Yang-Ming University, Taipei, Taiwan*
[f] *Faculty of Medical Laboratory and Biotechnology Science, College of Health Science, Kaohsiung Medical University, Kaohsiung, Taiwan*
[g] *Department of Pathology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

Received 6 October 2010; accepted 26 November 2010
Available online 21 March 2011

**KEYWORDS**
Breast cancer;
Ductal carcinoma *in situ*;
Invasive ductal carcinoma;
Manganese superoxide dismutase;
Immunohistochemical stain

**Abstract** Breast cancer has become the second leading cancer among females in Taiwan. Even though the etiology of breast cancer is multifactorial, oxidative stress plays an important role in the carcinogenesis. The purpose of this study was to investigate the expression of manganese superoxide dismutase (MnSOD), one of the major antioxidant enzymes that is involved against oxidative stress, in adjacent cancer-free breast tissues and neoplasm tissues within the same patient. Sixty-five breast cancer patients' formalin-fixed tissue blocks, including ductal carcinoma *in situ* (DCIS) tissues, invasive ductal carcinoma (IDC) tissues, and adjacent cancer-free tissues, were evaluated by immunohistochemical stain. Meanwhile, their demographic and clinical information was also collected. The combined scores of MnSOD-positive cell proportion and MnSOD staining intensity were compared for different tissues within the same patient. The results showed that the mean combined scores of MnSOD expression in adjacent cancer-free tissues (6.33), IDC (5.30), and DCIS (3.78) were significantly different when assessed by repeated-measurement analysis of variance ($F = 14.17$,

* Corresponding author. Faculty of Medical Laboratory and Biotechnology Science, College of Health Sciences, Kaohsiung Medical University, No. 100, Shih-Chuan 1st Road, Kaohsiung 807, Taiwan.
*E-mail address:* tsliyu@kmu.edu.tw (L.-Y. Tsai).


doi:10.1016/j.kjms.2010.11.003

關鍵詞
乳癌;
乳腺管原位癌;
浸潤性乳腺管癌;
錳超氧化物歧化酶;
免疫組織化學染色

$p < 0.001$). Additionally, the results revealed that the distribution of strong MnSOD protein expression was 80.0%, 72.3%, and 52.3% in adjacent cancer-free tissues, IDC, and DCIS, respectively. However, there was no statistically significant relationship between the expression of MnSOD and grades of breast cancer or other clinicopathologic variables. We suggest that the expression of MnSOD in neoplasm tissues, independent of the clinicopathologic characters, plays a critical role in breast cancer biology.

**摘要** 乳癌目前已經位居台灣女性癌症的第二位，根據文獻報導，乳癌雖然有許多的致病因子，然而氧化性傷害在其致癌機轉中扮演重要的角色，因此本研究擬探討生物體中最重要的抗氧化酵素-錳超氧化物歧化酶(Manganese superoxide dismutase; MnSOD)在乳癌組織中之表現。本實驗共收集65位診斷為乳癌病人(年紀51.5±10.7歲)之乳房組織為實驗材料。經由免疫組織化學染色法分析同一病人之乳腺管原位癌(DCIS)、浸潤性乳腺管癌(IDC) 及其鄰近無癌化乳房組織之MnSOD蛋白表現。而MnSOD蛋白表現之綜合分數表示方法乃結合細胞染色定性強度及定量評分價數來呈現。結果顯示：於鄰近之無癌化乳房組織、IDC與DCIS等乳房癌組織之MnSOD蛋白表現之平均綜合分數分別為6.33、 5.30與3.79。以重複測量變異數分析，發現：這些平均值具有統計上的差異 ($F = 14.17$, $p < 0.001$)。此外，本研究也指出：於同一病人之鄰近無癌化乳房組織、IDC、DCIS等乳房癌組織其MnSOD蛋白之極強免疫染色反應比例分別為80.0%、 72.3% 和 52.3%。然而在本研究中也發現MnSOD蛋白表現之平均綜合分數與其他臨床指標值之間均無統計學上的相關性。綜合以上結果，MnSOD蛋白表現在乳癌致癌過程中可能扮演重要的角色。


## Introduction

Breast cancer is the most frequent cancer in women (22.9% of all cancers), with an estimated 1,384,155 new cases in 2008 according to the global cancer statistics from the Web site of The International Agency for Research on Cancer [1]. Breast cancer is the most prevalent tumor in women in the West and is the second leading cause of cancer deaths among females in Taiwan. It is second only to cervical cancer, and there has been an annual steady increase in the past 20 years [2,3]. The incidence has rapidly increased in other areas of Asia in the past decade [3]. The research of epidemiology in Taiwan indicated that this increase was more apparent in the younger age groups rather than the older age groups, and the age-specific incidence rates peaked between ages 45 years and 49 years [3,4].

The etiology of breast cancer is multifactorial. Hormonal, genetic, and environmental factors appear to interplay in the pathogenesis of breast cancer [5]. Increased lifetime exposure to endogenous or exogenous hormone is recognized as a major risk factor in the development of breast cancer [6]. Several genes, for example, BRAC1, BRAC2, HER-2/neu, and p53, are linked to breast cancer susceptibility and development [7,8]. In our previous research, it was demonstrated that overexpression of the HER-2/neu gene might induce nuclear factor-kappaB activity in human breast cancer cells [9]. Reactive oxygen species (ROS) are byproducts generated endogenously by all aerobic cells as the result of oxygen metabolism. High concentrations of ROS, which are highly reactive, exert harmful effects on living organisms, inducing oxidative damage to their DNA and cell membranes. Numerous reports have implicated the association of free-radical generation with the pathogenesis of a wide variety of diseases [10—16]. The accumulation of DNA damages is believed to contribute to carcinogenesis [17].

Superoxide dismutases (SODs) decompose superoxide radicals to hydrogen peroxide, and then hydrogen peroxide and other peroxides of the cell are consumed by multiple enzymes, such as catalase and glutathione peroxidase [17]. There are three kinds of SODs in human tissues; manganese SOD (MnSOD) in the mitochondria, copper/zinc-superoxide dismutase (CuZnSOD) in the cytosol, and extracellular SOD in the extracellular matrix [18]. MnSOD is considered to be one of the most important antioxidant enzymes that acts against endogenous and exogenous superoxide radicals [19]. The basal expression of MnSOD is usually low, almost undetectable, but the enzyme is induced by hyperoxia; irradiation; cytokines, such as tumor necrosis factors, interleukins, and interferon; and changes in the cellular redox state [20].

Several studies have suggested that the level of MnSOD in tumor cells is low [21]. Furthermore, MnSOD activity has been shown to correlate with the degree of differentiation of nonmalignant and malignant cells, which suggested that more differentiated cells, such as the normal cells, have higher MnSOD activity [21,22]. To evaluate the role of MnSOD in malignancy, we investigated the expression of MnSOD in invasive and *in situ* breast carcinomas and compared the reactivity with that of cancer-free breast tissues. We would like to find out if there are any correlations between the expressions of MnSOD and sex hormones [estrogen receptor (ER) and progesterone receptor (PR)] using the immunohistochemical (IHC) assay in the studies.

## Methods

### Patients and study materials

Sixty-five newly diagnosed breast cancer patients (age, $51.5 \pm 10.7$ years) from Kaohsiung Medical University Hospital were recruited according to the criterion stating

**Table 1** Demographic and clinical characteristics of patients with breast ductal carcinoma ($n=65$)

| Parameters[a] | $n$ (%) |
|---|---|
| Ages, yr | 51.5 ± 10.6 |
| Menopausal statuses | |
| Premenopausal | 38 (58.5) |
| Postmenopausal | 27 (41.5) |
| Cancer sites | |
| Left breast | 36 (55.4) |
| Right breast | 29 (44.6) |
| Tumor sizes, cm | |
| <2 | 28 (46.0) |
| 2–5 | 26 (42.6) |
| >5 | 7 (11.4) |
| Axillary node involvement | |
| No | 34 (52.3) |
| Yes | 31 (47.7) |
| Histological grades | |
| I | 8 (12.3) |
| II | 40 (61.5) |
| III | 17 (26.2) |
| HER-2/neu | |
| Positive | 29 (44.6) |
| Negative | 36 (55.4) |
| Estrogen receptor | |
| Positive | 38 (58.5) |
| Negative | 27 (41.5) |
| Progesterone receptor | |
| Positive | 37 (56.9) |
| Negative | 28 (43.1) |

[a] All of the variables listed were not statistically related to the expression of manganese superoxide dismutase in invasive ductal carcinoma.

that they were willing to accept the operation treatment and gave their approvals to be the study subjects in this study. The information about the clinical features, such as age, menopausal status, tumor size, and others, and the pathological profiles, including HER-2/neu, ER, and PR status, was obtained from Kaohsiung Medical University Hospital medical records for analysis. The detailed descriptive statistics for those variables were listed in Table 1.

All the breast tissues, including the breast cancer tissues and the adjacent cancer-free tissues, after the surgery had been fixed in 10% buffered formalin and embedded in paraffin wax before the IHC assay. The hematoxylin and eosin–stained sections from each tumor sample were examined by an experienced pathologist to confirm histological diagnosis and to assess tumor content. However, if the DCIS component was hardly examined in the collected breast cancer tissues, the collected tissues would be excluded from this study. This study was approved by the Ethics Committee of Kaohsiung Medical University Hospital.

## IHC staining

A polyclonal rabbit antibody for human MnSOD was used for the IHC stain. Sections of 3 μm were cut from appropriately selected paraffin blocks containing the breast tissues. These were mounted on glass slides coated with ploy-L-lysine and dried overnight at 60°C. The sections were deparaffinized in xylene and rehydrated by a series of graded alcohols. To enhance immunoreactivity, the sections were incubated in a 0.1 M citrate buffer (pH 6.0) and autoclave-treated at 121°C for 10 minutes. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 5 minutes at room temperature. After being washed with Tris buffer solution (TBS) and incubation with 5% bovine serum albumin, the sections were incubated with rabbit polyclonal primary antibody MnSOD (06-984, 1:50; Upstate Biotechnology, Charlottesville, VA, Virginia, USA) for 1 hour at room temperature. The sections were rinsed off with TBS at pH 7.6 and incubated with the linking biotinylated antibody (DAKO LSAB2 kit, K0675; DAKO Glostrup, Denmark) for 20 minutes. Subsequently, the sections were rinsed off with TBS followed by incubation with the peroxidase-conjugated streptavidin complex (DAKO LSAB2 kit, K0675) for 20 minutes. Freshly prepared 3, 3'-diaminobenzidine tetrahydrochloride (DAB) solution (Sigma K3468, St. Louis, USA) was applied on the slides for 5 minutes after it had been rinsed off with TBS. DAB was removed by rinsing with distilled water. The slides were counterstained with hematoxylin, dehydrated in increasing grades of ethanol, and cleared in xylene. The negative controls were obtained by replacing the primary antibody with nonimmune serum.

Even though the pathological profiles, including ER, PR, and HER-2/neu, were all obtained from the patients' medical records, the methods for such assessments will be illustrated briefly. The IHC stain method of ER, PR, and Her-2/neu was the same as that of MnSOD. The concentration of the primary anti-ER antibody, anti-PR antibody, and anti-HER-2/neu were 1:50 (DAKO, DAKO, and Biogenex (San Francisco, USA), respectively). The positive criterion for ER and PR is greater than 10% tumor cells with nuclear staining, and the positive criterion for HER-2/neu is uniform intense membranous staining in at least 30% of cells.

The IHC stain of MnSOD expression was evaluated as follows [20]: (1) The qualitative intensity of the IHC stain with all the antibodies was evaluated by dividing the staining reactions into four groups: 1 = weak cytoplasmic staining intensity, less than 25% of the intensity of histiocytes; 2 = moderate cytoplasmic staining intensity, 25–50% of the intensity of histiocytes; 3 = strong cytoplasmic staining intensity, 50–75% of the intensity of histiocytes; 4 = very strong cytoplasmic staining intensity, 75–100% of the intensity of histiocytes. (2) The quantity of the IHC stain was divided into five groups according to the staining reactions: 0 = no positive immunostaining; 1 = less than 25% of tumor cells showing cytoplasmic positivity; 2 = 25–50% of tumor cells showing cytoplasmic positivity; 3 = 50–75% of tumor cells showing cytoplasmic positivity; 4 = greater than 75% of tumor cells showing cytoplasmic positivity. (3) A combined score for the IHC staining, based on both qualitative and quantitative IHC staining, was adopted in this study. The combined scores were

then divided into four main groups: x = no immunostaining, score 0; + = weak immunostaining, scores 1–2; ++ = moderate immunostaining, scores 3–4; +++ = strong immunostaining, scores 5–8. To obtain more objective scores, two pathologists (also the coauthors of this study) who were blinded to the clinical outcome evaluated the immunostaining patterns independently. If a discrepancy was present, the pathologists reanalyzed the slides together and reached a consensus regarding the final score.

### Statistical analysis

SPSS for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The expression of MnSOD in the invasive carcinomas, *in situ* carcinomas, and adjacent cancer-free epithelium cells was evaluated by repeated-measurement analysis of variance (ANOVA). The associations between the expression of MnSOD and those of binary variables and nominal variables in the patients with breast cancer were evaluated by Student *t* test and ANOVA, respectively. The probability *p* value less than 0.05 was considered statistically significant.

## Results

There were 65 women with newly diagnosed breast cancer in this research, ranging in ages from 28 years to 83 years, with a mean age of 51.5 years. General characteristics of the study subjects were presented in Table 1, including the distribution frequencies of their menopausal statuses and clinical manifestations, such as histological grades, cancer sites, tumor sizes, involvement of axillary nodes, and the pathological profiles, including the statuses of HER-2/neu, ER, and PR. The distribution frequency of the postmenopausal status was 59%. Our analyses revealed the following: higher proportions of tumors in the left breast (55%), tumor size less than 2 cm (46.0%), no axillary node involvement (52.3%), Grade II tumors (62%), negative HER-2/neu status (55%), and positive ER (59%) and PR statuses (57%). However, all of the aforementioned variables did not significantly correlate with the combined scores of MnSOD expression in invasive carcinoma tissues assessed by Student *t* test or ANOVA. Moreover, the *p* value for tumor sizes, axillary node involvement, histograde, and statuses of Her-2/neu, ER, and PR, were 0.23, 0.34, 0.06, 0.20, 0.34, and 0.75, respectively. Even though the *p* value for histograde statuses is close to a significant level, we believe that the results of the MnSOD expression in different tissues and the aim of this research could not be biased by the aforementioned variables.

Regarding MnSOD expression, we found that the immunoreactivity of MnSOD was stronger in the adjacent cancer-free epithelium cells than in the breast cancer cells (Fig. 1A). Because of the inflammatory cells and fibroblastic cells of the desmoplastic stroma surrounding the tumor cells as well as tissue histiocytes and endothelial cells, a relatively strong immunoreactivity was observed. As a result, we also noticed that the cytoplasmic immunostaining of MnSOD expressed around the invasive lesions (Fig. 1B) and the *in situ* lesions (Fig. 1C). The quantities of the MnSOD immunoreactivity in invasive carcinoma, *in situ* breast lesions, and the adjacent cancer-free tissues are shown in Table 2. The frequencies of strong MnSOD protein expression were 80.0%, 72.3% and 52.3% in the adjacent cancer-free tissues, invasive carcinomas tissues, and *in situ* carcinomas tissues, respectively. Furthermore, the mean combined scores were 6.33, 5.30, and 3.79, in the adjacent cancer-free tissues, invasive carcinoma tissues, and *in situ* carcinoma tissues, respectively; they differed significantly from each other when assessed by repeated-measurement ANOVA ($F = 14.17$, $p < 0.001$).

## Discussion

Tumorigenesis is a multistep process that requires the acquisition of certain properties common to all tumors [23]. The increasing incidence of breast cancer urges people the need to understand the various mechanisms involved in breast tumorigenesis. Oxidative DNA damage, including mutagenic and cytotoxic lesions, is implicated in the initiation phase of carcinogenesis. The interaction between oxygen radicals and DNA produces base adducts, deletions, frameshifts, strand breaks, and DNA–protein crosslinks [24]. Oxygen free radicals also attack breast epithelium and lead to fibroblast proliferation, epithelial hyperplasia, cellular atypia, and breast cancer [25]. Biologically, a living body has to develop an efficient protection against harmful effects of ROS by both enzymatic and nonenzymatic antioxidant mechanisms. SOD, the most important antioxidant enzyme, catalyzes the dismutation of highly reactive $O_2^{-\cdot}$ to $O_2$ and $H_2O_2$, a less ROS. In the human body, MnSOD was responsible for the detoxification of ROS in the mitochondria [26], which

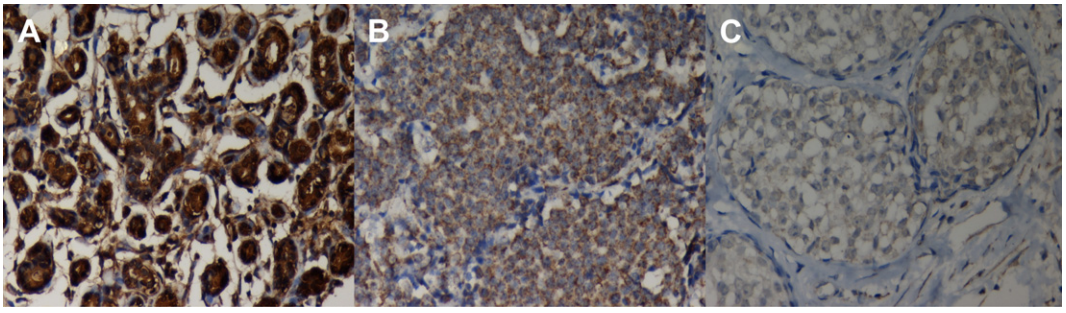


**Figure 1.** Manganese superoxide dismutase staining: (A) adjacent cancer-free tissue (400×), (B) invasive carcinoma tissue (400×), and (C) *in situ* carcinoma tissue (400×) of the same patient with breast ductal carcinoma.

**Table 2** Manganese superoxide dismutase immunoreactivity in the invasive carcinomas, the *in situ* carcinomas, and the adjacent cancer-free tissues of the same patient with breast ductal carcinoma

| Sites | Frequencies for expression | | | | Combined score[a] |
|---|---|---|---|---|---|
| | Negative | Weak | Moderate | Strong | |
| | *n* (%) | *n* (%) | *n* (%) | *n* (%) | Mean ± SD |
| Invasive carcinoma | 7 (10.8) | 1 (1.5) | 10 (15.4) | 47 (72.3) | 5.30 ± 5.76 |
| *In situ* carcinoma | 26 (40.0) | 1 (1.5) | 4 (6.2) | 34 (52.3) | 3.79 ± 11.47 |
| Cancer-free tissue | 10 (15.4) | 1 (1.5) | 2 (3.1) | 52 (80.0) | 6.33 ± 8.47 |

[a] There were significant differences in the means among these tissues when assessed by repeated-measurement analysis of variance ($F = 14.17$, $p < 0.001$).
SD = standard deviation.

rendered it to play a key role in the antioxidative system. However, epidemiologically, the role of MnSOD was ambiguous in cancer research. Mokenge Malafa et al. [27] showed that MnSOD expression, which was evaluated by Western blot analysis, was upregulated in the primary tumors of gastric cancer patients with lymph node metastases and in lung carcinomas [28]. In contrast, MnSOD levels [29] and MnSOD immunoreactive protein [30] have been found to be decreased in human pancreatic cells from chronic pancreatitis specimens when compared with those in normal pancreas. Other types of cancers, including gastric tumor [31,32] and prostatic cancer [32], also showed the same consequences. In breast cancer research regarding the enzymatic activities of SOD, R. Kumaraguruparan et al. [33] suggested that upregulation of antioxidants induced by oxidative stress confers a selective growth advantage to tumor cells over their adjacent normal counterparts. Conversely, a significant decrease in the activities of SOD of erythrocyte lysate in breast cancer patients as compared with those of the control subjects and fibroadenoma patients was reported in another study [34]. Our previous research revealed that the activities of antioxidant enzymes in the blood of the patients with breast cancer were significantly higher than those of the controls [35].

To clarify the role of MnSOD, Li et al. [36] proposed that the activity of MnSOD correlated with the degree of differentiation in human breast cancer cells. Portakal et al. pointed out that activities of MnSOD and the total SOD enzymes in breast tumor tissues were significantly higher than those in the corresponding cancer-free tissues [37]. Other evidences revealed by Liu and Zhong et al. showed that activities of MnSOD and total SOD were increased in human tumor cell lines [38,39], and Soini et al. [20] pointed out that MnSOD expression assayed by IHC was less frequent in invasive breast carcinomas than in *in situ* carcinomas or nonneoplastic breast epithelial cells. In the present study, we investigated the expression of MnSOD by IHC in invasive breast carcinomas, *in situ* carcinomas, and in adjacent cancer-free tissues with a unique statistical method, repeated-measurement ANOVA, which was not adopted in previous studies, highlighting the within-subject difference. Therefore, it is hard to elucidate the results of this study compared with other previous studies. However, the results showed that the expression of MnSOD was decreased in the *in situ* carcinoma tumor tissues in comparison with that in the invasive carcinoma or the adjacent cancer-free tissues, which could be explained as follows. First, MnSOD might act as a tumor suppressor in normal condition; thus, MnSOD might protect the normal tissues from the attack of free radical damages. Second, lower expression of MnSOD enhanced the development of tumorogenesis; as a result, the expression of MnSOD was decreased in the *in situ* carcinoma. Finally, the expression of MnSOD was increased in the invasive carcinoma to help tumors infringe the surrounding cells. Hence, the expression of MnSOD was higher in invasive carcinoma than in *in situ* carcinoma. Based on the evidence revealed by this present study, we deem that $H_2O_2$ could be the key point to explain why there was a higher expression of MnSOD in breast cancer tissues than in *in situ* carcinoma tissues. $H_2O_2$ was generated with the help of MnSOD, which has a special property to penetrate cell membranes freely, and could attack the surrounding normal cells and then help cancer cell invasion. Taken together, we propose that the expression of MnSOD protein in neoplasm tissues, independent of the tumor grade or stage, plays a critical role in breast cancer biology.

## Acknowledgments

The study was supported by a grant from the Research Fund of Cancer (KMU-QC-093001) at Kaohsiung Medical University, Taiwan.

## References

[1] IARC. Globocan, http://globocan.iarc.fr/factsheets/populations/factsheet.asp; 2008. accessed 16.12.10.
[2] WHO. The world health report; 2010. WHO, Geneva, Switzerland.
[3] Department of Health, Executive Yuan, Taiwan. Cancer registry annual report, 2007, Taiwan. 2010, Republic of China, pp. 80.
[4] Hou MF, Tsai KB, Fan HM, Wang CY, Lin WC, Liu CS, et al. Familial breast cancer in southern Taiwan. Kaohsiung J Med Sci 2000;16:414—21.
[5] Russo J, Hu YF, Yang X, Russo IH. Developmental, cellular, and molecular basis of human breast cancer. J Natl Cancer Inst Monogr 2000;27:17—37.
[6] Clemons M, Goss P. Estrogen and the risk of breast cancer. N Engl J Med 2001;344:276—85.
[7] Carter RF. BRCA1, BRCA2 and breast cancer: a concise clinical review. Clin Invest Med 2001;24:147—57.
[8] Li SS, Tseng HM, Yang TP, Liu CH, Teng SJ, Huang HW, et al. Molecular characterization of germline mutations in the

BRCA1 and BRCA2 genes from breast cancer families in Taiwan. Hum Genet 1999;104:201—4.
[9] Hou MF, Lin SB, Yuan SS, Tsai SM, Wu SH, Yang FO, et al. The clinical significance between activation of nuclear factor kappa B transcription factor and overexpression of HER-2/neu oncoprotein in Taiwanese patients with breast cancer. Clin Chim Acta 2003;334:137—44.
[10] Tsai LY, Tsai SM, Lee KT, Yu HS. Levels of plasma lipid peroxides before and after choledocholithotomy on patients with obstructive jaundice. J UOEH 1992;14:261—9.
[11] Tsai LY, Lee KT, Tsai SM, Yu HS. Changes of lipid peroxide levels in blood and liver tissue of patients with obstructive jaundice. Clin Chim Acta 1993;215:41—50.
[12] Tsai LY, Lee KT, Liu TZ. Evidence for accelerated generation of hydroxyl radicals in experimental obstructive jaundice of rats. Free Radic Biol Med 1998;4:732—7.
[13] Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A, Tsai LY. Free radical triggered hepatic injury of experimental obstructive jaundice of rats is mediated via the overproduction of proinflammatory cytokines and enhanced activation of nuclear factor κB. Ann Clin Lab Sci 2001;31:383—90.
[14] Tsai SM, Lee KT, Tsai LY. Effects of S-adenosyl-L-methionine on liver damage of experimental obstructive jaundice of rats. Kaohsiung J Med Sci 2001;17:455—60.
[15] Jao HC, Lin YT, Tsai LY, Wang CC, Liu HW, Hsu C. Early expression of heme oxygenase-1 in leukocytes correlates negatively with oxidative stress and predicts hepatic and renal dysfunction at late stage of sepsis. Shock 2005;23:464—9.
[16] Yeh CC, Hou MF, Wu SH, Tsai SM, Lin SK, Hou LA, et al. A study of glutathione status in the blood and tissues of patients with breast cancer. Cell Biochem Funct 2006;24:555—9.
[17] Vendemiale G, Grattagliano I, Altomare E. An update on the role of free radicals and antioxidant defense in human disease. Int J Clin Lab Res 1999;29:49—55.
[18] Oury TD, Day BJ, Crapo JD. Extracellular superoxide dismutase: a regulator of nitric oxide bioavailability. Lab Invest 1996;75:617—36.
[19] Wong GH. Protective roles of cytokines against radiation: induction of mitochondrial MnSOD. Biochim Biophys Acta 1995;1271:205—9.
[20] Soini Y, Vakkala M, Kahlos K, Pääkkö P, Kinnula V. MnSOD expression is less frequent in tumour cells of invasive breast carcinomas than in in situ carcinomas or non-neoplastic breast epithelial cells. J Pathol 2001;195:156—62.
[21] Oberley LW, Oberley TD. Antioxidant enzymes in the cancer. Histol Histopathol 1997;12:525—53.
[22] Li JJ, Oberley LW, St Clair DK, Ridnour LA, Oberley TD. Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. Oncogene 1995;10:1989—2000.
[23] Chen WT, Huang CJ, Wu MT, Yang SF, Su YC, Chai CY. Hypoxia-inducible factor-1 alpha is associated with risk of aggressive behavior and tumor angiogenesis in gastrointestinal stromal tumor. Jpn J Clin Oncol 2005;35:207—13.
[24] McCord JM. Human disease, free radicals, and the oxidant/antioxidant balance. Clin Biochem 1993;26:351—7.
[25] Hochstein P, Atallah AS. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. Mutat Res 1988;202:363—75.
[26] Cai Q, Shu XO, Wen W, Cheng JR, Dai Q, Gao YT, et al. Genetic polymorphism in the manganese superoxide dismutase gene, antioxidant intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Breast Cancer Res 2004;6: 647—55.
[27] Malafa M, Margenthaler J, Webb B, Neitzel L, Christophersen M. MnSOD expression is increased in metastatic gastric cancer. J Surg Res 2000;88:130—4.
[28] Svensk AM, Soini Y, Paakko P, Hiravikoski P, Kinnula VL. Differential expression of superoxide dismutases in lung cancer. Am J Clin Pathol 2004;122:395—404.
[29] Cullen JJ, Mitros FA, Oberley LW. Expression of antioxidant enzymes in diseases of the human pancreas: another link between chronic pancreatitis and pancreatic cancer. Pancreas 2003;26:23—7.
[30] Cullen JJ, Weydert C, Hinkhouse MM, Ritchie J, Domann FE, Spitz D, et al. The role of manganese superoxide dismutase in the growth of pancreatic adenocarcinoma. Cancer Res 2003; 63:1297—303.
[31] Korenaga D, Yasuda M, Honda M, Nozoe T, Inutsuka S. MnSOD expression within tumor cells is closely related to mode of invasion in human gastric cancer. Oncol Rep 2003;10:27—30.
[32] Baker AM, Oberley LW, Cohen MB. Expression of antioxidant enzymes in human prostatic adenocarcinoma. Prostate 1997; 32:229—33.
[33] Kumaraguruparan R, Subapriya R, Viswanathan P, Nagini S. Tissue lipid peroxidation and antioxidant status in patients with adenocarcinoma of the breast. Clin Chim Acta 2002;325: 165—70.
[34] Kumaraguruparan R, Subapriya R, Kabalimoorthy J, Nagini S. Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast. Clin Biochem 2002;35:275—9.
[35] Yeh CC, Hou MF, Tsai SM, Lin SK, Hsiao JK, Huang JC, et al. Superoxide anion radical, lipid peroxides and antioxidant status in the blood of patients with breast cancer. Clin Chim Acta 2005;361:104—11.
[36] Li Z, Khaletskiy A, Wang J, Wong JY, Oberley LW, Li JJ. Genes regulated in human breast cancer cells overexpressing manganese-containing superoxide dismutase. Free Radic Biol Med 2001;30:260—7.
[37] Portakal O, Ozkaya O, Erden Inal M, Bozan B, Koşan M, Sayek I. Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. Clin Biochem 2000;33: 279—84.
[38] Liu R, Oberley TD, Oberley LW. Transfection and expression of MnSOD cDNA decreases tumor malignancy of human oral squamous carcinoma SCC-25 cells. Hum Gene Ther 1997;8: 585—95.
[39] Zhong W, Oberley LW, Oberley TD, St Clair DK. Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. Oncogene 1997;14:481—90.