

## A study of glutathione status in the blood and tissues of patients with breast cancer

Chih-Ching Yeh<sup>1,2</sup>, Ming-Feng Hou<sup>3</sup>, Szu-Hsien Wu<sup>4</sup>, Shih-Meng Tsai<sup>5</sup>, Shu-Kai Lin<sup>6</sup>, Linda Ann Hou<sup>7</sup>, Hsu Ma<sup>4</sup> and Li-Yu Tsai<sup>6,8\*</sup>

<sup>1</sup>Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

<sup>2</sup>Department of Pathology and Laboratory Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

<sup>3</sup>Department of Surgery, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

<sup>4</sup>Division of Plastic Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan

<sup>5</sup>Department of Public Health, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

<sup>6</sup>Division of Clinical Biochemistry, Department of Clinical Laboratory, Kaohsiung Medical University Affiliated Chung-Ho Memorial Hospital, Kaohsiung, Taiwan

<sup>7</sup>Keck School of Medicine, University of Southern California, USA

<sup>8</sup>Department of Clinical Biochemistry, Faculty of Biomedical Laboratory Science, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan

Glutathione plays an important role in the antioxidant system that is required for the maintenance of the redox status of the cell, defence against free radicals and detoxification of toxic compounds. Reduced glutathione (redGSH) can be converted to oxidized glutathione (GSSG) during oxidative stress. The ratio of redGSH/total glutathione can be regarded as an index of the redox status and a useful indicator of disease risks. We conducted experiments by the capillary zone electrophoresis method to investigate the alterations of the glutathione status in the blood and tissue samples from patients with breast cancer. The results showed that the levels of redGSH, GSSG, total glutathione and the ratio of redGSH/total glutathione were significantly decreased in the blood of the patients with breast cancer compared to those of the control subjects. The levels of various forms of glutathione were lower and more pronounced in stage III. In contrast, the levels of redGSH, GSSG, total glutathione and the redGSH/total glutathione ratio in breast cancer tissues were significantly increased relative to those of the adjacent cancer-free tissues, especially in stage II. We suggest that the high redGSH levels are associated with the enhancement of cell proliferation and resistance to apoptosis in the cancer cells, and the loss of the large amount of erythrocyte redGSH may be due to increased detoxification capacities and defence against oxidative stress. We propose that redGSH should be regarded as an important biochemical parameter for detecting breast malignancy. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS — breast cancer; glutathione status

ABBREVIATIONS — GSSG, oxidized glutathione; redGSH, reduced glutathione

### INTRODUCTION

Glutathione is the most abundant intracellular low-molecular-mass thiol, which plays a key role in cell biochemistry and physiology.<sup>1</sup> It is the predominant

defence against free radicals and peroxides. It also plays a role in the detoxification of a variety of xenobiotic electrophilic compounds, the metabolites of drugs and other toxic compounds in conjunction with glutathione peroxidase (GPx) and glutathione-S-transferase (GST).<sup>2</sup> In addition, it also regulates protein and DNA synthesis, cell differentiation, gene expression and apoptosis.<sup>3</sup> Glutathione can exist in either a reduced (redGSH), or oxidized (GSSG) state. Within the cell it is present mainly in the reduced

\* Correspondence to: Li-Yu Tsai, Department of Clinical Biochemistry, Faculty of Biomedical Laboratory Science, College of Health Sciences, Kaohsiung Medical University, No. 100, Shi-Chuan 1st Road, San Ming District, Kaohsiung, Taiwan. Tel: 886 7 312 1101, Ext. 2350. Fax: 886 7 237 0544. E-mail: tsliyu@kmu.edu.tw

form, which can be converted to the oxidized form during oxidative stress. Therefore, the measurement of the redGSH levels or the ratio of redGSH/total glutathione can be regarded as an important parameter to evaluate the redox status in biological systems.

Levels of redGSH are maintained by two systems. One system is the synthesis of the redGSH from its three amino acid precursors, i.e. glutamate, cysteine and glycine by the ATP-requiring enzymes  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ GCS) and glutathione synthetase in the cytosol of the cell. The other constitutes a recycling system involving glutathione reductase (GRx), which reduces the GSSG back to the redGSH at the expense of NADPH.<sup>4</sup>

Changes in glutathione homeostasis have been implicated in the aetiology and progression of a variety of human diseases, including cancer, neurodegenerative disorders, cystic fibrosis, HIV infection, liver disease, Parkinson's disease and aging.<sup>5</sup> Maintaining an optimal balance of the glutathione status in the cell is critical to survival. Studies have indicated that high intracellular redGSH levels prevented cell death, whereas depletion of intracellular redGSH has been reported to occur with the onset of apoptosis.<sup>3</sup> Therefore, the measurement of the various forms of glutathione in biological samples is important for the understanding of glutathione homeostasis in health and disease. The aim of this study is to investigate the alterations of reduced and oxidized glutathione and the redGSH/total glutathione ratio in the blood and tissues of patients to assess whether oxidative stress is involved in breast cancer.

## METHODS

### *Subjects*

The present study was based on 112 women with diagnosed breast cancer, ranging in ages from 27 to 83 years with a mean age of  $48 \pm 10$  years, 36% of them being menopausal. There were healthy volunteers (age- and sex- matched) serving as the control subjects. The 112 patients without previous therapy for breast tumours were chosen randomly for the study. The patients and the controls were non-smokers and did not use hormones or oral contraceptives. None of the subjects had concomitant diseases such as diabetes mellitus, rheumatoid arthritis or liver disorders. None of them showed any distal metastasis at the diagnosis of malignancy, whereas axillary lymph nodes were detected in 83% of the patients. Most of the tumour samples (79%) were of the ductal carcinoma histological type. Fifty-Six, 37 and 26% of the

tumours exhibited estrogen receptors, progesterone receptors and HER-2/neu (human epidermal growth factor receptors), respectively. The patients were clinically classified as stage I (19 patients), stage II (86 patients) and stage III (7 patients) according to the tumour-nodes-metastasis (TNM) system. The study was approved by the ethical committee of Kaohsiung Medical University and informed consent was obtained from all the participants.

### *Glutathione status analysis*

Fresh tumour tissues and adjacent normal tissues were washed extensively with PBS solution to remove erythrocytes. The tissues were blotted on filter paper, then stored at  $-80^{\circ}\text{C}$  until analysis. Just prior to assay, the breast samples were cut into small pieces and weighed. Tissues were mixed with 1% MPA (Metaphosphoric acid) and incubated on ice and then homogenized. After centrifugation for 20 min at 12 000 rpm at  $4^{\circ}\text{C}$ , the supernatants were filtered through a  $0.2\text{-}\mu\text{m}$  filter and injected into an automated capillary electrophoresis system (Beckman Coulter, USA), equipped with a fixed wavelength UV detector. Throughout all the experiments, uncoated fused-silica capillaries ( $75\text{ }\mu\text{m}$  I.D., 50 cm effective length) from Beckman were used. The sample was injected by the application of 0.5-psi pressure for 15 s. The capillary was thermostated at  $28^{\circ}\text{C}$ . Before each run, the capillary was rinsed and filled with the running buffer [300 mM boric acid (pH 7.8)]. The electrophoresis was performed with a constant voltage of 25 kV for 8 min. Beckman P/ACE MDQ software was used for instrument control. Data were quantified on the basis of corrected peak areas with migration times.

Venous blood samples from the breast cancer patients and control group were collected in EDTA-containing tubes. After the separation of plasma, the buffy coat was removed and the packed cells were washed three times with physiological saline. Aliquots  $100\text{ }\mu\text{l}$  of washed RBC were treated with  $300\text{ }\mu\text{l}$  of 5% MPA. To precipitate proteins completely, the samples were vortex-mixed and incubated on ice for 10 min. After centrifugation at 12 000 rpm for 10 min at  $4^{\circ}\text{C}$ , the supernatants were then filtered through a  $0.2\text{-}\mu\text{m}$  filter and diluted five times before being injected into the capillary electrophoresis system.

### *Statistical analysis*

Data were expressed as mean  $\pm$  standard deviation (SD). Statistical significance was assessed by using the paired *t*-test to compare the tumour tissue and

surrounding cancer-free tissue of the same patient; an unpaired Student's *t*-test was used to compare the mean values in blood. Correlation was established by the Pearson method. Values of  $p < 0.05$  were considered significant.

## RESULTS

In the study, we found that the levels of redGSH, GSSG, total glutathione and the ratio of redGSH/total glutathione were significantly decreased in the blood of the patients with breast cancer compared to those of the controls ( $p < 0.05$ ) (Table 1). The extent of the decrease was 2.57, 1.29 and 2.26-folds for redGSH, GSSG and total glutathione, respectively. In addition, the levels of redGSH and total glutathione were found significantly depressed at all clinical stages in the blood of the patients with breast cancer compared to those of the control subjects ( $p < 0.05$ ). The GSSG levels in stages II and III were significantly lower than those of the controls ( $p < 0.05$ ). The redGSH/total glutathione ratio in stages I and II was markedly decreased in the breast cancer patients ( $p < 0.05$ ) (Table 1). On the other hand, the levels of redGSH and the ratio of redGSH/total glutathione in the cancer tissues were higher than those of the adjacent cancer-free tissues in the breast cancer patients, especially in

stage II. The levels of GSSG and total glutathione in the breast tumour tissues were significantly elevated in stages I and II compared to those of the adjacent cancer-free tissues ( $p < 0.05$ ). The levels of redGSH, GSSG and total glutathione showed 8.43, 2.81 and 5.43-fold increases, respectively, in tumour tissues compared to those of the adjacent tissues (Table 2).

In the correlation analysis, there were positive correlations between the redGSH levels and both the total glutathione and the ratio of redGSH/total glutathione, GSSG levels and the total glutathione levels, and a negative correlation between the GSSG levels and the ratio of redGSH/total glutathione in the blood of the patients with malignant breast tumours ( $p < 0.05$ ). Meanwhile, there were positive correlations between the redGSH, GSSG, total glutathione and the ratio of redGSH/total glutathione in the breast tumour tissues ( $p < 0.05$ ).

## DISCUSSION

Glutathione is a multifunctional tripeptide that is an important antioxidant in protecting cellular integrity from oxidative stress. It is present in high concentrations in various body fluids and tissues. Glutathione in the diet can be partly absorbed from the small intestine and can also be synthesized *de novo*; therefore,

Table 1. Levels of redGSH, GSSG, total glutathione and the ratio of redGSH/total glutathione in the blood of patients with breast cancer in different stages<sup>#</sup>

Parameters	Controls ( $n = 40$ )	Total patients ( $n = 112$ )	Stage I ( $n = 19$ )	Stage II ( $n = 86$ )	Stage III ( $n = 7$ )
RedGSH ( $\mu\text{M}$ )	1289 $\pm$ 317	502 $\pm$ 230*	504 $\pm$ 230*	507 $\pm$ 234*	434 $\pm$ 195*
GSSG ( $\mu\text{M}$ )	103 $\pm$ 51.1	79.6 $\pm$ 60.8*	92.9 $\pm$ 88.8	79.4 $\pm$ 54.4*	46.7 $\pm$ 31.4*
Total glutathione ( $\mu\text{M}$ )	1496 $\pm$ 365	661 $\pm$ 276*	689 $\pm$ 318*	665 $\pm$ 272*	528 $\pm$ 201*
RedGSH/total glutathione (%)	86.5	75.8*	75.0*	75.5*	81.7

<sup>#</sup>Values are expressed as mean  $\pm$  SD.

\* $p < 0.05$ ; as compared to the controls.

Table 2. Levels of redGSH, GSSG, total glutathione and the ratio of redGSH/total glutathione in the tissues of patients with breast cancer in different stages<sup>#</sup>

Stages		RedGSH ( $\mu\text{M/g}$ )	GSSG ( $\mu\text{M/g}$ )	Total glutathione ( $\mu\text{M/g}$ )	RedGSH/total glutathione (%)
I ( $n = 19$ )	Tumour tissue	498 $\pm$ 862	109 $\pm$ 108*	716 $\pm$ 1010*	36.4
	Adjacent tissue	164 $\pm$ 476	52.2 $\pm$ 143	269 $\pm$ 747	22.2
II ( $n = 86$ )	Tumour tissue	902 $\pm$ 2084*	167 $\pm$ 320*	1236 $\pm$ 2644*	49.1*
	Adjacent tissue	82.9 $\pm$ 236	56.4 $\pm$ 97.8	196 $\pm$ 385	36.1
III ( $n = 7$ )	Tumour tissue	230 $\pm$ 285	89.8 $\pm$ 112	409 $\pm$ 474	31.9
	Adjacent tissue	38.3 $\pm$ 70.8	31.4 $\pm$ 40.5	101 $\pm$ 150	31.8
Total ( $n = 112$ )	Tumour tissue	792 $\pm$ 1869*	152 $\pm$ 286*	1096 $\pm$ 2367*	45.9*
	Adjacent tissue	93.9 $\pm$ 284	54.1 $\pm$ 104	202 $\pm$ 455	33.5

<sup>#</sup>Values are expressed as mean  $\pm$  SD.

\* $p < 0.05$ ; as compared to adjacent tissues.

glutathione is an exogenous and endogenous antioxidant.

Tumour cells can generate large amounts of hydrogen peroxide that may contribute to mutation and damage of normal tissues, and therefore facilitate tumour growth and invasion.<sup>6</sup> Many cells which protect against oxidative stress are associated with high intracellular levels of glutathione. Previously, it has been reported that the elevation of the intracellular glutathione content is associated with mitogenic stimulation,<sup>7</sup> and that the compound controls the rate of tumour-cell proliferation and inhibition of cancer growth by regulating protein kinase C activity and intracellular pH.<sup>8</sup> Obrador *et al.*<sup>9</sup> also indicated that there was a close association of high rates of cellular proliferation with increased intracellular glutathione content in tumour cells. In addition, the cellular glutathione redox status is also an important factor during apoptosis mediated by reactive oxygen species. Armstrong<sup>10</sup> suggested that redGSH depletion act as an early activator of apoptotic signalling. In breast tumours, the glutathione concentration is more than twice the levels found in normal breast tissues.<sup>11</sup> Overproduction of glutathione has been reported in human tumours by other research;<sup>12–15</sup> however, a contrary result has also been reported.<sup>16</sup> In the present study, we found that redGSH, GSSG, total glutathione and the ratio of redGSH/total glutathione were elevated in malignant tissues. We suggest that a large increase of redGSH levels of a cell should make it more resistant to oxidative damage. The elevation of redGSH concentration in malignant cells may be associated with cell growth and resistance to apoptosis.

Glutathione is a putative indicator of health. Blood levels are believed to be predictors of morbidity and mortality.<sup>17</sup> Measurements of the oxidation—reduction status of blood glutathione have been considered as an index of oxidative stress and a useful indicator of disease risks.<sup>18</sup> In humans, low redGSH levels and low redGSH/GSSG ratios have been found in the blood of patients with various disease states, including cancer, diabetes mellitus, HIV infections and the low redGSH/GSSG ratio is considered to be the cause of increased oxidative stress in these patients.<sup>1</sup> Our results also showed that the redGSH/GSSG ratio was significantly lower in the breast cancer patients (data not shown). We found that redGSH levels and redGSH/total glutathione ratio in the blood of the patients with breast cancer were significantly decreased compared to those of the controls. The change of the glutathione redox status in the blood is mainly due to the loss of a large amount of erythrocyte redGSH levels. We suggest that this could be due

to both the increased redGSH detoxification capacities, which can lead to redGSH depletion within the red blood cells, and lower efficacy in the reduction of GSSG to redGSH. The findings support the idea of the protective role of redGSH against reactive oxygen species-mediated oxidative stress in cancer patients.

To summarize, in the present study, we observed that there were differential responses for glutathione levels in the blood and tissue samples. There was an imbalance in the glutathione redox status in the breast tumour patients. We suggest that the high redGSH levels of the breast cancer tissues could be associated with the enhancement of cell proliferation and resistance to oxidative stress, thereby conferring a selective growth advantage for tumour cells as compared to their normal counterparts. In addition, the low redGSH levels of the blood may be due to increased detoxification in the circulation. The study revealed that measurement of the glutathione status might be of important clinical value in breast cancer.

#### ACKNOWLEDGEMENT

The study was supported by the grant (NSC 90-2320-B-037-016) from the National Science Council in Taiwan.

#### REFERENCES

1. Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. *Clin Chim Acta* 2003; **333**: 19–39.
2. Jefferies H, Coster J, Khalil A, Bot J, Mccauley RD, Hall JC. Glutathione. *ANZ J Surg* 2003; **73**: 517–522.
3. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. *J Hepatol* 2001; **34**: 946–954.
4. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition* 2002; **18**: 872–879.
5. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 2003; **57**: 145–155.
6. Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumour cells. *Cancer Res* 1991; **51**: 794–798.
7. Shaw JP, Chou I-N. Elevation of intracellular glutathione content associated with mitogenic stimulation of quiescent fibroblasts. *J Cell Physiol* 1986; **129**: 193–198.
8. Terradez P, Asensi M, Lasso de la Vega MC, Puertes I, Vina J, Estrela JM. Depletion of tumour glutathione in vivo by buthionine sulfoximine: modulation by the rate of cellular proliferation and inhibition of cancer growth. *Biochem J* 1993; **292**: 477–483.
9. Obrador E, Navarro J, Mompó J, Asensi M, Pellicer JA, Estrela JM. Glutathione and the rate of cellular proliferation determine

- tumour cell sensitivity to tumour necrosis factor in vivo. *Biochem J* 1997; **325**: 183–189.
10. Armstrong JS, Steinauer KK, Hornung B, *et al.* Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. *Cell Death Differ* 2002; **9**: 252–263.
  11. Perry RR, Mazetta J, Levin M, Barranco SC. Glutathione levels and variability in breast tumours and normal tissue. *Cancer* 1993; **72**: 783–787.
  12. 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**: 165–170.
  13. Perquin M, Oster T, Maul A, Froment N, Untereiner M, Bagrel D. The glutathione-related detoxification system is increased in human breast cancer in correlation with clinical and histopathological features. *J Cancer Res Clin Oncol* 2001; **127**: 368–374.
  14. Azza H, Abou G, Iman MF. Glutathione and its metabolizing enzymes in patients with different benign and malignant diseases. *Clin Biochem* 2000; **33**: 657–662.
  15. Perquin M, Oster T, Maul A, Froment N, Untereiner M, Bagrel D. The glutathione-related detoxification pathway in the human breast: a highly coordinated system disrupted in the tumour tissues. *Cancer Letter* 2000; **158**: 7–16.
  16. Skrzydlewska E, Stankiewicz A, Sulkowska M, Sulkowski S, Kasacka I. Antioxidant status and lipid peroxidation in colorectal cancer. *J Toxicol Environ Health A* 2001; **64**: 213–222.
  17. Lang CA, Shkin SN, Schneder DL, Mills BJ, Lindeman RD. Low blood glutathione levels in healthy aging adults. *J Lab Clin Med* 1992; **120**: 720–725.
  18. Navarro J, Obrador E, Carretero J, *et al.* Change in glutathione status and the antioxidant system in blood and in cancer cells associate with tumour growth in vivo. *Free Rad Biol Med* 1999; **26**: 410–418.