

Correlation of the Biological Traits of Cancers with Its Redox Status

Zhuhua Li Department of Pathophysiology, Luzhou Medical College, Luzhou 646000, Sichuan, China
 Xiaqing Han Department of Pathology, Wuhan Hospital for Infectious Diseases, Wuhan 040022, Hubei, China
 Qiuling Wang The First Affiliated Hospital of Chengdu Medical College, Chengdu 610500, Sichuan, China
 Yilun Liu The First Affiliated Hospital of Chengdu Medical College, Chengdu 610500, Sichuan, China
 Chenghen Wu Department of Pathophysiology, West China College of Preclinical and Forensic Medical Sciences, Sichuan University, Chengdu 610041, China
 Yaorong Ma Department of Pathology, Luzhou Medical College, Luzhou 646000, Sichuan, China

Abstract: Background The Redox status is a fundamental element for homeostasis, its deviation may be deeply involved in the pathogenesis of cancers. Here we investigate the deviation of representative redox pair of GSH/GSSG in cancers and its impacts on its biological traits. **Materials and Methods** The deviation of representative redox pair of GSH/GSSG in cancers was measured, including its changes in plasma of cancer patients and in tumor tissues, and its impacts on apoptosis, drug resistance of tumor cells and the tumor-neoangiogenesis. The state of GSH/GSSG in plasma and tumor tissues of cancer patients vs their control counterparts was examined by fluorometric analysis. The correlation of apoptotic factors of tumors with GSH/GSSG redox status were examined by immunohistochemical method in tissue microarray, the impact of GSH/GSSG redox status on proliferation of endothelial cells and on drug resistance of tumor cell were explored by MTT. **Results** The GSH/GSSG redox status in plasma of cancer patients deviated to pro-oxidative direction, while the GSH/GSSG redox status in cancer tissues deviated to reductive direction, which showed an opposite deviation vs that in plasma. The proliferation of endothelial cells stimulated by tumor-conditioned medium was totally reversed by GSH depletion. Depletion of intracellular GSH increased the adriamycin sensitivity in both MCF-7/ADM and MCF-7/S cells, and at the background of GSH depletion, the adriamycin exerted a significant reducing effect on intracellular GSH content in a dose-dependent manner. **Discussions** These results suggest that the GSH/GSSG redox status in cancer's plasma and cancer's tissues were differently deviated, which may be deeply involved in some unique traits of tumor cells, including the apoptosis, drug resistance of tumor cells and the tumor-neoangiogenesis. [Life Science Journal 2010;7(3):81-90]. (ISSN: 1097-8135).

Key words: Redox status; Cancer; Glutathione; Neoangiogenesis; Apoptosis; Drug resistance

Abbreviations

BSO	buthionine sulfoxine
CML-K562	chronic myelogenous leukemia K562 cell line
DEM	diethylmaleate
GSH	reduced glutathione
GSSG	oxidized glutathione
KCM	K562 tumor cell conditioned medium
MAPK	mitogen-activated protein kinase
MCF-7/ADM	adriamycin-resistant breast cancer cells
MCF-7/S	adriamycin sensitive breast cancer cells
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphe tetrazolium bromide (thiazolyl blue)
ROS	reactive oxygen species

Introduction

Some unique traits of tumor cells give the cancer strong surviving advantage over the normal cells, such as the immortality, the anti-apoptosis, the capable of angiogenesis, even the drug resistance. These traits may involve a series of pathogenic mechanism, but the homeostasis of cancer must be different from normal cells in some aspects, and the altered homeostasis may be deeply involved in the occurrence and development of cancer, no matter as a attacking factor or as a consequences. Investigation of these alteration could possibly expose some intrinsic mechanism and clue some novel anti-tumor targets.

The Redox status, which describes the balance of oxidative-reductive biochemical reaction in the cells, is a fundamental element for homeostasis. Accumulating evidences have showed that the deviation of redox status might associated with the occurrence and development of tumors[1,2]. So the purpose of the present study was to

investigate the potential involvement of the deviation of redox status in the regulation of some tumor's biological properties. Particularly in the influence of redox status on apoptosis of tumor cells, the tumors angiogenesis, and the drug resistance, which are very important factors for tumor's proliferation and anti-tumor therapy.

The GSH/GSSG redox pairs was selected as the marker of the redox status, the GSH is a essential intrinsic antioxidant protection mechanism and maintained at high concentration of millimolar level in tissues, which provide the intracellular milieu in a highly reductive state. The GSH/GSSG also functions in maintenance of tissue and plasma thiol-disulfide redox balance, the disulfide band, buffered by GSH/GSSG redox status, is extremely important for protein construction, the change-over of thiol-disulfide has been considered as a molecular switcher in regulation of gene expression, activities of enzymes, etc[3,4]. The GSH is also released from tissues,

and in the steady state, plasma GSH/GSSG redox values provide a means to distinguish between upstream oxidative events and downstream antioxidant events of oxidative stress.

So in the present study, the deviated degree of GSH/GSSG redox status in plasma of cancer patients compared with health subjects, and the deviation of GSH/GSSG redox status in cancer tissues compared with their counterpart paratumor tissues were examined, which would provide the background state of GSH/GSSG redox status in cancers. Then the anti-apoptosis, the capable of angiogenesis, and the drug resistance of cancer cells were investigated, and their relationship with the deviation of GSH/GSSG redox status were analyzed.

Materials and Methods

Materials

Adriamycin(ADM)-resistant human breast cancer cell line (MCF-7/ADM) and ADM-sensitive breast cancer cells(MCF-7/S), chronic myelogenous leukemia K562 cell line (CML-K562) were kindly gifted by Transplant and Immunity Laboratory of West China Hospital, Sichuan University, P.R.China. The umbilical cords for umbilical vein endothelial cell primary culture were obtained from health Caesarean birth in The Second West China Hospital, Sichuan University.

Assay of GSH/GSSG Redox Status in Plasma of Healthy Individuals and Cancer Patients

Subjects

120 healthy individuals (mean age 43.3 years old) from Sichuan University students and volunteers undergoing normal physical examination and 29 patients with cancer (mean age 43.5 years old) in digestive system from Department of Chemotherapy of West China Hospital, Sichuan University from 2003 to 2004 participated in the study. All participants gave their informed consent prior to their inclusion in the study and the study performed in accordance with the ethical standards of Declaration of Helsinki.

Measurement of GSH and GSSG in Homo Sapiens Plasma

The GSH and GSSG was measured by fluorescent-spectrophotometry modified from Hissin PJ[5,6].

Preparation of the GSH (Sigma) and GSSG (Sigma) standard curves

A series diluted GSH,GSSG mixed standard solution was prepared with the concentrations as 40、20、10、5、2.5nmol/ml of GSH and 20、10、5、2.5、1.25 nmol/ml of GSSG.

Table 1. Assay Protocols for GSH and GSSG

	standard GSH GSSG	&	NEM (2umol)	PBS	DTT (0.25umol)	PBS	'	PBS	OPA (1umol)	
A'	50μL		50μL	0	0	150	A	100	1300	100μL
B'	50μL		0	50μL	0	150	B	100	1300	100μL
C'	50μL		0	0	50μL	150	C	100	1300	100μL

The GSH and GSSG standard curves was plotted with the GSH、GSSG concentrations as independent variable and measured fluorescent values as the function.

Measurement of plasma GSH、GSSG contents

Fasting blood samples were collected in the morning, the plasma was collected, deproteinized by addition of 1 volume of ice-cold 10% (w/v) metaphosphoric acid (MPA) and centrifuged at 10,000 g for 3 min. The following procedure was same with the preparation of GSH and GSSG standard curves, just replace the standard GSH & GSSG with 50μl deproteinized plasma, and the contents of GSH and GSSG in plasma were calculated from the standard curves.

Measurement of GSH/GSSG, the Apoptosis-related Factors in Tumor Tissues and Paratumor Tissues and Their Relationship Analysis

Collection of tissue specimens and preparation of tissue homogenates

Tumor tissue and corresponding paratumor tissue were obtained from surgery excised specimens in 42 patients with digestive system cancer. The tumor type included esophageal cancer (13 cases), gastric cancer (14 cases) and colon cancer (15 cases). Corresponding paratumor tissues were taken from the resected specimens where it appeared healthy under light microscope, about

5-10 cm distant from the tumors tissue. Parts of tissue specimens were immediately frozen in liquid nitrogen after excision and kept at -70°C refrigerator until analysis for GSH/GSSG.

0.05g tissue specimen was homogenized with 0.75ml sodium phosphate-EDTA (pH 8.0) and 0.2 ml 25% MPA at ice-water bath, then centrifuged at 4000rpm for 15 min, the supernatant was collected for assay of tissues GSH and GSSG contents.

Determination of GSH and GSSG in tissues

0.2ml tissue homogenates supernatant were diluted for 2000 times with sodium phosphate-EDTA, the measurement for tissue's GSH/GSSG is similar to which in plasma.

Determination of apoptosis-related factors

Parts of the cancer and paratumor tissues were paraffin wax embedded, and arranged in a tissue chip (tissue microarray) for immunohistochemical staining.

The tissue chip was prepared by cybrdi biotech Ltd (China), the first antibody was mouse polyclonal antibodies (Wuhan Boster biotech, China) for Bcl-2, Bax, NF- κ B and rabbit polyclonal antibody (Wuhan Boster biotech, China) for caspase-9, and the second antibodies were from sheep serum. The routine immunohistochemical procedure was carried out with the negative control by PBS for replacement of the first antibodies. The immunohistochemical images were

analyzed by software of IMAGE-PRO plus4.1. Then the correlations between the expression of apoptosis-related factors and the indexes of redox status were analyzed impacts of GSH/GSSG redox status deviation on the proliferation of endothelial cells potentiated by tumor cell-conditioned medium

This part of the study is aimed to investigate whether the deviation of GSH/GSSG redox status correlates with the proliferation of endothelial cells potentiated by tumor cells, and hereby to explore the anti-tumor possibility through regulation of GSH/GSSG redox status.

Preparation of tumor-conditioned medium and Cell culture

CML-K562 cell (Chronic myelogenous leukemia line K562) were seeded at 2.5×10^5 /ml in RPMI-1640 medium (RPMI-1640) supplemented with newborn calf serum (Beijing Baolanbo Biol-Tech) for 24 hours, the post-culture medium were harvested and centrifuged at 1500rpm for 20 min to remove cell debris, and the collected supernatant was assigned as K562 cells conditioned medium (tumor-conditioned medium KCM) and stored at -20°C until use.

Endothelial cells were isolated from Homo sapiens umbilical cord and cultured in medium-199 (GIBCO) supplemented with 20% newborn calf serum. The cell identifying test with antibody to Factor VIII-related antigen showed that more than 95% of cells were positively stained, and the cells grew to a typical cobblestone morphology when confluent..

Experiment groups

The endothelial cells in exponential phase were harvested and then cultured for 24 h with RPMI-1640 medium contained 4% newborn calf serum to reach synchronization. The synchronized cells were divided into three groups: (a) RPMI medium without any more treatment as control; (b) various concentrations of KCM ($v:v = 0\%, 20\%, 40\%$ respectively) were added into the culture; (c) various concentrations of KCM (same with b) and 1.5mmol/L BSO (Sigma) were added into the culture. All cells continued culture for another 48 h.

Evaluation of the viability of endothelial cells in KCM and their intracellular GSH/GSSG redox status

After 48 h treating, one bolus of endothelial cells was used for assay of cells viability by MTT test (Sigma). Another bolus of endothelial cells were treated by trypan blue for viable cell counts, and other cells were lysed by repeated freeze (at -70°C) and thaw (four times) and centrifuged. The supernatants were used for GSH and GSSG assay.

Impact of Depletion of Intracellular GSH Content on Drug-resistance of Tumor Cells

First, the human breast cancer resistant line cells to ADM (Pharmacia & Upjohn Company, Italy) (MCF-7/ADM) and sensitive cell lines (MCF-7/S) were treated by different concentration adriamycin (ADM) respectively, cell viability were measured by MTT, and the IC₅₀ (the drug dose for 50% survival rate) and multiple coefficient for the drug-resistibility between the two kinds of cells were calculated.

The MCF-7/ADM cells were cultured to exponential phase, then transferred to serum-free medium for 24 h to reach synchronization, the synchronized cells were divided into four groups: (a) MCF-7/ADM cells were cultured in RPMI-1640 medium as control; (b) MCF-7/ADM cells were treated with different concentrations of ADM; (c) MCF-7/ADM cells were treated with 0.1 $\mu\text{mol/L}$ DEM for 3 h, then the medium was renewed with RPMI-1640 medium ; (d) MCF-7/ADM cells were pretreated with 0.1 $\mu\text{mol/L}$ DEM for 3 h, medium was then renewed with RPMI-1640 medium and ADM was added with different concentrations as (b). All cells were cultured for further 24 h.

The survival rate before and after DEM was measured by MTT, and different concentration ADM was then added for observing the changes of drug-resistance after DEM. The GSH concentration in MCF-7/ADM cells was examined simultaneously by fluorescent-spectrophotometry, the correlation between the changes of resistance to ADM and the intracellular GSH concentration was analyzed.

The MCF-7/S cells were also treated by DEM for 3 hours, and the changes of sensitivity to ADM and intracellular GSH content were examined as in MCF-7/ADM cells.

The diethylmaleate (DEM, Beijing Baolanbo Biol-Tech, China) is a pro-oxidant, glutathione-depleting agent, which can decrease intracellular GSH level in a dose-dependant manner.

Statistical Analysis

Data are expressed as means \pm standard error of means (SEM). Data were analyzed using one-way analysis of variance (ANOVA), t-test and linear correlation analysis by SPSS 12.0 software, and the difference was considered significant when $P < 0.05$.

Results

GSH/GSSG Redox Status in Cancers Plasma and Tissues Deviated Differently from Healthy Plasma and Paratumor Tissues.

The results in table 2 displayed that the GSH content and GSH/GSSG ratio in plasma of cancer's patients declined compared with healthy individuals ($P < 0.05$), which indicated a deviation of GSH/GSSG redox status to pro-oxidative direction. The cancers tissues, however, expressed an opposite deviation of GSH/GSSG redox status to reductive direction, the GSH content and GSH/GSSG ratio in cancer's tissues were significantly higher than their counterparts of paratumor tissues ($P < 0.01$).

The redox status in plasma usually represented a general condition of cancer's patients, while the redox status in cancer tissues may more directly reflect the actual state in cancer's itself. The direction-different deviation of GSH/GSSG redox status in the two sources of cancer samples could imply some underlain intrinsic mechanisms involved in the tumor's biological properties, which will be further referred in discussion.

The Different Expression of Apoptosis-related Factors in Cancer and Paracancer Tissues and Their Correlation with the Deviation of GSH/GSSG Redox Status

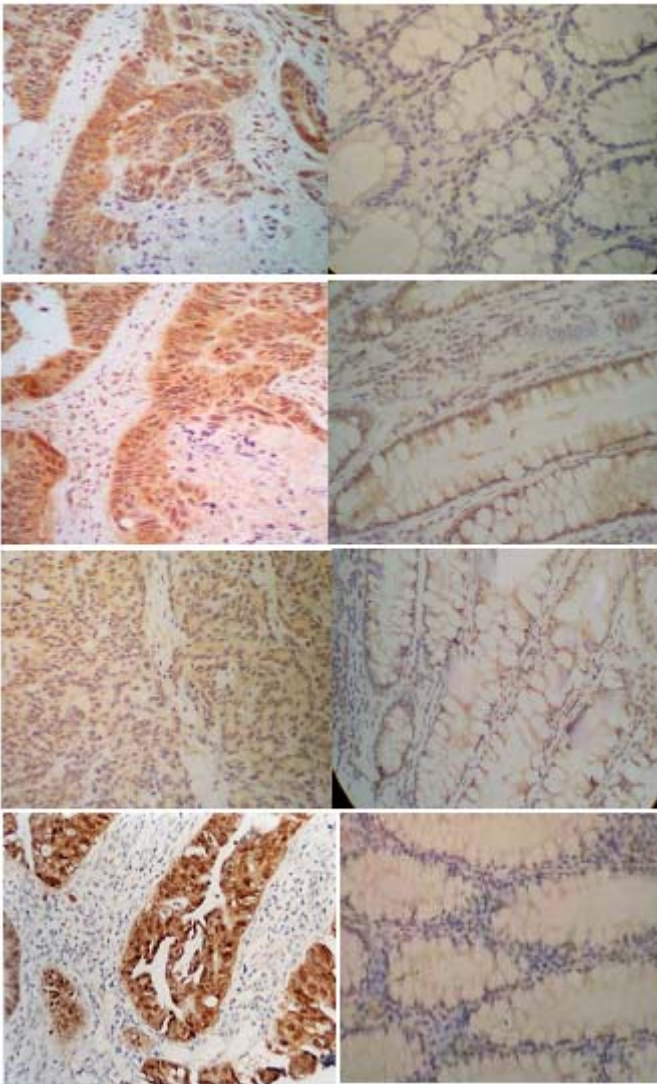


Fig 1. The Immunohistochemical Staining of Bcl-2、 Bax、 caspase-9 and NF- κ B in Sequence from Top to Bottom, the left column is cancer tissues and the right column is corresponding paracancers tissues. The images in left column showed much stronger positive staining.

Owing to the positive- proliferating effects of NF- κ B activation on cell, the increased Bcl-2 expression and the reduction-deviated redox status in carcinoma tissues, which furnishes the cancer anti-apoptotic trait, and the malignant cells showed a great proliferation capability. Results from correlations analysis with redox status showed a obvious correlations between the expression of Bcl-2 and the GSH/GSSG ratios (Fig. 2).

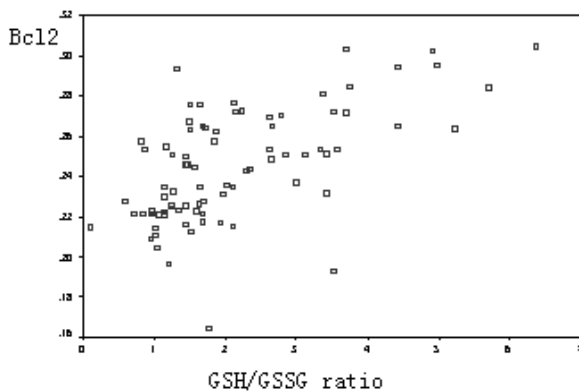


Fig 2 Correlation between Expression of Bcl-2 and the GSH/GSSG Ratio in Cancers and Corresponding Paracancers Tissues ($r=0.603$. $P<0.01$)

Table 2. GSH/GSSG Redox Status in Plasma and Tissues of Healthy Subjects and Cancer Patients

Samples	subjects	n	GSH(nmol/ml)	GSSG(nmol/ml)	GSH/GSSG ratio
Plasma	Cancer patients	29	6.01±0.65*	0.74±0.11	8.39±1.81*
	Healthy individuals	120	7.47±1.50	0.66±0.05	11.44±2.45
Tissues	Cancer tissues	42	584.88±207.93#	62.91±94.94	9.78±1.38#
	Paratumor tissues	42	322.49±88.81	64.99±114.80	5.51±0.66

* P<0.05 vs healthy individuals, # P<0.01 vs corresponding paratumor tissues

Table 3. The Expression of Bcl-2, Bax, Caspase-9 and Nuclear Positive Rate of NF-κB in Cancers and the Corresponding Paratumor Tissues ($\bar{x} \pm s$)

Groups	N	Bcl-2	Bax	Bcl-2/ Bax	Caspase-9	nuclear positive rate of NF-κB (%)
cancer	42	0.264±0.020*	0.275±0.013*	0.963±0.080*	0.262±0.017*	21.63±3.16*
paratumor	42	0.225±0.019	0.263±0.020	0.862±0.098	0.246±0.019	4.13±0.95

*P<0.01 vs the corresponding paratumor tissues

The expression of Bcl-2 as one of suppression factors of apoptosis, the Bcl-2/Bax ratios and the nuclear positive rate of NF-κB obviously increased in cancer tissues compared with their corresponding paratumor tissues (Fig.1).

These results suggested that the increased antioxidative capability in cancer tissues is one of the important reasons for their great anti-apoptosis and proliferation capability. The expression of Bax and caspase-9, pro-apoptotic factors, increased too. The oxidative stress in cancer tissues is probably contributed to this increase.

Depletion of Intracellular GSH Reverses the Proliferating Activity of Endothelial Cells Inspired by Tumor-conditioned Medium

A promising tumor-treating remedy has been designed to antagonize the neoangiogenesis of tumor through inhibiting tumor-secreted proliferating factors, such as the HIF α , VEGF, FGF, PDGF etc, and their receptors, which were thought to be the initiating and developing engine in angiogenesis of tumor microvasculature. But how is the response of endothelial cells, which were not the malignant cells, to these proliferating factors? as the essential constituent of the tumor microvasculature, endothelial cell may also be deeply involved in the regulation of this neoangiogenesis, some biological properties of endothelial cells in this proliferating process under the influence of tumor tissues could be hence a potential novel target in anti-neoangiogenesis for tumor therapy.

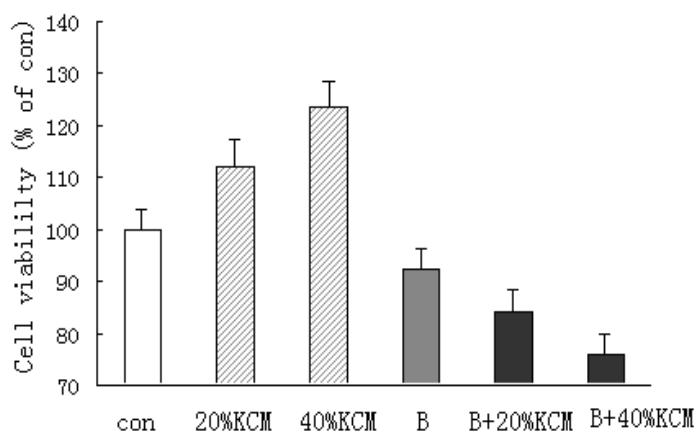


Figure 3 Impacts of KCM and BSO on Proliferating Activity of Endothelial Cells

KCM : tumor conditioned medium;
BSO: buthionine sulfoxine, inhibitor of GSH synthesis

The result in figure 3 illustrated the inspiring effects of tumor conditioned medium KCM on endothelial proliferation, and the reversing effects on this inspiring effects by BSO treatment, which is a specific inhibitor of de novo GSH synthesis. The proliferation of endothelial cells was inspired by KCM in a dose-dependent manner without BSO treatment, but adding the BSO into the culture system, which decreased the endothelial intracellular GSH content, the situation was totally turned over, the promoting effect on proliferation of endothelial cells by KCM was reversed even in a negative dose-dependent manner, the endothelial viability showed a downhill trend along with the increasing concentration of KCM in BSO treating groups (Figure 4).

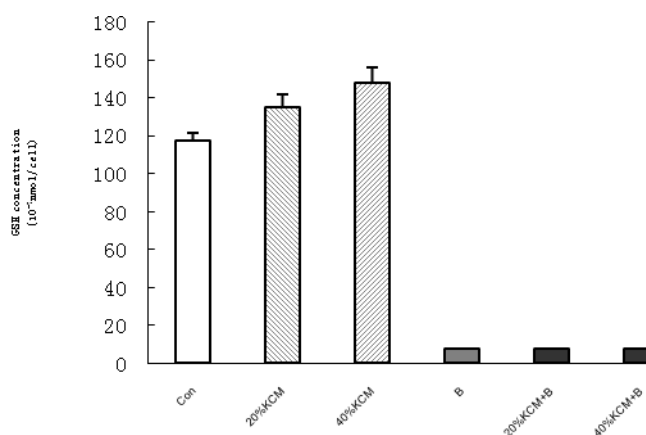


Figure 4 Impacts of KCM and BSO on Intracellular GSH Concentration of Endothelial Cells (P<0.01 without BSO vs with BSO, and between different KCM groups)

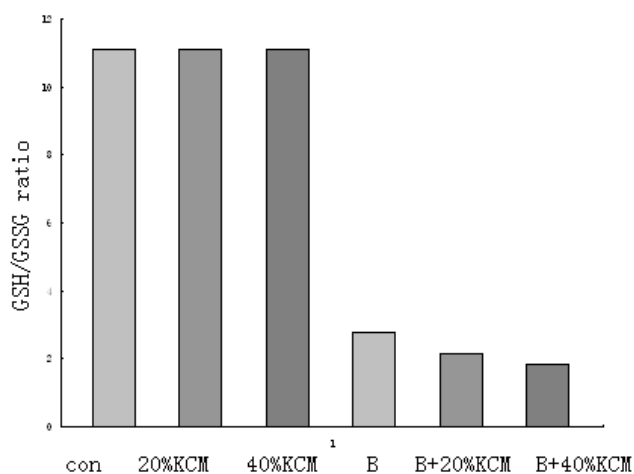


Figure 5 Impacts of KCM and BSO on GSH/GSSG ratio of Endothelial Cells (P<0.01 without BSO vs with BSO, and between different KCM groups)

Figure 4 and 5 illustrated the impacts of KCM on GSH/GSSG redox status of endothelial cells with and without BSO. Figure 6 analyzed the correlation between the proliferating activity of endothelial cells and its intracellular GSH/GSSG redox status with the KCM and BSO treatment.

The result from figure 4 showed that the intracellular GSH concentration in endothelial cells was enhanced by KCM treatment in a dose-dependent manner. After BSO treatment, however, the GSH concentration greatly declined, and no enhancing effect by KCM was observed along with the increased KCM concentration.

The interesting result in figure 5 was that if we take the GSH/GSSG ratio, but not the GSH content as the parameter, the KCM didn't change the GSH/GSSG ratio, which means the GSH/GSSG redox status of endothelial cells was maintained under the KCM stimulation, different from the result in figure 4, where the GSH content was enhanced by KCM. This fact however, may suggest a concert with the results in the above part of GSH/GSSG redox status in tissues and plasma of cancers patients, where a coexist of increased GSH content in cancer tissue and oxidative stress in plasma may suggest that the tumor could maintain a balanced redox status via increased GSH content in face of oxidative stress. Similar with this, the endothelial cells was also maintained a balanced redox status during the stimulation of tumor conditioned medium KCM. But if we depleting the intracellular GSH in BSO groups, the KCM was exhibited a pro-oxidant effect on GSH/GSSG redox status in endothelial cells, the GSH/GSSG ratio in endothelial cells displayed a potential dose-dependent decline with the increased KCM concentration (figure 5).

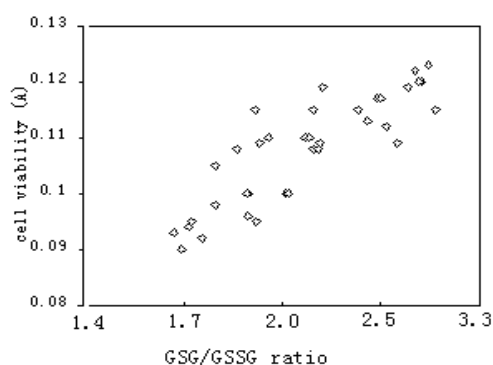


Figure 6 The Correlation of Proliferating Activity of Endothelial Cells with Its Intracellular GSH/GSSG Ratio ($r=0.865$, $P<0.01$)

Analyzing the correlation of proliferating activity of endothelial cells with its intracellular GSH/GSSG redox status displayed a close relationship between the two properties, the coefficient was $r=0.865$ ($P<0.01$) as in Figure 6.

Depletion of Intracellular GSH Dose-dependently Reverses the Drug Resistance of Cancer Cells

Another important characteristics of cancer cells, which may involve the intracellular redox status, was the drug resistance. In the fourth part of the study, we measured the intracellular GSH contents in cancer cells of human breast cancer cell line MCF-7 with adriamycin resistant(MCF-7/ADM) or adriamycin sensitive(MCF-7/S), the MCF-7/ADM cells showed a higher GSH concentration ($10.70\pm 1.58\text{nmol}/106\text{cells}$) than MCF-7/S have ($6.29\pm 0.67\text{nmol}/106\text{cells}$). The IC₅₀ (the drug dose for 50% survival rate) for adriamycin in MCF-7/ADM cells was much higher than the IC₅₀ value in MCF-7/S cells.

Pretreatment of MCF-7/ADM cells with DEM, which is a intracellular GSH depleting agent, the IC₅₀ value for adriamycin in MCF-7/ADM cells downed from $1075.72\pm 30.20\text{mg/L}$ to $314.11\pm 30.17\text{mg/L}$ as shown in table 3.

Table 3 The Intracellular GSH (nmol /106cells) Content and the IC₅₀(mg/L)for ADM in Breast Cancer MCF-7 Cells

Human breast cancer cell line	MCF-7/S	MCF-7/ADM	MCF-7/ADM treating with $0.1\mu\text{mol/L}$ DEM for 3 h
IC ₅₀ (mg/L)for ADM	28.10 ± 0.98	1075.72 ± 30.20	314.11 ± 30.17
Intracellular GSH (nmol /106cells)	6.29 ± 0.67	10.70 ± 1.58	Dose-dependent declined along with the increased ADM concentration (see figure 5)

The figure 7 showed that the treatment with DEM or ADM alone just had a mild to moderate effects on GSH decline. After DEM pretreatment however, the ADM exerted a significant dose-dependent reducing impact on intracellular GSH content, which was even much stronger than the simple added effects of DEM+ADM. The cancer cell viability exposed a close relationship with the intracellular GSH content as shown in figure 8.

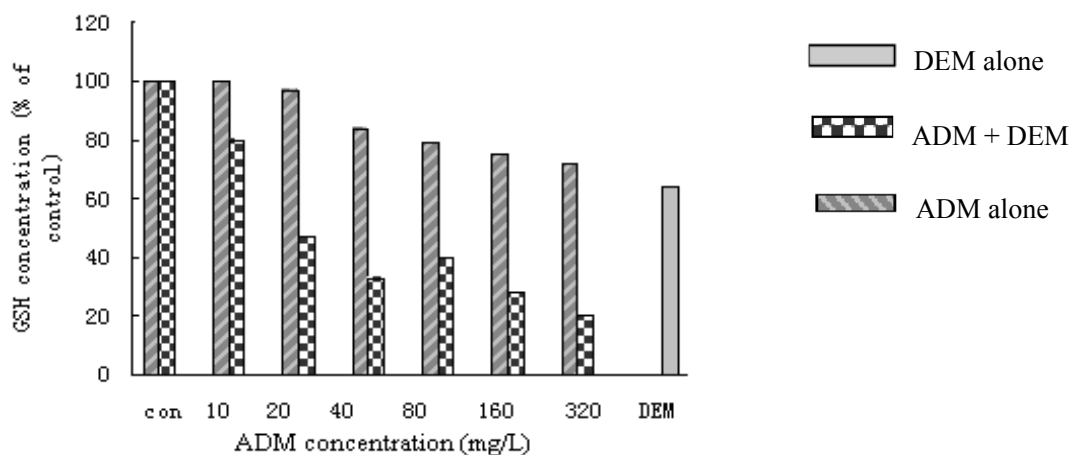


Figure 7. Changes of GSH Concentration in MCF-7/ADM Cells with or without DEM Pretreatment. MCF-7/ADM cells were pretreated with $0.1\mu\text{mol/L}$ DEM for 3 hr. Medium was renewed and ADM was added at increasing concentrations for further 24 hr incubation, GSH concentration was then assayed. The GSH content exhibits a significant dose-dependent decline along with the increased ADM concentration in group with pretreatment of DEM, while the GSH content didn't show significant changes in group without DEM pretreatment along with the increased ADM concentration.

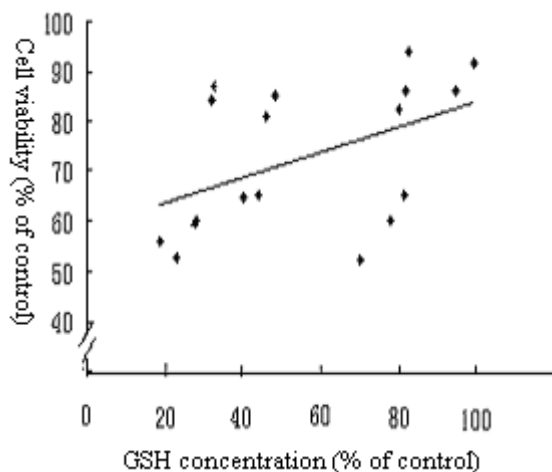


Fig 8 Correlation between the Cell Viability of MCF-7/ADM and Its Intracellular GSH Concentration ($r=0.90$, $P<0.05$)

Discussion

Apparent Oxidative Deviation of GSH/GSSG Status in Plasma of Cancer Patients, but Potential Reductive Deviation in Tumor Tissues

Our data on GSH/GSSG status in plasma of cancer patients showed a obvious pro-oxidant deviation compared with healthy subjects, which was consistent with previous reports[7,8,9,10]. The oxidative stress may come from increased reactive oxygen species (ROS) generation by tumor cells. In 70 years ago, a landmark research by Warburg[11] pioneered the fact that the mitochondrial defects of malignant cells played an important role in the development and progression of cancer, which rendered the cancer to satisfy their energy needs by producing a large portion of their ATP through glycolysis rather than through oxidative phosphorylation, but the mitochondrial defects would compromise the normal electron flow and result in an increase generation of superoxide radicals, which are subsequently converted into other ROS. Studies in Zhou Y et al have demonstrated that primary leukemia cells from patients with chronic lymphocytic leukemia contain significantly higher levels of cellular ROS compared to normal lymphocytes [12].

The increased ROS generation may also come from anticancer immune response and hypoxic microenvironment in solid tumors, mounting experimental and clinical evidences has demonstrated a obvious increased production of ROS in numerous pathophysiological settings including immune reaction、inflammation、hypoxia etc[13,14,15].

The pro-oxidant deviation of GSH/GSSG status in plasma reflected a general oxidative stress in cancer patients. The GSH/GSSG status in cancer tissue assayed by this study, however, displayed a significant higher level of GSH and GSH/GSSG ratio in tumor tissues than their counterparts of paratumor tissues, although the tumor cells produced numerous ROS, the significant increased level of GSH maintains an apparent reductive deviation in tumor tissues, this bifurcation of GSH/GSSG status in cancer tissue and plasma may represent a somewhat unique characteristics of malignant cells.

Oxidative stress possesses ambilateral functions,

mild-moderate oxidative stress is able to promote proliferation of tumor cell. Lots of signal pathway such as endothelial growth factor receptor, platelet derived growth factor receptor and MAPKs, which are related with proliferation, differentiation and migration of cells, are all redox-sensitive. The increase in endogenous ROS thus provides a constant stimulus for cell proliferation, and most importantly, may cause further damage to both mtDNA and nDNA, leading to cancer development, genetic instability, and disease progression[1,2].

On the other hand, oxygen free radicals are potent inducer of apoptosis for tumor cells [12,16]. One of the anticancer mechanisms of radiotherapy and chemotherapy was the production of ROS to kill tumor cells. The significant increased GSH provides the tumor cells a strong anti-oxidative stress and anti-apoptosis capacity, this nature may be even related with its drug resistance mechanisms.

So, the bifurcation of GSH/GSSG status in plasma and cancer tissues may render the malignant cells a great advantage over the double-edged effects of ROS, and a potent growth/survival potentials.

Proliferation-promoting Effect of Malignant Cells on Endothelial Cells is Partial GSH/GSSG Status-dependent

Since Dr. Judah Folkman first proposed the hypothesis in 1971 that tumor growth was angiogenesis dependent[18], the role of angiogenesis in tumor growth and progression has been firmly established, the tumor's angiogenesis is widely recognized been deeply involved in tumor progression and metastasis[19]. The process of tumor angiogenesis is thought to be primarily determined by the pro-angiogenic regulators released from malignant cells, such as hypoxia inducible factor- α , vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) etc, and considerable effort has been directed to anti-angiogenic therapy against these angiogenic growth factors and their receptors to treat human cancers[20].

But how does the endothelial cell function in its own proliferation? In the present study, the proliferation of endothelial cells was inspired by tumor conditioned medium KCM in a dose-dependent manner, which suggested the existence of pro-angiogenic substances

secreted by tumor cells. In this regard, various angiogenic growth factors such as HIF α , PDGF, VEGF, bFGF, etc have been frequently found in high concentrations in tumors, it thus is not surprising why the KCM showed significant promoting effect on proliferation of endothelial cells. Meanwhile, the present data also showed that the KCM treatment enhanced GSH content in endothelial cells, but did not change the GSH/GSSG ratio, which suggested maintenance of a constant redox status of GSH/GSSG by endothelial cells under the KCM stimulation.

Treatment of endothelial cells with BSO, a specific inhibitor of de novo GSH synthesis, reduced the endothelial intracellular GSH content down to approximately 6.5% of control, which but just showed a mild impact on cell viability. Under the background of depletion of GSH by BSO, the proliferation-promoting effect on endothelial cells by KCM was totally reversed even in a negative dose-dependent manner, the endothelial viability showed a downhill trend along with the increasing concentration of KCM, and the redox state of GSH/GSSG couple deviated to more and more oxidized with the increase of concentration of KCM. Correlation analyzing of proliferating activity of endothelial cells with its intracellular GSH/GSSG redox status displayed a close relationship ($r=0.865$, $P<0.01$).

These results showed that if the endothelial redox state of GSH/GSSG was maintained constant, the malignant cells stimulated endothelial proliferation, and GSH synthesis; But if inhibiting the GSH synthesis of endothelial cells, the stimulating effects of the malignant cells on endothelial cells was totally reversed, the effects of malignant cell on endothelial cell became anti-survival on endothelial viability and pro-oxidant on GSH/GSSG redox status. In summary, our data showed that the proliferation-promoting effect of malignant cells on endothelial cells exhibits a trait of partial dependent on GSH/GSSG redox status

Increased GSH Level in Malignant Cell Correlated with It's Drug Resistance

Several lines of evidence have indicated that block of GSH synthesis could reverse the drug resistance in assorted cancer cells[17,21], the effect may involve the multidrug-resistance-associated protein (MRP), which is a plasma membrane glycoprotein that can lower intracellular drug concentration by transport a complex of drugs and GSH out of cells. Attempts have been made to modify drug sensitivity with BSO, the inhibitor of GSH synthesis, and this compound has even been tested in some humans[22]

But the dependence of drug resistance on intracellular GSH was not conclusively ascertained. [Franzini M](#) et al[23] recently reported that the cancer cells transfected by GGT, the gamma-glutamyltransferase, which is regarded as critical for the maintenance of intracellular levels of glutathione, exhibited reduced sensitivity to cisplatin, but a decrease rather than an increase of intracellular GSH levels.

We show here that the intracellular GSH content in breast cancer cells with adriamycin resistant (MCF-7/ADM) is much higher than adriamycin sensitive cells(MCF-7/S); depletion of intracellular GSH by DEM increases the adriamycin sensitivity in both MCF-7/ADM

and MCF-7/S cells; and the adriamycin exerted a significant dose-dependent reducing effect on intracellular GSH content in DEM pretreated cells, the decrease of intracellular GSH level was even stronger than the simple added effects of DEM with adriamycin. All the data suggested that the intracellular GSH level was an important factor in regulating the drug-sensitivity, which supported the view of the dependence of drug resistance on intracellular GSH level. This result was also in accordance with our finding in the first part, that a significant higher level of GSH and GSH/GSSG ratio was found in tumor tissues, the potential reductive deviation in tumor tissues renders the tumor cells a privilege over oxidative stress and cytotoxic drugs.

In summary, the present study indicated that the GSH/GSSG redox status is an important determinant in regulating some of the tumors biological properties, further investigation for regulating ways of GSH/GSSG redox status may be very worthwhile in anticancer study.

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Corresponding author:

Yaorong Ma Department of Pathology, Luzhou Medical College, Luzhou 646000, P.R.China phone: 86-13808288096 Email: myr666@163.com

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