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Glutathione Levels in Human Tumors

Michael P. Gamcsik[*,1], **Mohit S. Kasibhatla**[2], **Stephanie D. Teeter**[1], and **O. Michael Colvin**[2]
[1]UNC/NCSU Joint Department of Biomedical Engineering, Raleigh, NC 27695

[2]Department of Medicine, Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, NC 27710

Abstract

This review summarizes clinical studies in which glutathione was measured in tumor tissue from patients with brain, breast, gastrointestinal, gynecological, head and neck and lung cancer. Glutathione tends to be elevated in breast, ovarian, head and neck and lung cancer and lower in brain and liver tumors compared to disease-free tissue. Cervical, colorectal, gastric and esophageal cancers show both higher and lower levels of tumor glutathione. Some studies show an inverse relationship between patient survival and tumor glutathione. Based on this survey, we recommend approaches that may improve the clinical value of glutathione as a biomarker.

Keywords

antioxidant; cancer; biomarker; patients; response; oxidative stress

INTRODUCTION

Glutathione is a tripeptide of glutamate, cysteine and glycine found at high concentration in virtually all mammalian tissue. The functionality of glutathione originates from the sulfhydryl (SH) group of the cysteinyl moiety. This sulfhydryl group is a powerful reducing agent and a strong nucleophile that is able to react with cellular toxicants directly or via the catalysis of the glutathione *S*-transferase family of enzymes. The detoxification capability of glutathione has inspired the clinical studies of its tissue levels in relation to cancer therapy. Many antineoplastic agents, in particular the alkylating agents, are designed to modify DNA and react with electrophilic sites. Glutathione therefore is thought to intercept and inactivate these agents before they can act to kill the cancer cell. Inactive conjugates of these drugs can be substrates for a number of membrane bound export pumps (e.g. MRP1) acting to reduce intracellular concentration of toxic species. In addition to inactivation by direct conjugation of drugs, cytotoxic reactive oxygen and nitrogen species generated by other antineoplastic agents and by ionizing radiation can also be neutralized by the glutathione metabolic system leading to another mechanism of therapeutic resistance (Conklin 2004). More recently, it has become apparent that the reduction-oxidation (redox) balance in tissue is controlled in part by the relative concentrations of reduced glutathione and its oxidized disulfide counterpart that can influence gene expression, cellular differentiation, proliferation and apoptosis (Burhans & Heintz 2009). Many of the chemotherapeutic agents and radiation treatments used today induce some form of oxidative stress in the cancer cell and alter this redox balance (Conklin 2004). Therefore, antioxidants play a key role in determining cellular

Corresponding Author: Michael P. Gamcsik, PhD, UNC/NCSU Joint Department of Biomedical Engineering, NC State University, Campus Box 7115, Raleigh, NC 27695-7115, Phone: 919-513-0786, Fax: 919-513-3814, mgamcsi@ncsu.edu.

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response to wide range of therapies. This has spurred extensive research into the role of the glutathione metabolic system in the development, diagnosis and treatment of cancer (Singh, Khan and Gupta 2012). Many clinical oncology studies have focused on the contribution of other aspects of glutathione metabolism such as the glutathione peroxidases or glutathione *S*-transferases. The latter enzyme has been the subject of a number of reviews (e.g. (Lo & Ali-Osman 2007)) but, as far as we are aware, there is no compilation of the clinical studies evaluating the role of glutathione itself. Since it plays a key role in many of these processes we sought to survey the oncology literature to assess whether glutathione has been shown to be a useful biomarker in diagnosis or prognosis to date and how it may best be applied in the future. We have divided this review into sections based on the tissue of tumor origin and subdivided each section into tumor tissue levels; glutathione in relation to histological or clinical assessment, and the possible relationship between observed levels of glutathione in cancer tissue and therapeutic outcome. In each section, we have focused upon studies in which tumor tissue is examined and have not attempted to cover the effect of tumor burden and/or therapy on plasma or blood levels of glutathione. The latter is another rather fertile area of research that shows great promise in diagnosis and monitoring of therapy but is really beyond what we can cover in a single review. In addition, alterations in glutathione levels in the circulatory system can be indicative of other disease processes (e.g. cardiovascular disease, diabetes (Jones 2006)), therefore this review focuses on the clinical implications of measuring glutathione in cancer tissue. In some studies presented herein, glutathione levels are compared between tumor tissue and healthy normal tissue, in other studies the levels of glutathione in the tumor tissue alone are reported. Studies in which single cell suspensions were isolated from clinical tumor samples (e.g. (Allalunis-Turner et al. 1992; Allalunis-Turner et al. 1991; Cook et al. 1991; Lee et al. 1989; Lewandowicz et al. 2002)) may offer some important insights into glutathione metabolism but were not included as the isolation procedure and culture of these cells may influence the glutathione levels. Although a number of studies find glutathione is a useful prognostic marker in hematological cancers (e.g. (Kearns et al. 2001)), often a normal control tissue is not available as a comparison so we decided to confine our studies to reports on clinical solid tumors. We have concentrated on tumors from brain, breast, ovary, cervix, colon and rectum, stomach, esophagus, liver, head and neck and lung as a number of studies were available in each of those anatomical sites.

The PubMed database of the National Library of Medicine was searched with keywords ‘glutathione’, ‘patient’ and ‘cancer’, back to 1980 and returned approximately 3000 results. Many of these are patient studies reporting levels of the enzymes glutathione *S*-transferase and glutathione peroxidase in human tumors. From these results, we gleaned approximately 120 clinical studies reporting glutathione levels in tumor tissue and these have been reviewed. Data for glutathione is often buried within reports and therefore will not be cited during the normal keyword search of the biomedical databases, so we may have missed papers that should have been covered. We apologize if we have overlooked any studies but hope this communication serves as a basis to assess the clinical importance of glutathione in oncological practice and emphasize the value of targeting the tumor’s redox machinery.

With a few noteworthy exceptions, tumor glutathione tends to be higher than found in disease-free tissue; i.e. the ratio of glutathione levels in tumor compared to disease-free tissue >1. As a visual aid in quickly identifying exceptions to this rule and the trends for particular tumor types, studies in which this ratio is found to be significantly <1 are shaded in the Tables.

Brain Cancers

Tissue Preparation and Analytical Methods

Normal brain tissue tends to have low activity of the glutathione-degrading enzymeγ-glutamyltranspeptidase but a number of brain tumors express high levels of this enzyme (Schafer et al. 2001). Precautions therefore should be taken in tissue processing to reduce the possibility of glutathione degradation prior to assay. Many of the studies treated rapidly thawing tissue with acid to precipitate proteins thereby inhibiting degradation and slowing oxidation. Constantin et al., (Constantin et al. 2012), Wright et al., (Wright et al. 2010) and Opstad et al., (Opstad et al. 2008) examined excised tissue by magnetic resonance methods that were not further processed and quickly analyzed samples at temperatures between 1° and 4 °C. Wright et al., (Wright, 2010) and (Opstad, 2008) noted the degradation of glutathione in such excised tissue samples after samples were left for extended periods at these temperatures. In contrast to the studies of excised tissue, magnetic resonance studies *in vivo* by Hazany et al., (Hazany et al. 2007) and Opstad et al., (Opstad et al. 2003) employ a noninvasive method to quantify this metabolite thereby avoiding procedures that can degrade or oxidize glutathione during typical *ex vivo* processing.

The assays used in most of the studies are specific for glutathione except for the that used by Zengin et al., (Zengin et al. 2009) which would not distinguish between glutathione and other cellular thiols and are therefore presented in italics in Table 1. However their reported levels although did not differ significantly from the levels reported using more specific assays.

Glutathione Levels in Brain Tumors and Disease-Free Brain Tissue

Brain glutathione levels in tumors range from approximately 10 – 50 nmol/mg-protein (Dudek et al. 2001; Dudek et al. 2002; Dudek et al. 2004; Dudek et al. 2005; Zengin, 2009), 600 – 4600 nmol/g-tissue wet weight (Kudo et al. 1990; Landolt et al. 1994; Louw et al. 1997; Suess et al. 1991) or 0.5 – 3 mM (Opstad, 2003; Wright, 2010). Whenever possible, reported values were converted to the units listed. The influence the normalization factor has on the data is illustrated in the study of Landolt et al., (Landolt, 1994) where normalization to tissue wet weight found no appreciable difference between tumor and peritumoral tissue glutathione levels However, when normalized to tissue DNA content, significantly lower levels were found in the astrocytoma tissue compared to peritumoral tissue levels (Table 1). They ascribe this difference to the observation that brain tumor tissue has more tightly packed cells per unit volume, and therefore higher DNA content, compared to normal brain tissue. Glutathione levels in disease-free brain tissue, either peritumoral or normal tissue from healthy controls, range from 25 – 50 nmol/mg-protein (Dudek, 2001; Dudek, 2002; Dudek, 2004; Dudek, 2005; Zengin, 2009), 1300 – 2700 nmol/g-tissue wet (Kudo, 1990; Landolt, 1994; Louw, 1997; Suess, 1991) or 1.2 mM (Opstad, 2003).

Almost all the studies compare tumor tissue levels to disease-free peritumoral tissue. The exception to this is the magnetic resonance study of Opstad et al., (Opstad, 2003) where data from brain tissue from healthy volunteers was obtained noninvasively. Therefore, whether histologically normal, disease-free peritumoral tissue is biochemically normal remains unresolved. Louw et al., (Louw, 1997) obtained ‘normal’ brain tissue from three cancer-free patients suffering from temporal lobe epilepsy. However, glutathione biochemistry does appear to be perturbed in epileptic patients and may be lower than in healthy tissue (Mueller et al. 2001). Lowe et al., (Louw, 1997) also obtained multiple tissue samples from the tumors to compare glutathione levels at the tumor surface and core. They did not observe statistically significant differences between core and surface levels of glutathione in these tumors.

In the studies comparing tumor types, meningiomas had higher glutathione levels than all other brain tumors. When normalized to tissue wet weight or volume, meningiomas appear to have increased levels of glutathione compared to healthy tissue (Opstad, 2003) or disease-free peritumoral tissue (Kudo, 1990; Suess, 1991). When normalized to protein (Dudek, 2001; Dudek, 2002; Dudek, 2004; Dudek, 2005; Zengin, 2009) or DNA content (Landolt, 1994) content, meningiomas, express lower levels of glutathione compared to disease-free tissue. In fact, glutathione levels in appear to be similar to or lower in the tumors than in disease-free tissue; i.e. T/C < 1 (Table 1). Meningiomas may be the exception to this conclusion.

Glutathione in Relation to Histological Grade or Clinical Stage of Brain Tumors

Two studies showed a relationship between glutathione levels in tumor tissue and malignant brain tumor grade. Zengin et al., (Zengin, 2009) found high grade (III and IV) gliomas had significantly lower glutathione levels compared to low grade (I and II) tumors. Similarly Louw et al., (Louw, 1997) found higher grade (II/IV) astrocytomas had lower glutathione levels than lower grade (II) astrocytomas. Wright et al., (Wright, 2010) found glutathione levels to be significantly higher in meningioma compared to gliomas. Pope et al., (Pope et al. 2012) found glutathione levels were lower in gliomas expressing a mutant form of the isocitrate dehydrogenase 1 enzyme characteristic of more aggressive tumors. This is consistent with the lower levels with higher grades found in the studies listed above. Interestingly, Constantin et al., (Constantin, 2012) found that grade 2 glioma tumors with higher levels of metabolites such as glutathione were more likely to progress to higher grades. Grade 2 tumors with lower levels of glutathione and other metabolites tended to remain grade 2. Although this appears inconsistent with the above studies noting lower glutathione with higher grade, this study did not actually compare glutathione levels in all tumor grades (Constantin, 2012).

Glutathione and Brain Cancer -- Conclusions

Glutathione levels in most brain tumors appear to be lower than found in to disease-free brain tissue. Meningiomas appear to have the highest level of glutathione compared to other brain tumor types. Indeed, for meningiomas, if tissue levels are normalized to tissue mass or volume, these tumors appear to have increased glutathione levels compared to disease-free tissue. More aggressive brain tumors appear to have the lowest levels of glutathione. As Kudo et al., (Kudo, 1990) suggested, low levels may make the tumors more susceptible to therapy.

Breast Cancers

Tissue Preparation and Analytical Methods

Both normal and breast cancer tissues express the glutathione degrading enzyme γ-glutamyltranspeptidase activity to varying degrees (Durham, Frierson and Hanigan 1997). Most studies treated isolated tissue with protein denaturing acids to remove this transpeptidase and slow oxidation. Two studies using histological preparations of tissue samples likely inactivated degradative enzymes during tissue fixation (Murray, Burke and Ewen 1987; Woolston et al. 2011).

Most assays used in these studies were specific for glutathione with the exception of the work of that reported total acid-soluble thiols as indicated by italics in Table 2. Kumaraguruparan et al., (Kumaraguruparan et al. 2002) noted that 80–90% of non-protein sulfhydryl measured by this assay is glutathione. This is reasonable generalization but there are tissues that fall outside this range so assays specific for glutathione and cysteine are always preferable.

Glutathione Levels in Breast Cancers and Disease-Free Breast Tissue

Glutathione levels in breast tumors range from approximately 10 – 40 nmol/mg-protein, 250 – 2000 nmol/g-tissue wet weight, or 500–700 μmol/g-DNA (Table 2). Whenever possible, reported values were converted to the units listed. For disease-free breast tissue, values range from approximately 1 – 10 nmol/mg-protein (Albin et al. 1993; Perquin et al. 2000; Perquin et al. 2001; Perry et al. 1993; Sreerama & Sladek 1997), 70 – 250 nmol/g-tissue (Coban et al. 1998; Honegger et al. 1988; Iscan et al. 1998; Perry, 1993; Sreerama, 1997; Sreerama & Sladek 2001) or 200 – 300 μmol/g-DNA (Honegger, 1988; Langemann et al. 1989). Three studies listed data that could not be converted or, if the given units listed are correct, yield glutathione levels three or more orders of magnitude greater than the highest values listed above (Abou Ghalia & Fouad 2000; El-Sharabasy et al. 1993; Rzymowska & Dyrda 1993). This suggests typographical errors in listing the units. Barranco et al., (Barranco et al. 1994) have performed an extensive study of tumor and normal tissue glutathione levels in 132 patients including 42 breast cancer patients. They sampled at least two sites in many of these tissues to assess heterogeneity of glutathione distribution. Disease-free peritumoral breast tissue averaged 7.16 ± 6.50 nmol/mg-protein.

In most studies of breast cancer patients, tumor glutathione levels are compared to histologically defined uninvolved peritumoral tissue (Table 2). In breast cancer, glutathione is localized to epithelial cells (Murray, 1987). Similar to the brain tumor studies, the ratio of glutathione in tumor to peritumoral tissue is influenced by the normalization factor used in calculating tissue concentrations. For example, Langemann et al., (Langemann, 1989) noted that normal breast tissue contains more fat and fewer cells per unit mass than tumor tissue; therefore glutathione levels should be normalized to DNA content. This results in differences in the T/C ratios when glutathione is normalized to DNA or protein rather than tissue mass in the studies of Honegger et al., (Honegger, 1988) or Perry et al., (Perry, 1993), respectively. Similarly, normalize glutathione to DNA or protein levels (Table 2). In a study of heterogeneity of glutathione in tumor biopsy specimens, Perry et al. (Perry, 1993) sampled at least 3 sites/tumor and found values ranging from below normal tissue levels to more than 11 times normal. Barranco et al. (Barranco, 1994) using multiple sampling in each tissue, found breast tumors to have a particularly high glutathione tumor-to-normal tissue ratio compared to other tumor types.

There is no clear relationship between menopausal status and glutathione levels. Perquin et al. (Perquin, 2001) noted a higher tumor-to-peritumoral glutathione ratio in post- compared to pre-menopausal women but others did not (Coban, 1998; Buser et al. 1997; Kumaraguruparan, Kabalimoorthy and Nagini 2005).

Yeh et al., (Yeh et al. 2006) and Perquin et al., (Perquin, 2001) found oxidized glutathione levels to be about 20% of the reduced glutathione levels. This indicates that there may be unusual levels of oxidative stress in breast cancer as oxidized glutathione in healthy tissue is normally <5% of the reduced from (Ballatori et al. 2009).

Most of the studies compared tumor glutathione levels to histologically normal peritumoral tissue. None determined if peritumoral tissue is biochemically ‘normal’ with respect to glutathione. Only the study of Sreerama et al., (Sreerama, 1997) compared tumor levels to normal breast tissue from cancer-free patients and found T/C ratios similar to the other studies (Table 2). Sreerama et al., (Sreerama, 2001) and Perry et al., (Perry, 1993) also reported lymph node metastatic breast tumor cells with increased glutathione levels relative to the primary tumor.

The study of Mourão de Farias et al., (Mourão de Farias et al. 2011) sought to determine whether a glutamine-supplemented diet would improve oxidative stress parameters in

women undergoing neoadjuvant chemotherapy. After chemotherapy, patients on a milk protein or glutamine-enriched diet showed no difference in glutathione levels in tumors compared to peritumoral tissues (Table 2). They concluded that the glutamine-supplemented diet offered no protection against oxidative stress in breast cancer patients.

Glutathione Levels in Relation to Histological Grade or Clinical Stage in Breast Cancer

Studies show mixed results in the relationship between glutathione levels and histological grade. Buser et al. (Buser, 1997) found higher glutathione levels in lower grade tumors, whereas Coban et al. (Coban, 1998) reported higher levels in grade 2 than grade 1, but found no general correlation with grade. In a semi-quantitative histofluorescence study, Murray et al., (Murray, 1987) indicated a weak, almost significant increase in glutathione with tumor grade. Woolston et al., (Woolston, 2011) and Perquin et al. (Perquin, 2001) found no relationship between glutathione levels and tumor grade but the latter study reported higher glutathione levels in c-ErbB-2 negative tumors. Langemann et al. (Langemann, 1989) found no correlation between glutathione and histological type or progesterone receptor but did detect a positive correlation to the estrogen receptor levels. This work included more patients than an earlier study from this same group (Honegger, 1988) that did not detect any relationship between hormone receptor status and glutathione levels. Other studies did not find a relationship between steroid receptor and glutathione levels (Murray, 1987; Perry, 1993; Perquin, 2001; Buser, 1997).

Kumaraguruparan et al., (Kumaraguruparan, 2005) found stage III levels higher than in stage I and II (Table 2). Yeh et al., (Yeh, 2006) noted an increase in tumor glutathione in stage II tumors compared to stage I but lower levels in stage III. Although Rzymowska and Dyrda (Rzymowska, 1993) found no difference between tumor and peritumoral glutathione levels (Table 2) before chemotherapy, they did find lower tumor levels after chemotherapy. In contrast, no differences were seen between glutathione levels in early breast cancer compared to locally advanced cancer (Buser, 1997) or clinical stage (Barranco, 1994; Coban, 1998; Yeh, 2006). As mentioned above, two studies found higher glutathione levels in lymph node metastases compared to the primary tumor (Perry, 1993; Sreerama, 2001) but no correlation was found between levels in the tumor and lymph node status (Perry, 1993; Buser, 1997; Perquin, 2001) or tumor size (Perry, 1993).

Glutathione in Relation to Prognostic Value in Breast Cancer

No differences in disease-free or overall survival were noted in patients with high levels of various drug resistance parameters including glutathione (Buser, 1997). In fact high levels of glutathione were associated with good prognosis. Woolston et al., (Woolston, 2011) found no correlation between glutathione levels and recurrence or overall survival.

Glutathione and Breast Cancer -- Conclusion

Glutathione levels were found to be higher in most breast tumors than in adjacent disease-free tissue. There is no clear relationship between histology, clinical stage or outcome.

Gastrointestinal Cancers

Table 3 lists the published reports of glutathione levels in tumors of the gastrointestinal tract. In this category we include tumors of the esophagus, stomach, liver, colon and rectum and are grouped according to tissue of origin.

Tissue Preparation and Analytical Methods

Gastrointestinal tissues and tumors express γ-glutamyltranspeptidase activity (Hanigan et al. 1999) that may affect assayed levels of glutathione if not inhibited or removed in samples

during tissue preparation. Most studies treated samples with acid or organic solvents to remove proteins and therefore prevent degradation and oxidation. The study of Schipper et al., (Schipper et al. 2000) provided no details on sample preparation.

For gastrointestinal tumors there was a mix of assay methods used. Some assays are specific for glutathione and others measure all non-protein thiols (see italics in Table 3).

Glutathione Levels in Colorectal Cancers and Normal Tissues

Obtaining a tumor biopsy specimen that is biochemically representative of the total tumor is a challenge. The study of Barranco et al., (Barranco et al. 2000) is noteworthy in that 3–7 samples of each colorectal tumor specimen were taken and averaged to address heterogeneity issues. This laboratory determined that multiple sampling is required in order to obtain an accurate measure of average glutathione content across a given tumor specimen (Barranco, 1994). Redmond et al., (Redmond et al. 1991) evaluated tumor variability in a subset of four patients in their study by listing tumor values from 3 separate samples from maximally distant sites within the same tumors. In this case, glutathione levels were consistent within the tumor with standard deviation ranging between 5–20% of the mean values in four patients. The studies of Berger et al., (Berger et al. 1994) and Eapen et al., (Eapen et al. 1998) averaged two samples per tissue specimen. Most other studies determined glutathione levels in one site from tumor or disease-free tissue.

Tumor tissue levels of glutathione in colorectal cancers range from approximately 10 – 50 nmol/mg-protein. When normalized to tissue mass, the variability in the tissue levels is pronounced. The study of Mekhail-Ishak et al., reports a level of 5700 nmol/g-tissue wet weight, approximately 5-times higher than the studies of Murawaki et al., (Murawaki et al. 2008) and one of three studies by Skrzydlewska et al., (Skrzydlewska et al. 2001) and nearly 40-times higher than two more recent studies by Skrzydlewska et al., (Skrzydlewska et al. 2003; Skrzydlewska et al. 2005).

For disease-free peritumoral colorectal tissue, glutathione levels range from approximately 5 – 50 nmol/mg-protein. Siegers et al., (Siegers et al. 1984) report some variation in levels in peritumoral tissue with colon (45 nmol/mg-protein) lower than in ileum, sigmoid or rectum (70–79 nmol/mg-protein) but did not report the statistical significance of these differences. These results are in contrast to glutathione levels of approximately 30 nmol/mg-protein found in normal colon mucosa from >200 healthy cancer-free subjects and was not dependent on gender, age or location; i.e. the proximal or distal colon (Hoensch et al. 2006). Barranco et al., (Barranco, 1994) sampled multiple peritumoral sites and obtained levels in the colon (8.48 ± 5.54 nmol/mg-protein) and rectum (13.04 ± 9.61). Interesting results from Grubben et al., (Grubben et al. 2006) examined levels of glutathione in disease-free tissue in the colon of patients with adenomas, familial adenomatous polyposis, patients with a family history of hereditary nonpolyposis cancer and carcinomas. They found a significant decrease of glutathione levels in disease-free tissue in all cases except for those from patients with adenomas (Grubben, 2006). The levels in disease-free tissue from adenoma patients (41 nmol/mg-protein) were similar to that of colon tissue from healthy, disease-free control subjects (44 nmol/mg-protein). Their conclusion was that low glutathione levels were correlated with high clinical risk for development of colon cancer (Grubben, 2006). These results suggest that the biochemistry of the peritumoral tissue from colorectal cancer patients is influenced by the presence of the tumor.

With this in mind, five studies (Barranco, 2000; Mekhail-Ishak et al. 1989; Ozdemirler et al. 1998; Redmond, 1991; Skrzydlewska, 2005; Butler et al. 1994) reported statistically significant increases in glutathione levels of colorectal cancer tissue compared to disease-

free peritumoral tissue and three reports (Baur & Wendel 1980; Berger, 1994; Skrzydlewska, 2003) showed trends to higher levels.

Two studies showed significant decreases in glutathione levels in colorectal tumors (Murawaki, 2008; Siegers, 1984). In several studies, Skrzydlewska et al, (Skrzydlewska, 2003; Skrzydlewska, 2001; Skrzydlewska, 2005) showed mixed results in tumors compared to peritumoral tissue that may, in part, be explained by tumor stage (see below). They also showed a trend toward lower levels in tumors of the colon and rectum. One study detected a trend toward lower levels in tumor tissue that was not statistically significant (Hengstler et al. 1998).

With the exceptions listed above, most of the results for colorectal cancer show higher glutathione levels in tumors compared to peritumoral disease-free tissue. As mentioned above, Grubben et al., (Grubben, 2006) noted that peritumoral tissue may be lower than normal glutathione levels contributing to higher T/C levels (Table 3). Differences in the data could also be due to groupings of colon and rectal tumors into one pool. Barranco et al., (Barranco, 2000) found that peritumoral tissue from patients with rectal cancer was significantly higher than in peritumoral tissue from in patients with colon cancer. This also was a trend seen by Siegers et al., (Siegers, 1984). Tumor heterogeneity is also a problem in that a single tissue sample may not accurately reflect the average value across a tumor mass. Barranco et al., (Barranco, 1994) determined multiple tissue samples are required to address this issue and may explain conflicting results where only single samples are available. For this reason, their later work listed in Table 3 (Barranco, 2000) is especially compelling as this is based on the average of 3 to 7 tissue samples from each tumor. Their results show a significant increase in both colon and rectal tumors compared to disease-free peritumoral tissue.

Glutathione Levels in Gastric Cancers and Normal Tissues

Where comparable, glutathione levels range from approximately 20 – 70 nmol/mg-protein in gastric tumor and disease-free tissue (Table 3). This is similar to the colorectal tissues. The exception is the study of Czeczot et al., (Czeczot et al. 2005) where levels appear to be much higher in both tumor and peritumoral tissues.. Eapen et al., (Eapen, 1998) and Hoppenkamps et al., (Hoppenkamps et al. 1984) reported increased nonprotein thiols in peritumoral tissue compared to gastric tissue obtained from cancer-free subjects suggesting the presence of the tumor does affect the biochemistry of nearby tissue. In contrast, Engin et al., (Engin & Ferahkose 1990) reported peritumoral gastric tissue to have lower levels of nonprotein thiols compared to gastric tissue from healthy, tumor-free subjects. The study of Eapen et al., (Eapen, 1998) however sampled ‘healthy’ tissue from 54 patients with non-ulcer dyspepsia. Although the cause of dyspepsia in these patients was not given, another study showed that both gastritis and peptic ulcer disease can significantly decrease glutathione levels in gastric mucosa (Demir et al. 2003).

One study (Eapen, 1998) found a higher level of glutathione in tumor compared to control tissue (T/C, Table 3) and two studies showing lower levels compared to control (Engin, 1990; Hoppenkamps, 1984). The two studies with T/C < 1, enrolled a total of 21 patients (Engin, 1990; Hoppenkamps, 1984) whereas the study showing significantly higher levels in the tumor sampled tissue from 49 patients. All three studies used similar tissue processing and analytical methods. As noted above, the comparison to healthy tissue using dyspeptic gastric tissue may skew the results of the larger study by Eapen et al., (Eapen, 1998). Other studies showed mixed results that were not found to be statistically significant. Therefore there is no discernible trend in T/C ratios for gastric tumors.

Glutathione Levels in Esophageal Cancers and Normal Tissues

Eight publications reporting esophageal tumor tissue glutathione levels are reviewed (Table 3). It appears that two separate publications of Kaur et al., (Kaur et al. 2008a; Kaur et al. 2008b) include data from the same patient population so these results are combined. In addition, the study of Sihvo et al., (Sihvo et al. 2003) may report updated data from an earlier study (Sihvo et al. 2002) and therefore have an overlapping patient population.

The range for glutathione levels in esophageal tissues is between 1 (Sihvo, 2003; Sihvo, 2002) and 125 (Kaur, 2008a; Kaur, 2008b) nmol/mg-protein; a much wider variation than found in colorectal or gastric tissues. This variability may obscure a trend in the data when comparing tumor to disease-free tissue. Skrzydlewska et al., (Skrzydlewska, 2003) reported a significant increase in esophageal tumor tissue compared to disease-free pertiumoral tissue whereas, Sihvo et al., (Sihvo, 2003; Sihvo, 2002) showed significantly lower glutathione levels in tumor tissue compared to healthy tissue. Levy et al., (Levy et al. 1999) reported lower glutathione levels in esophageal cancer tissue compared to tissue from healthy controls but provided no details on measured levels or whether these differences were statistically significant. Similar to the colorectal tumor results, no laboratory has compared peritumoral tissue to control healthy esophageal tissue from cancer-free controls. The other studies are mixed with some showing some slight, statistically insignificant differences in levels between tumor and normal tissues.

Glutathione Levels in Liver Cancer and Normal Tissues

The range in glutathione levels in the limited amount of liver tumor studies that can be compared is 6 – 4600 nmol/mg-protein (Corrocher et al. 1986; Czeczot et al. 2003). The low end of this range is in line with most of the other studies of gastrointestinal tissue listed in Table 3.

The studies of Abel et al., (Abel et al. 2009), Corrocher et al., (Corrocher, 1986), Czeczot et al., (Czeczot et al. 2006) and Lee et al., (Lee et al. 2007) all report a decrease in glutathione in hepatocellular carcinoma when compared to peritumoral or control liver tissue. The report by Lee et al., (Lee, 2007) involves 24 patients with hepatitis virus originated hepatocellular carcinoma and, in this respect, the peritumoral tissue may not be considered healthy. However, the observed decrease in glutathione content in the cancer tissue is consistent with the other studies. Abel et al., (Abel, 2009) showed glutathione levels higher in hepatitis B infected peritumoral tissue compared to virus-free peritumoral tissue. Jungst et al., (Jungst et al. 2004) reported a slight trend toward decreased glutathione in hepatocellular carcinoma tissue compared to adjacent disease-free tissue. Abou Ghalia and Fouad (Abou Ghalia, 2000) found lower levels of glutathione in liver cancer than in benign liver lesions. The results of Corrocher et al., (Corrocher, 1986) imply that peritumoral tissue glutathione levels are significantly affected by the presence of the tumor when a comparison is made to tissue from control, cancer-free patients.

Glutathione in Relation to Histological Grade or Clinical Stage in Gastrointestinal Cancers

Only the study of Skrzydlewska (Skrzydlewska, 2005) report glutathione data for colorectal cancer patients grouped by histological grade and clinical stage. A decrease in glutathione is seen with increasing clinical stage but this was not reported to be statistically significant. These differences show how colorectal tumor glutathione levels can vary from slightly higher than peritumoral tissue (T/C >1) to lower (T/C <1) depending upon the stage of the tumor. Barranco et al., (Barranco, 2000) noted no significant correlation between glutathione levels and any pathologic parameters however glutathione-to-normal tissue ratio and clinical stage were seen to be highly predictive of patient survival (see below). In the study by Lee et al., (Lee, 2007) of viral-associated hepatocellular carcinoma, patient data was limited but

well differentiated (n = 8), moderately differentiated (n = 16) and poorly differentiated (n = 2) tumors exhibited glutathione levels of 513 ± 238, 465 ± 194 and 210 ± 110 μM/g-protein, respectively. In contrast, no clear trend with clinical stage was observed with Stage I (n = 17), Stage II (n = 2), Stage III (n = 5) patient tumors contained 460 ± 219, 234 ± 76 and 442 ± 139 μM/g-protein, respectively.

Glutathione Levels in Relation to Therapy Response and Prognosis in Gastrointestinal Cancers

The studies of Kaur et al., (Kaur, 2008a; Kaur, 2008b) noted a decrease in both tumor glutathione levels and peritumoral tissue glutathione levels in response to chemotherapy. No data was collected in relation to patient outcome.

The study of Schipper et al., (Schipper, 2000) did not report specific glutathione levels in gastric cancer tissue samples but relative changes in levels in stage III and IV patients after 2 and 6 courses of chemotherapy. Patients with a partial response and stable disease showed increased tumor glutathione after 2 and 6 courses of therapy. Patients showing progressive disease initially displayed a decrease in glutathione after 2 courses that later reverted to pretreatment levels after 6 courses of chemotherapy. The authors concluded that glutathione levels were not predictive for response to chemotherapy.

Barranco et al., (Barranco, 2000) studied glutathione levels in relation to patient survival. Patients were divided into two groups; eleven patients in the group with glutathione levels greater than the mean, and 32 patients in the low group, less than the mean. For patients with high glutathione, the survival level at 24 months was 54% compared with 89% for the low group. The glutathione tumor-to-normal tissue ratio was found to be a significant predictor of survival. Only tumor stage proved to be a better predictor. They noted that for the first time, a significant association was found between colorectal tumor levels at the time of diagnosis and patient survival (Barranco, 2000).

Glutathione and Gastrointestinal Cancer -- Conclusions

Colorectal tumors generally appear to have increased glutathione levels compared to peritumoral tissue samples and these levels may have important implications for patient survival. For other gastrointestinal tumors, there are conflicting results but in general it appears that tumors of the esophagus, stomach and liver tend to exhibit lower glutathione levels compared to disease-free tissues. The clinical significance of glutathione levels in these other sites has not been probed but the promising results of Barranco et al., (Barranco, 2000) support further clinical studies in evaluating glutathione as a prognostic marker. Their results though emphasize the need for multiple sampling of tumor tissue to obtain an accurate assessment of glutathione content.

Gynecological Cancers

The published reports concerning glutathione levels in clinical samples of gynecological cancer are presented in Table 4 and divided between studies of ovarian and cervical cancers. The study of Osmak et al., (Osmak et al. 1997) assayed glutathione in tumor and peritumoral tissues of corpus uteri and is included in the cervical cancer section.

Tissue Preparation and Analytical Methods

The glutathione degrading enzyme γ-glutamyltranspeptidase is found in human gynecological cancers (Hanigan, 1999) so, ideally, tissue processing prior to analysis should take measures to reduce loss of glutathione by inactivating this enzyme. Most of the studies, processed the tissue at early stages with concentrated acid solutions to precipitate proteins

and one processed histological tissue sections with organic solvents (Hedley et al. 2005). Both these processes would inactivate γ-glutamyltranspeptidase. Four studies did not report specific precautions taken in regard to inactivating γ-glutamyltranspeptidase (Britten, Green and Warenius 1992; Guichard et al. 1990; Osmak, 1997; Sprem et al. 2001). One of these, noted a 25% decrease in tissue glutathione content upon standing 3 h at room temperature (Britten, 1992) but this time period was much longer than their typical sample processing time.

Most of the papers reviewed in this section utilized glutathione quantitative methods specific for this metabolite. Guichard et al., (Guichard, 1990) found cervical tumors contained cysteine, at approximately 14% of the glutathione levels. Only one paper (Djuric et al. 1990) reported non-protein sulfhydryl content rather than glutathione levels (italics Table 4).

Glutathione Levels in Gynecological Cancers

The levels of glutathione found in the gynecological tumor specimens are consistent across the studies. Most report glutathione levels between 0.3 (Kigawa et al. 1998b) to 126 nmol/mg-protein (Sprem, 2001) in ovarian cancer. This range is similar for four of the cervical cancer studies (Ahmed et al. 1999; Chang, Chang and Hsueh 1993; Guichard, 1990; Osmak, 1997). However, Jadhav et al, (Jadhav et al. 1998) and Balasubramaniyan et al., (Balasubramaniyan, Subramanian and Govindaswamy 1994) reported 10 to 500 times higher levels, respectively. Limited data is available on normal tissue levels but normal ovarian tissue ranges from 5 (Britten, 1992) to 50 (Sprem, 2001) nmol/mg-protein. The ranges for normal, healthy cervical tissue, like the tumor tissue, shows a wide range from approximately 40 nmol/mg-protein reported by Ahmed et al., (Ahmed, 1999) to more than 4000 nmol/mg-protein (4 μmol/mg-protein), detected by Balasubramaniyan et al., (Balasubramaniyan, 1994). Whether this reflects real differences in tissue content or reporting errors is unknown.

There are eleven studies listed in Table 4 in which glutathione levels are compared between gynecological tumors and peritumoral tissue (6 studies), or from tissue obtained from cancer-free patients (5 studies). One study compared tumor tissues to benign lesions (Abou Ghalia, 2000). No studies have determined whether peritumoral tissue glutathione levels differ from that of tissue from a tumor-free patient. Three of the five ovarian cancer studies showed higher levels of glutathione in tumor tissue compared to disease-free tissue (Abou Ghalia, 2000; Joncourt et al. 1998; Sprem, 2001). One study showed a trend toward higher glutathione in tumor tissue but was not considered statistically significant (Djuric, 1990). In three studies, benign ovarian lesions exhibited lower levels than malignant (Abou Ghalia, 2000; Sprem, 2001) or normal tissue (Sprem, 2001) but no significant difference between benign and peritumoral tissue (Djuric, 1990). Only Britten et al., (Britten, 1992) did not follow the trend toward increased levels of glutathione in tumors however, the relative levels depended on whether glutathione is normalized to protein or cell number (Table 4).

For the studies of tumors of the cervix and uterus, there is no clear trend as to whether glutathione in tumor tissue is higher or lower than peritumoral or healthy tissue. One study surveyed samples from 238 patients and 55 controls and is one of the largest in terms of patient enrollment (Balasubramaniyan, 1994). In this case, the glutathione levels were no different or lower in patient tumors compared to healthy tissue.

The greater inconsistency in the cervical cancer results may be due to real differences in the biochemistry of the cervix and ovary. Although the number of studies is relatively limited, this conclusion may be supported by results showing vastly different trends in glutathione levels in cervical and ovarian cancer with clinical stage (see below). Other inconsistencies may result from differences in sampling of the heterogeneous tumor tissue. Only two studies

(Chang, 1993; Vukovic, Nicklee and Hedley 2000) reported taking multiple samples from each tumor specimen to address this concern. The study of Chang et al., (Chang, 1993) showed the margins of the tumors had higher levels of glutathione when compared to the tumor tissue nearer the center.

Glutathione in Relation to Histological Grade or Clinical Stage in Gynecological Cancers

Three studies report a tendency for a glutathione increase with histological grade in ovarian tumors but none were statistically significant (Joncourt, 1998; Tanner et al. 1997; Hengstler et al. 2001). Studies of tumors of the cervix (Guichard, 1990) or uterus (Osmak, 1997) found no correlations with grade or degree of differentiation. One study found tumor glutathione levels lower in poorly differentiated versus well- and moderately-differentiated tumors (Ahmed, 1999).

Two studies involving a total of 178 patients, report an increase in glutathione levels in ovarian tumors with increase in FIGO stage (Joncourt, 1998; Tanner, 1997). In cervical cancer, two studies involving a total of 265 patients show that tumor glutathione levels markedly decreased in FIGO Stage III and IV tumors compared to Stages I and II (Ahmed, 1999; Balasubramaniyan, 1994). One study involving 18 patients ranging in FIGO Stage I to Stage III detected no correlation between stage and glutathione levels (Guichard, 1990). The latter study included only three Stage I tumors with the rest Stage II (7 patients) and Stage III (7 patients). Significant differences may not be apparent between these two stages in this limited sampling. This is borne out by the results of the Balasubramaniyam study (Balasubramaniyan, 1994) showing little difference between glutathione levels between Stage II and III in a more extensive study. The overall trend in the highest enrollment studies suggest that ovarian cancers show an increase in glutathione with increasing FIGO stage whereas the opposite is true for cervical cancer. This may help explain why ovarian tumor glutathione levels are consistently increased compared to disease-free tissue whereas cervical cancers show no clear trend.

Glutathione in Relation to Therapy Response and Prognosis in Gynecological Cancers

In a study of 139 patients with ovarian cancer, median glutathione levels were found to be 4.9μg/mg protein (Tanner, 1997). For 60 patients with tumor glutathione levels below this value, median survival time following chemotherapy was 2058 days compared to 839 days for patients with glutathione levels above 4.9 μg/mg protein. This study also reported glutathione levels increasing with increasing FIGO stage. This same group reported a larger study several years later (Hengstler, 2001). In the newer study, high levels of glutathione in combination with thiol-rich protein metallothionein was associated with poor outcome in patients with grade 1 tumors but not grades 2 or 3 (Hengstler, 2001). In other studies, there was no correlation between pretreatment glutathione levels and therapy response in ovarian (Ghazal-Aswad et al. 1996; Joncourt, 1998) or cervical (Hedley, 2005) tumors. In addition, no differences in mean glutathione levels were observed between a group of patients responding to therapy and non-responders (Kigawa et al. 1998a). However, these authors noted differences in gene expression for γ-glutamylcysteine synthetase, a key enzyme controlling glutathione biosynthesis, between responders and non-responders. Buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcysteine synthetase, was tested in a Phase I study by O'Dwyer et al., (O'Dwyer et al. 1996) on patients that had failed all other treatment options. In a limited study, they showed BSO did lower glutathione levels in 4 patients with ovarian cancer. One of these patients showed a partial response to melphalan treatment. Similarly, three of eight previously chemotherapy-resistant ovarian cancer patients treated with BSO showed some clinical response to melphalan (Bailey 1998). Although these initial results were promising, BSO also enhanced myelosuppression and no updated trials have been reported.

These results illustrate a key point in that biosynthetic capacity rather than steady-state levels likely plays a significant role in therapy response. For example, Cheng et al., (Cheng et al. 1997) monitored tumor levels before and after chemotherapy in ten patients responding to therapy and ten non-responders. There was *no difference* in the mean concentration of glutathione in the tumors from these two groups before and after combination chemotherapy. This would seem to support a conclusion that glutathione does not play a role in therapy response. However, when comparing the ratio of glutathione before and after therapy in each individual patient, nonresponders had significant increases in this ratio (3.0 ± 1.3) compared to responders (0.6 ± 0.3). In cervical cancer, a ≥70% decrease in glutathione levels were observed in patients with a complete response to radiotherapy whereas non-responders averaged <50% decrease (Jadhav, 1998). Two studies noted increased glutathione levels after therapy (Lewandowicz, 2002; Britten, 1992) and one study a decrease (Djuric, 1990). In a study of relapsed ovarian cancer patients prior to second line chemotherapy, glutathione content in non-responders (18.4 ± 9.7 μg/mg-protein) was more than twice that of responders (8.1 ± 11.4) (Kigawa, 1998b).

Glutathione and Gynecological Cancers -- Conclusions

The prognostic value of measuring glutathione in relation to response is not clear. Although one study sees significant differences in patient survival in relation to glutathione levels (Tanner, 1997) others do not. This does not appear to be due to differences in intracellular distribution of glutathione (Hedley, 2005) as some studies report multiple biopsy samples taken in a given tumor. There appears to be more consistency with the dynamic behavior of glutathione in response to therapy (Jadhav, 1998; Kigawa, 1998b). In the key study by Cheng et al., (Cheng, 1997) no difference was observed in average glutathione levels between responders and non-responders. However, tracking changes in individual patients with therapy showed that glutathione levels tend to increase in non-responders and decrease in responders. This points to the value of determining both glutathione levels and its regenerative capacity in tissue in response to therapy.

Head and Neck Cancers

The 20 published reports covering 19 studies reviewed here most commonly concern head and neck tumors originating in the oral cavity or the larynx (Table 5).

Tissue Preparation and Analytical Methods

Tumors of the oral, pharyngeal and laryngeal mucosa express varying activities of the glutathione degrading enzyme γ-glutamyltranspeptidase (Calderon-Solt & Solt 1985). Most of the published reports included a processing step in which enzymes and proteins were acid-denatured and precipitated from solution (Table 5). One study (Lafuente et al. 1998) did not appear to include such precautions and in three other studies (Guichard, 1990; Patel et al. 2007; Patel et al. 2008; Saroja, Balasenthil and Nagini 1999) it is unclear as to whether such steps were taken. A magnetic resonance study of untreated, excised tissue showed unchanged levels of metabolites after one hour at 25 C (Somashekar et al. 2011) supporting their conclusion that significant loss of metabolites, including glutathione, did not occur during their analyses.

A combination of glutathione-specific assays and total non-protein thiols (italics Table 5) are reported. Since cysteine levels are reported at approximately 25% of glutathione levels in head and neck cancers (Guichard, 1990), assays measuring total thiols should only be directly compared to the glutathione-specific results with caution.

Glutathione Levels in Head and Neck Cancers

The levels of glutathione in head and neck cancers mostly range between 10 – 90 nmol/mg-protein. One reported value is much higher than this range (Kacakci et al. 2009) and may be due to a typographical error in units reported. Despite the variation in anatomic sites all appearing under the classification of head and neck cancers, most clinical studies show statistically significant increases in glutathione levels in tumors compared to either normal controls or peritumoral tissue. Only one study (Patel, 2007), detected a statistically significant decrease in oral squamous cell carcinoma tissue compared to peritumoral tissue. This decrease of about 25% in total tissue thiol levels does not necessarily reflect glutathione content as these authors used an assay measuring total non-protein thiols. Since cysteine is present in relatively high concentration in oral cancer tissue (Guichard, 1990), the difference in thiol content may or may not reflect a difference in glutathione. In thyroid tissue, elevated glutathione levels were found in adenomas and papillary carcinomas but not follicular carcinoma (Sadani & Nadkarni 1996).

Whether peritumoral tissue glutathione levels are influenced by the presence of the tumor remains unresolved as the study of Fiaschi et al. (Fiaschi et al. 2005) showed that glutathione levels in disease-free peritumoral tissue from oral cancer patients was higher than from healthy tissue taken from cancer-free patients but Wong et al., (Wong et al. 1994) found no difference. It is not clear in these studies if the clinical stages of the patients were similar.

Glutathione in Relation to Grade, Stage or Response in Head and Neck Cancers

Histological grading in relation to glutathione was not presented in any of the works. Two studies (Inci et al. 2003; Lafuente, 1998) found no correlation between glutathione levels and the degree of differentiation. Wong et al (Wong, 1994) found a tendency toward higher levels of glutathione in well-differentiated oral carcinoma compared to tumors classified as moderately-differentiated that was not statistically significant.

Kolanjiappan et al., (Kolanjiappan, Ramachandran and Manoharan 2003) and Parise et al., (Parise et al. 1994) reported increasing glutathione levels with tumor stage. In addition the latter authors noted that glutathione increased with tumor size and nodal status. Guichard et al., (Guichard, 1990) found no correlation between glutathione level and clinical stage or degree of differentiation but was limited to primarily T2 and T3 patients. Similarly, Inci et al., (Inci, 2003) found no difference in glutathione levels when comparing patients grouped as either Stage I/II or Stage III. Wong et al., (Wong, 1994) found no correlation level with tumor size in oral carcinoma and Lafuente et al., (Lafuente, 1998) found no correlation with the extent of tumor penetration in larynx tumors.

No studies evaluated the relationship between patient outcome and glutathione measurements.

Glutathione and Head and Neck Cancers Conclusions

The general trend observed is that head and neck cancer tissue tends to have higher levels of glutathione than in disease-free tissue and tends to increase with clinical stage.

Lung Cancer

Tissue Preparation and Analytical Methods

Normal lung and lung tumors express activity of the glutathione-degrading enzyme γ-glutamyltranspeptidase (Blair et al. 1997). Activity of this enzyme is increased in a number of lung cancers (Blair, 1997; Korotkina et al. 2002) so this enzyme must be inactivated

during preparation of the tissues prior to glutathione analysis. Most studies incorporated a protein-precipitation step to prevent enzyme-catalyzed degradation. One study (Duarte et al. 2010) used unprocessed excised tissue for magnetic resonance analysis and in one other study, the tissue processing methods were less detailed as to whether proteins were removed prior to analysis (Ilonen et al. 2009).

Cysteine levels were found to be almost 30% of glutathione levels in lung carcinoma and 40% of glutathione levels in non-cancerous peritumoral tissue (Krepela et al. 1997). Krepela et al., also found that the cysteine levels were also significantly increased in tumor compared to noninvolved lung tissue (Krepela, 1997). This high level of cysteine emphasizes the need for caution when compare glutathione-specific assay levels to those reporting non-protein thiol levels (italics in Table 6).

Glutathione Levels in Lung Cancers

The levels of glutathione in lung parenchymal specimens is about four-fold higher than that found in tissue samples from the bronchial tree (Petruzzelli et al. 1988). Two studies concluded that glutathione levels did not differ between parenchymal tissue sampled from healthy patients and uninvolved parenchymal tissue from lungs with tumors (Bartsch et al. 1992; Petruzzelli, 1988). Glutathione levels in lung tumors range from 2 – 60 nmol/mg-protein or 1 – 3 μmol/g-tissue (Table 6). Ten of the twelve studies listed in Table 6 report glutathione levels in lung cancer and ‘normal’ disease-free peritumoral lung tissue. Most tissues samples were obtained from patients before therapy and many were collected from patients with a history of smoking. Smoking affects glutathione levels in both tumor and normal tissues (see below).

Tumor tissue glutathione levels were compared to disease-free peritumoral tissue with the exception of the study of Korotkina et al (Korotkina, 2002) in which tissue from disease-free lungs were used as controls. Another study has shown that the origin of control tissue is not critical as similar results were obtained when tumor levels were compared to normal peritumoral lung tissue from the same patient and normal tissue from disease-free patients (Saydam et al. 1997). The study of Ilonen et al., (Ilonen, 2009) also presented control data from patients treated for benign lung disease. These data fell within the range of values found for the non-involved peritumoral tissue from cancer patients in the same study.

Of the 10 studies where comparisons are made, most showed higher levels of glutathione in tumor tissue compared to disease-free lung tissue. Not included in the table are two patients from the study of Melloni et al., (Melloni et al. 1996) with squamous cell carcinoma showing increased levels of glutathione in the tumor tissue compared to peritumoral tissue. One factor that may affect results is whether patients are smokers, ex-smokers or non-smokers. Although many of the studies are from patients with a history of smoking, disease-free lung tissue from patients who are smokers or ex-smokers is lower than that of non-smokers (Oberli-Schrammli et al. 1994). Conversely, tumors from these patients show higher glutathione levels than tumors from patients with no smoking history (Oberli-Schrammli, 1994).

Glutathione Levels in Epithelial Lining Fluid

The availability of alveolar epithelial lining fluid (ELF) for sampling *via* bronchoalveolar lavage offers a unique window to study the biochemistry of lung tissue. Glutathione levels are at least 100-fold higher in ELF than in blood plasma in disease-free patients (Cantin et al. 1987). Plasma levels of glutathione are no different between smokers and non-smokers but ELF levels are almost two-fold higher in smokers (Neurohr et al. 2003; Cantin, 1987). In a comparison of glutathione levels in the ELF of non-smokers, disease-free smokers and

smokers with non-small cell lung cancer, Melloni et al., (Melloni, 1996) observed a 1.6-fold increase in glutathione in ELF from disease-free smokers as compared to non-smokers. In addition, they found a substantial 2.7-fold increase in glutathione in ELF from smokers with non-small cell lung cancer compared to the disease-free smokers. Similarly, Kontakiotis et al., (Kontakiotis et al. 2011) found a 1.6-fold increase in glutathione in ELF in lung cancer patients compared to healthy controls.

Glutathione Levels in Relation to Histological Grade or Clinical Stage in Lung Cancer

In many cases the literature reports on glutathione levels in lung cancer are classified by tumor histology. The results are presented in Table 6, with non-small cell cancers divided into adenocarcinoma (AC) and squamous cell carcinoma (SQ). Undefined large cell carcinoma and bronchoalveolar carcinoma specimens were place in the ‘other’ category as there were fewer reports of these tumor types.

The majority of patients enrolled presented with non-small cell lung cancer with AC or SQ. Of the 6 studies reporting glutathione levels grouped by histological type, three showed SQ levels distinctly higher than AC levels (Cook, 1991; Oberli-Schrammli, 1994; Rowell et al. 1989), one showed slightly higher levels (Blair, 1997) or equal levels (Saydam, 1997). Two studies reported lower levels of glutathione in SQ compared to AC (Ilonen, 2009; Korotkina, 2002). Studies on glutathione levels in small cell cancer (SCC) are less common. The largest sample, 6 of 55 total patients reported with SCC exhibited glutathione levels greater than AC but slightly less than SQ (Oberli-Schrammli, 1994). One other study identified a single patient (out of 7 total) with SCC and found this tumor to have lower levels than either AC or SQ (Rowell, 1989). Korotkina et al., (Korotkina, 2002) compared found glutathione levels in benign tuberculomas and hamartomas higher than in control tissue from disease-free lungs and similar to levels found in broachoalveolar carcinoma but much lower than levels in AC and SQ.

The relationship between glutathione and clinical stage is unclear. Krepela et al., (Krepela, 1997) in samples of SQ, found higher levels of glutathione in Stage III compared to Stage I tumors when normalized to tissue mass but no significant difference when normalized to total protein content. In a mixture of histological tumor types, Ferruzzi et al., (Ferruzzi et al. 2003) reported that glutathione levels may be higher in Stage I than in advanced stages but no data was presented likely due to the limited sample numbers. The data of Blair et al., (Blair, 1997) shows no clear differences in glutathione levels with stage across many histological types. Similarly Oberli-Schrammli et al., found no correlation between glutathione levels, extent of disease, tumor size or grade or patient age (Oberli-Schrammli, 1994). One study of 190 patients with bronchial carcinoma did not measure tumor glutathione but compared glutathione levels in disease-free peritumoral tissue from patients grouped by clinical stage (Bluhm & Greschuchna 1989). There was a slight but not significant increase in glutathione levels in the lung tissue of patients with stage II disease compared to stage I (Bluhm, 1989). However in stage III disease there was a statistically significant decrease in glutathione levels observed (Bluhm, 1989).

Glutathione in Relation to Clinical Outcome in Lung Cancer

Only the study Oberli-Schrammli et al., (Oberli-Schrammli, 1994) investigated the relationship between glutathione levels and other parameters in relation to clinical outcome. No relationship was found between these parameters and overall survival but the authors were hesitant to draw conclusions in this regard due to the low number of patients undergoing therapy.

Glutathione and Lung Cancer Conclusions

Lung cancer tissue was found to have increased glutathione levels compared to disease-free tissue.

Conclusions

Not surprisingly in human tissue samples, glutathione levels vary greatly. This inherent heterogeneity can be due to the different levels of protection required by the tissue during normal function. Accurately assessing glutathione concentrations in tissue however, is not straightforward. If possible, multiple tissue samples should be procured that will accurately reflect the average tissue content. Once samples are obtained, they should immediately be processed or frozen at −80 °C until analysis. Processing tissue under conditions that minimize degradation and/or oxidation is critical. Glutathione in tissue can be degraded by the enzyme γ-glutamyltranspeptidase. The data in the Tables indicates that the investigators, for the most part, took measures that would reduce or inhibit γ-glutamyltranspeptidase-catalyzed degradation. However the Table data reflects only that the authors stated or referenced procedures that included an acid-deproteinization step that would remove this enzyme from the assay mixture. Unfortunately, in some studies this deproteinization step does not occur early in the tissue processing procedure and therefore is open to potential losses in glutathione. Since this degradative activity varies substantially between tissues, the effect of the variability in tissue processing is unknown. To pre-empt any questions on enzyme-catalyzed degradation, de-proteinization should occur as quickly as possible or an inhibitor such as acivicin should be included during tissue processing (Roberts & Francetic 1993). None of the studies reported inclusion of acivicin or otherγ-glutamlytranspeptidase inhibitors in there processing methods. A caveat on acid denaturants must be included in that some strong acid denaturants may oxidize glutathione thereby this lowering the measured level of the reduced peptide (Hansen & Winther 2009). Inclusion of metal chelators also slows oxidation in tissue lysates. Lysates should be used as quickly as possible in assays dependent upon measuring reduced glutathione. Alternatively, blocking glutathione in its reduced form by reacting with tissue-permeable, highly reactive reagents early in the tissue processing steps avoids many problems commonly encountered in processing and analyzing tissue samples (Hansen, 2009). These blocked glutathione conjugates are not compatible with the widely used reductase-recycling assays for glutathione (e.g. (Roberts, 1993)) but can be tailored to HPLC-based analytical methods (e.g.(Guichard, 1990)). Future clinical studies should strive to consistently treat biopsy specimens with the goal in mind of reducing sample degradation and oxidation during the tissue processing steps.

Even with the wide variability in tissue processing to date, some conclusions can be drawn in regard to glutathione levels in human solid tumors. Compared to disease-free peritumoral or healthy tissue, glutathione levels tend to be elevated in breast, ovarian, head and neck and lung cancers. Conversely, brain and liver tumors, for the most part exhibit lower tissue levels of glutathione compared to healthy tissue. Other cancers show no clear trend as there are a significant number of studies reporting mixed results when comparing tumor tissue to disease-free peritumoral tissue or healthy tissue from cancer-free patients. An important question to ask is whether the tumor induces changes in glutathione, or other tissue biochemistry in nearby disease-free peritumoral tissue. Unfortunately only a few studies address this issue and those that do, draw conflicting conclusions.

Because there were few studies addressing this question, there is no clear trend as to differences in glutathione levels in relation to histological grade or clinical stage. Some studies did show intriguing relationships between high glutathione levels and poor patient outcome (Barranco, 2000; Hengstler, 2001; Kigawa, 1998b; Tanner, 1997), these results were not observed in other studies. In some cases trends are obscured when a tissue levels

from a patient population are averaged and therefore there is some value in following biochemical changes in individual patients in order to observe consistent changes in glutathione metabolism reflecting therapeutic response (Cheng, 1997). The value of assessing the metabolic capacity of a cancer cell to maintain glutathione in response to therapy is apparent. Patients with high glutathione levels and the capacity to maintain these levels would tend to be more resistant to therapy than those lacking the synthetic capability. Future studies evaluating both the level of glutathione and the presence of key enzymes in its synthesis and metabolism may offer the best prognostic factors.

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Table 1

Clinical Studies of Glutathione Levels in Brain Cancers

Study	Patients / Tumor[a]	Tumor Glutathione[b]	γGT[c]	Control[d]	Ratio T/C[e]
(Constantin et al. 2012)	53 Grade 2 glioma		N		
(Dudek et al. 2001)	20 AST	15.61 ± 4.90 nmol/mg-protein	Y	P	0.31*
	26 GBM	16.30 ± 4.29			0.32*
	16 MEN	29.36 ± 8.46			0.57*
	10 Other	16.90 ± 4.16			0.33*
(Dudek et al. 2002)	23 AST	14.2 ± 5.3 nmol/mg-protein	Y	P	0.27*
	31 GBM	14.72 ± 3.2			0.29*
	7 Other	16.11 ± 4.9			0.32*
(Dudek et al. 2004)	30 AST	26.42 ± 5.83 nmol/mg-protein[f]	Y	P	0.50*
	37 GBM	12.52 ± 3.91			0.24*
	22 MEN	31.45 ± 8.52			0.59*
	16 Other	31.46–20.69 ± 5.19			0.39*
(Dudek et al. 2005)	45 MET	3.6 – 28.4 nmol/mg-protein	Y	P	0.07 – 0.53*
(Hazany et al. 2007)	5 MEN	MEN > Other			
	20 Other				
(Kudo et al. 1990)	7 AST	321.9 ± 11.8* μg/g-tissue	Y	P	0.78
	10 GBM	195.2 ± 57.1*			0.46*
	5 MEN	614.4 ± 237.4			1.5
	9 Other				
	5 MET	271.6 ± 143.8			0.66
(Landolt et al. 1994)	11 AST	431 ± 71 μg/g-tissue	Y	P	0.90
		918 ± 135 μg/g-DNA			0.53*

Study	Patients / Tumor[a]	Tumor Glutathione[b]	γGT[c]	Control[d]	Ratio T/C[e]
(Louw et al. 1997)	6 AST Grade II	4580 ± 1675 nmol/g-tissue	Y		
	Grade III/IV	1131 ± 108 *			
(Opstad et al. 2003)	6 AST Grade II	1.0 ± 0.26 mM		H	0.83
	6 MEN	3.3 ± 1.5 *			2.8 *
(Pope et al. 2012)	27 glioma		Y		
(Suess et al. 1991)	14 glioma	1.547 ± 0.306 nmol/mg-tissue	Y	P	0.57
	8 MEN	3.861 ± 0.793 *			1.49 *
(Wright et al. 2010)	9 AST Grade II	0.52 ± 0.15 mM	N		
	Grade III	0.55 ± 0.11			
	12 GBM	0.93 ± 0.11			
	20 MEN	2.04 ± 0.25			
	8 MET	0.96 ± 0.30			
(Zengin et al. 2009)	16 AST/GBM	*4.923 ± 0.23 μg/mg-protein*	Y	P	0.61 *
	14 MEN	*5.749 ± 8.609*			0.67 *
	13 Other	*6.051 ± 0.63*			0.69 *
	11 MET	*5.117 ± 0.25*			0.63 *

[a] Number of patients with cancer in the study. Tumor type: AST, astrocytoma; GBM, gliobastoma multiforme; MEN, meningioma; MET, metastases to brain.

[b] Concentration of glutathione or thiols in tissue. Numbers in italics indicates reports of non-protein sulfhydryls rather than glutathione.

[c] Denotes whether degradation by γ-glutamyltranspeptidase was prevented (Y) or not (N) by sample processing methods.

[d] Disease-free brain tissue used as control: Peritumoral (P) or tissue from healthy patient (H).

[e] Ratio of tumor glutathione to control tissue levels. Gray shading indicates statistically significant T/C<1

[f] Units given in (Dudek, 2005) are IU/mg-protein; however, based on previous publications by this author, assumed to be nmol/mg-protein.

* Asterisk denotes statistically significant differences between tumor and control levels

Table 2

Clinical Studies of Glutathione Levels in Breast Cancers

Study	Pts[a]	Tumor Glutathione Level[b]	γGT[c]	Control[d]	Ratio[e] (T/C)
(Abou Ghalia & Fouad 2000)	26	4.6 ± 0.7 mM/g-protein	Y	B	2.1*
(Albin et al. 1993)	12	22 ± 23 nmol/mg-protein	Y	P	2.0
(Barranco et al. 1994)	42		Y	P	>1
(Buser et al. 1997)	85		Y	P	>1*
	Grade 1	40.1 nmol/mg-protein			
	Grade 2	24.6			
(Coban et al. 1998)	39		Y	P	4.0*
	Grade 1	*725 ± 555 nmol/g-tissue*			
	Grade 2	*1018 ± 1845*			
	Grade 3	*1218 ± 365*			
(El-Sharabasy et al. 1993)	23	*204.5 ± 33.11 mg/g-tissue*	Y	P	1.4*
(Honegger et al. 1988)	20	553.8 ± 153.9 μmol/g-DNA	Y	P	1.8*
		618.6 ± 388 μg/g-tissue			10.5*
(Iscan et al. 1998)	40	*257 ± 218 nmol/g-tissue*	Y	P	3.7*
(Kumaraguruparan et al. 2002)	30	17.45 ± 0.26 mg/100g-tissue	Y	P	2.3*
(Kumaraguruparan, Kabalimoorthy and Nagini 2005)	50	18.89 ± 4.21 mg/100g-tissue	Y	P	2.0*
	Stage I	18.17 ± 4.10			
	Stage II	18.60 ± 1.03			
	Stage III	20.63 ± 4.78			
(Langemann et al. 1989)	25	677 ± 242 μmol/g-DNA	Y	P	2.9*
	43[f]				2.3

Study	Pts[a]	Tumor Glutathione Level[b]	γGT[c]	Control[d]	Ratio[e] (T/C)
(Mourão de Farias et al. 2011)	20	*~75 μmol/g-tissue (post chemo)*	Y	P	~1
(Murray, Burke and Ewen 1987)	116	histological study			
(Perry et al. 1993)	35	14.9 ± 1.5 nmol/mg-protein	Y	P	2.1*
		913 ± 110 nmol/g-tissue			6.7*
(Perquin et al. 2000; Perquin et al. 2001)	41	8.3 ± 5.7 nmol/mg-protein	Y	P	12.4*
(Rzymowska & Dyrda 1993)	25		Y	P	0.88
	Stage I	*0.18 μmol/μg-protein*			
	Stage II	*0.19*			
	Stage III	*0.28*			
(Sreerama & Sladek 1997)	82		Y	H	4.3*
	primary	1130 ± 1990 nmol/mg-protein			
	metastases	2120 ± 3360			
(Sreerama & Sladek 2001)	16		Y		
	primary	265 ± 245 nmol/mg-protein			
	metastases	414 ± 406			
(Woolston et al. 2011)	224	histological study			
(Yeh et al. 2006)	112		Y	P	8.4*
	Stage I	498 ± 862 μM/g-tissue			
	Stage II	902 ± 2084			
	Stage III	230 ± 285			

[a] Number of patients, and histological grade or clinical stage.

[b] Concentration of glutathione or thiols in tissue. Numbers in italics indicates reports of non-protein sulfhydryls rather than glutathione.

[c] Denotes whether degradation by γ-glutamyltranspeptidase was prevented (Y) or not (N) by sample processing methods.

[d] Disease free tissue from healthy controls (H) or peritumoral tissue (P), or benign lesion (B).

[e] Ratio of tumor glutathione to control tissue levels. Gray shading indicates statistically significant T/C <1.

[f] Total patient data analyzed when combined with results of an earlier study (Honegger, 1988).

[*] Asterisk indicates statistically significant differences in glutathione levels.

Table 3

Clinical Studies of Glutathione Levels in Gastrointestinal Tumors

Study	#Pts[a]	Tumor Glutathione Level[b]	γGT[c]	Control[d]	Ratio[e] (T/C)
Colorectal					
(Barranco et al. 2000)	41 Colon	15.33 ± 1.4 nmol/mg-protein	Y	P	1.9 *
	24 Rectum	16.56 ± 23			1.3 *
(Baur & Wendel 1980)	8 Colon	12.6 nmol/mg-protein	Y	P	1.3
(Berger et al. 1994)	5 Colon	11.1 ± 1.7 nmol/mg-protein	Y	P	1.5
(Butler et al. 1994)	25 Polyp	~26.8 nmol/mg-protein	Y	P	1.4 *
	58 Colon	~25.1			1.3 *
(Grubben et al. 2006)	37 Colon		Y	H	0.75[f]
(Hengstler et al. 1998)	23 Colon	5.6 μg/mg-protein	Y	P	0.77
(Mekhail-Ishak et al. 1989)	17 Colon	5.7 ± 1.1 μmol/g-tissue	Y	P	1.9 *
(Murawaki et al. 2008)	41 Colorectal	~1.3 μmol/g-tissue	Y	P	~0.4 *
(Oliva et al. 1997)	43 Colorectal	11.1 ± 4 nmol/mg-protein	Y	P	3.1 *
(Ozdemirler et al. 1998)	10 Colorectal	*39.9 ± 11.0 nmol/mg-protein*	Y	P	1.4 *
(Redmond et al. 1991)	23 Colon	57.47 ± 5.06 nmol/mg-protein	Y	P	1.3 *
(Siegers et al. 1984)	10 Colon	*39 ± 13 nmol/mg-protein*	Y	P	0.87
	8 Sigmoid	*34 ± 4*			0.45 *
	113 Rectum	*52 ± 12*			0.65
(Skrzydlewska et al. 2001)	55 Colorectal	~1.1 μmol/g-tissue	Y	P	0.79 *
(Skrzydlewska et al. 2005)	81 Colorectal		Y	P	

Study	#Pts[a]	Tumor Glutathione Level[b]	γGT[c]	Control[d]	Ratio[e] (T/C)
	Stage II	174 ± 36 nmol/g-tissue			1.04*
	Stage III	156 ± 39			0.93*
	Stage IV	150 ± 48			0.89*
(Skrzydlewska et al. 2003)	62 Colorectal	175 ± 42 nmol/g-tissue	Y	P	1.1
Gastric					
(Czeczot et al. 2005)	10	*1.81 ± 0.83 μmol/mg-protein*	Y	P	0.69
(Eapen et al. 1998)	49	*68.2 ± 24.2 nmol/mg-protein*	Y	P	0.94
				H	1.5*
(Engin & Ferahkose 1990)	9	*0.791 ± 0.072 mg/g-tissue*	Y	P	1.1
				H	0.46*
(Hoppenkamps et al. 1984)	12	*31.60 ± 8.83 nmol/mg-protein*	Y	P	0.54*
				H	0.67*
(Mun et al. 2004)	13				<1
(Schipper et al. 2000)	15		?		
(Skrzydlewska, 2003)	18	392 ± 87 nmol/g-tissue	Y	P	1.2
Esophageal					
(Evans et al. 2002)	20 AC,12 SQ	2.2 mM	Y	P	1.0
(Kaur et al. 2008a; Kaur et al. 2008b)	40	*38.4 ± 11.9 μg/mg-protein*	Y	P	1.2
(Levy et al. 1999)	31			H	<1
(Peters et al. 1993)	6 SQ, 3 AC	36 ± 12 nmol/mg-protein	Y	P	0.95
(Sihvo et al. 2003; Sihvo et al. 2002)	21 AC	*1.30 ± 1.30 nmol/mg-protein*	?	H	0.45*
(Skrzydlewska, 2003)	18 SQ	315 ± 75 nmol/g-tissue	Y	P	1.4*
Liver					

Study	#Pts[a]	Tumor Glutathione Level[b]	γGT[c]	Control[d]	Ratio[e] (T/C)
(Abel et al. 2009)	13	0.60 ± 0.05 μmol/g-tissue	Y	P	0.41 *
(Abou Ghalia, 2000)	5	0.8 ± 0.04 mM/g-protein	Y	B	0.57
(Corrocher et al. 1986)	7	*6.57 ± 6.27 μmol/g-protein*	Y	P	0.31 *
				H	0.091 *
(Czeczot et al. 2006)	*15*	*4.62 ± 2.94 μmol/mg-protein*	Y	P	0.84 *
(Jungst et al. 2004)	23	*0.99 μmol/g-tissue*	Y	P	0.64
(Lee et al. 2007)	24	439.8 ± 198.4 μM/g-protein	Y	P	0.61 *

[a] Number of patients in the study with AC, adenocarcinoma; SQ, squamous cell carcinoma; or as designated.

[b] Concentration of glutathione or thiols in tissue. Numbers in italics indicates reports of non-protein sulfhydryls rather than glutathione.

[c] Denotes whether degradation by γ-glutamyltranspeptidase was prevented (Y) or not (N) by sample processing methods.

[d] Disease free tissue from healthy controls (H); peritumoral tissue (P) or benign lesion (B).

[e] Ratio of tumor glutathione to control tissue levels. Gray shading indicates statistically significant T/C<1

[f] Ratio of peritumoral to control healthy tissue

[*] Asterisk indicates statistically significant differences in glutathione levels.

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Table 4

Clinical Studies of Glutathione Levels in Gynecological Cancers

Study	Pts [a]	Tumor Levels[b]	γGT[c]	Cntrl[d]	Ratio[e] (T/C)	Clinical Response
Ovarian						
(Abou Ghalia, 2000)	13	11.6 ± 2.4 mM/g-protein	Y	B	2.1 *	
(Bailey 1998)	8	225–325 [f] ng/mg-tissue	Y			Levels ↓ with BSO
(Britten, Green and Warenius 1992)	16	1.9 ± 0.1 nmol/mg-protein 1.11 ± 0.08 nmol/10^6 cells	N	H	0.35 4.1	Levels ↑ with therapy
(Cheng et al. 1997)	20	0.2 – 52.8 μg/mg-protein	Y			Levels ↑ in non-responders after therapy
(Djuric et al. 1990)	37	*379 ± 67 nmol/ml-cytosol*	Y	P	1.7	Levels ↓ after therapy
(Ghazal-Aswad et al. 1996)	39	3758 – 8351 nmol/g-dry wt	Y			No correlation pretreatment levels and response
(Hengstler et al. 2001)	189		Y			Some correlation pretreatment levels and response
(Joncourt et al. 1998)	39	16.8 Stage I–II nmol/mg-protein 35.3 Stage III 49.2 Stage IV	Y	P	>1 *	No correlation pretreatment levels and response
(Kigawa et al. 1998a)	32	2.1 – 53.8 μg/mg-protein	Y			No correlation pretreatment levels and response
(Kigawa et al. 1998b)	26	0.1 – 52.8 μg/mg-protein	Y			Levels ↑in non-responders
(O'Dwyer et al. 1996)	4	64.0 ± 89.5 [f] nmol/mg-protein	Y			Levels ↓ with BSO
(Sprem et al. 2001)	15	126.3 ± 12.8 * nmol/mg-protein	N	H	2.6 *	
(Tanner et al. 1997)	139	4.4 Stage I–II μg/mg-protein 6.0 Stage III–IV *	Y			↑pretreatment levels correlate with ↓survival
Cervical						
(Ahmed et al. 1999)	27	All 6.72 ± 2.68 μg/mg-protein Stage I 9.12 ± 2.1	Y	H	0.50 *	

Study	Pts [a]	Tumor Levels[b]	γGT[c]	Cntrl[d]	Ratio[e] (T/C)	Clinical Response
		Stage II 8.58 ± 2.27				
		Stage III 4.85 ± 1.12				
		Stage IV 4.1 ± 0.85				
(Balasubramaniyan, Subramanian and Govindaswamy 1994)	238	64.72 ± 9.71 Stg I μmol/mg-protein	Y	H	1.0	
		49.41 ± 11.40 Stage II			0.77*	
		43.65 ± 10.14 Stage III			0.69*	
		32.73 ± 7.26 Stage IV			0.51*	
(Chang, Chang and Hsueh 1993)	2	38.1±10.1 nmol/mg-prot Case 1	Y	P	2.0*	
		39.0 ± 9.0 Case 2			1.0	
(Guichard et al. 1990)	18	25.0 nmol/mg-protein	N	P	~1	
(Hedley et al. 2005)	58		Y			No correlation pretreatment levels and response
(Jadhav et al. 1998)	45	1.6–5.9 μmol/mg-protein	Y	H		Responders show greater↓ than non-responders
(Osmak et al. 1997)[g]	30	110.1 ± 46.4 nmol/mg-protein	N	P	0.87	
(Vukovic, Nicklee and Hedley 2000)	10	2.86 ± 0.15 mM	Y	P	~2	

[a] Number of patients with cancer in the study

[b] Concentration of glutathione or thiols in tissue. Numbers in italics indicates reports of non-protein sulfhydryls rather than glutathione.

[c] Denotes whether degradation by γ-glutamyltranspeptidase was prevented (Y) or not (N) by sample processing methods.

[d] Disease free tissue from healthy controls (H); peritumoral tissue (P) or benign lesion (B).

[e] Ratio of tumor glutathione to control tissue levels. Gray shading indicates statistically significant T/C<1

[f] Tumor levels from chemotherapy-resistant ovarian cancer patients.

[g] Tumor and normal tissue from corpus uteri.

* Asterisk indicates statistically significant differences in glutathione levels.

Table 5

Clinical Studies of Glutathione Levels in Head and Neck Cancers

Study	Pts[a]	Cancer Site / Type[b]	Tumor Glutathione Levels[c]	γGT[d]	Control[e]	Ratio[f] (T/C)
(Balasenthil et al. 2000)	10	Oral SQ	26.5 ± 2.6 mg/100g-tissue	Y	H	1.3*
(Caglar et al. 2001)	28	Larynx SQ	*0.69 ± 0.73 μmol/g-tissue*	Y	P	1.0
(Ferruzzi et al. 2003)	23	Oral, Oropharynx, Larynx SQ	34.8 ± 17.4 nmol/mg-protein	Y	P	1.4*
(Fiaschi et al. 2005)	18	Oral SQ	25.71 ± 4.976 nmol/mg-protein	Y	P	2.2*
					H	5.9*
(Guichard, 1990)	17	Oral, Oropharynx	21.5 ± 9.4 nmol/mg-protein	?	H	0.92
(Inci et al. 2003)	20	Larynx SQ	*23.39 ± 5.8 nmol/mg-protein All tumors*	Y	P	1.5*
			22.63 ± 7.51 Stage I/II			
			24.23 ± 3.45 Stage III			
(Janot et al. 1993)	9	Sinus SQ	47 ± 20 nmol/mg-protein	Y	P	2.4*
(Kacakci et al. 2009)	30	Larynx SQ	*30.14 ± 6.37 mg/mg-protein*	Y	H	1.0
(Kolanjiappan, Ramachandran and Manoharan 2003)	48	Oral SQ	*10.90 ±1.30 nmol/mg-protein Stage II*	Y	H	1.6*
			15.8 ± 1.30 Stage III			2.3*
			20.90 ± 4.20 Stage IV			3.1*
(Lafuente et al. 1998)	37	Larynx SQ	*93.05 ± 68.93 nmol/mg-protein*	N	P	1.9*
(Mulder et al. 1995)	25	Oral/Oropharynx	26.9 ± 1.6 nmol/mg-protein (Oral)	Y	P	0.88
		Larynx SQ	40.9 ± 4.9 (Larynx)			1.7*
(Nagini, Manoharan and Ramachandran 1998)	24	Oral SQ	*17.40 ± 4.27 nmol/mg-protein*	Y	H	2.8*
(Parise et al. 1994)	18	Oral, Oropharynx; Larynx. Hypopharynx	15.2 ± 8.2 nmol/mg-protein All tumors	Y	P	1.8*
			13 ± 6 Stage II			1.3

Study	Pts[a]	Cancer Site / Type[b]	Tumor Glutathione Levels[c]	γGT[d]	Control[e]	Ratio[f] (T/C)
			16 ± 9 Stage III			2.2
			17 ± 10 Stage IV			2.1*
(Patel et al. 2007; Patel et al. 2008)	32	Oral SQ	*0.0112 ± 0.00086 mmol/dL*	?	P	0.75*
(Sadani & Nadkarni 1996)	44	Thyroid	*3.4 ± 0.6 F Adenoma*[g] *μM/g-tissue*	Y	H	1.2*
			2.8 ± 0.1 F Carcinoma			0.97
			3.4 ± 0.4 P Carcinoma			1.2*
(Saroja, Balasenthil and Nagini 1999)	33	Oral SQ	*29.56 ± 3.87 mg/100g-tissue*	Y	H	1.4*
(Somashekar et al. 2011)	22	Oral, Larynx		N	P	≫1*
(Subapriya et al. 2002)	24	Oral SQ	*~28 mg/100g-tissue*	?	P	1.4*
(Wong et al. 1994)	26	Oral Epidermoid	24.36 ± 18.03 nmol/mg-protein	Y	P	8.0*
		Carcinoma			H	5.0*

[a] Number of patients.

[b] SQ, squamous cell carcinoma.

[c] Concentration of glutathione or thiols in tissue. Numbers in italics indicates reports of non-protein sulfhydryls rather than glutathione.

[d] Denotes whether degradation by γ-glutamyltranspeptidase was prevented (Y) or not (N) by sample processing methods.

[e] Disease free tissue from healthy controls (H); peritumoral tissue (P) or benign lesion (B).

[f] Ratio of tumor glutathione to control tissue levels. Gray shading indicates statistically significant T/C<1

[g] This study reported levels in Follicular Ademona, Follicular Carcinoma, Papillary Carcinoma

* Asterisk indicates statistically significant differences in glutathione levels

Table 6

Clinical Studies of Glutathione Levels in Lung Cancer

Study	Pts[a]	Type[b]	Tumor Glutathione Levels[c]	γGT[d]	Control[e]	Ratio[f]
(Blair et al. 1997)	20	AC, SQ, Other	20.8 ± 9.4 nmol/mg-protein	Y	P	1.8*
(Bluhm & Greschuchna 1989)	190		3.1 ± 0.3[f] Stage I μg/ml	Y		
			3.9 ± 0.4 Stage II			
			2.3 ± 0.4 Stage III			
(Duarte et al. 2010)	24	AC, other		N	P	>1*
(Ferruzzi, 2003)	29	AC, SQ, Other	24.1 ± 12.0 nmol/mg-protein	Y	P	1.8*
(Ilonen et al. 2009)	22	AC	*20.8 nmol/mg-protein*	?	P	1.8*
		SQ	*6.90*			1.4
(Korotkina et al. 2002)	72	AC	5.166 ± 0.630 nmol/mg-protein	Y	H	2.9*
		SQ	4.650 ± 0.323			2.6*
		other	2.836 ± 0.521			1.6*
		benign	2.75			1.5*
(Krepela et al. 1997)	21	SQ	63.2 ± 7.0 nmol/mg-protein	Y	P	2.5*
			1.80 ± 0.23 μmol/g-tissue			2.3*
(Oberli-Schrammli et al. 1994)	55	AC	27.1 nmol/mg-protein	Y	P	3.5*
		SQ	47.2			3.9*
		LCC	57.5			4.3
		SCC	41.4			4.6*
(O'Dwyer, 1996)	4		1.91 – 33.0 nmol/mg-protein			
(Rowell et al. 1989)	7	AC	*1.79 mM*	Y	P	2.1
		SQ	*3.54*			3.3

Study	Pts[a]	Type[b]	Tumor Glutathione Levels[c]	γGT[d]	Control[e]	Ratio[f]
		Other	*4.31*			4.7
		SCC	*0.84*			0.59
(Saydam et al. 1997)	38	AC, SQ	3.10 ± 0.45 μmol/g-tissue	Y	P	0.86
(Toussaint et al. 1993)	12	AC, SQ, LCC	10 ± 8 nmol/mg-protein	Y	P	1.0

[a] Number of patients in the study.

[b] Tumor type AC, adenocarcinoma; CONT, control, cancer-free patients; SCC, small cell carcinoma; SQ, squamous cell carcinoma; LCC, large cell carcinoma.

[c] Concentration of glutathione or thiols in tissue. Numbers in italics indicates reports of non-protein sulfhydryls rather than glutathione.

[d] Denotes whether degradation by γ-glutamyltranspeptidase was prevented (Y) or not (N) by sample processing methods.

[e] Disease free tissue from healthy controls (H); peritumoral tissue (P).

[f] Ratio of tumor glutathione to control tissue levels. Gray shading indicates statistically significant T/C<1

[*] Asterisk indicates statistically significant difference in levels of glutathione between tumor and nontumor tissue.