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Glutathione as Potential Target for Cancer Therapy; More or Less is Good? (Mini-Review)

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Abstract

Glutathione (GSH) plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular processes, including cell differentiation, proliferation and apoptosis. Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and pathogenesis of many diseases, one of which is cancer. The GSH content of cancer cells is associated with multidrug and radiation resistance. Just as low intracellular GSH levels decrease cellular antioxidant capacity, elevated GSH levels generally increase antioxidant capacity and resistance to oxidative stress, and this phenomenon is observed in may cancer cells as compared to normal ones. The present review will address the following questions: what is cancer-glutathione relation? Can glutathione play a role in treating or preventing cancer?

Keywords: Glutathione, antioxidant, cancer, prevention.

1. Introduction

It is amazing how a tripeptide composed of cysteine, glutamic acid and glycine can be of this importance for cellular function. Glutathione (L-g-glutamyl-L-cysteinylglycine) is the principal tripeptide thiol involved in the antioxidant cellular defense (Clark et al., 1984; Vojislav et al., 2001; Ganesaratnam et al., 2004). The most two important structural features of GSH are: γ-glutamyl linkage and sulphydryl group (–SH). It is the thiol of cysteine residue that composes the active group (Figure 1) (Kaplowitz et al., 1985).

GSH is a tripeptide produced by the liver and is able to detoxify the lungs, RBCs, liver and the intestinal tract; it also removes a wide range of toxins, including those produced by heavy metals, cigarette smoke, alcohol, radiation and cancer chemotherapy. Glutathione neutralizes oxygen molecules before they cause damage to cells. It is found in two forms: free or bound to proteins. Free form is present mainly in its reduced form (GSH), which can be converted to the oxidized form (GSSG) during oxidative stress, and can be reverted to the reduced form by the action of the enzyme glutathione reductase (Ames 1989; Ames et al., 1993) (Figure 2).

In normal conditions, the GSH concentrations in mammalian cells can range between 1 and 10 mM, with the reduced GSH predominating over the oxidized form (Hassan and Fridovich, 1980). Maintaining optimal GSH:GSSG ratios in the cell are critical for survival, and a deficiency of GSH can result in oxidative damage. This ratio exceeds 100 in a normal resting cell, whereas in various models of oxidative stress, this ratio was reported to decrease to values between 10 and 1 (Hassan and Fridovich, 1980).

Glutathione is synthesized in the cell by the sequential actions of γ-glutamylcysteine synthetase (GCS) and glutathione synthetase (GS) in a series of six-enzyme-catalysed reactions (Meister and Anderson, 1983). This review will highlight the importance of GSH homeostasis in cancer therapy.

2. Reactive Oxygen Species and Human Diseases

Due to different roles of reactive oxygen species (ROS) in cell signaling and many human pathological processes, imbalance of GSH is observed in a wide range of pathologies, including cancer, neurodegenerative disorders, cystic fibrosis (CF), HIV, and aging (Townsend and Tew, 2003; Ganesaratnam *et al.*, 2004; Ken *et al.*, 2004; Hayes *et al*., 2005). Maintaining proper GSH levels and oxidation state are important for cell function and their disruptions are observed in many human diseases. GSH deficiency leads to an increased susceptibility to oxidative stress and, thus, progression of many disease states (Townsend and Tew, 2003; Ganesaratnam *et al.*, 2004; Ken *et al*., 2004; Hayes *et al*., 2005). On the other hand, elevated GSH levels increase antioxidant capacity and resistance to oxidative stress and this is observed in many types of cancer (Townsend and Tew, 2003; Ganesaratnam *et al*., 2004; Ken *et al*., 2004; Hayes *et al*., 2005).

Free radicals produced by normal cellular metabolism can lead to extensive damage to DNA, protein, and lipid (Olinski *et al*., 1992; Okamoto *et al*., 1994; Devi *et al*., 2000; Wu *et al*., 2004).

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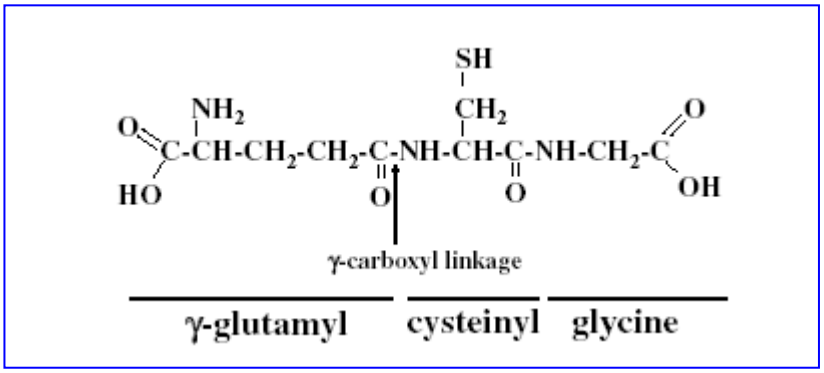

Figure 1. Structure of GSH or γ-glutamylcysteinyl glycine. The N-terminal glutamate and cysteine are linked by the γ-carboxyl group of glutamate (Kaplowitz, *et al.*, 1985).

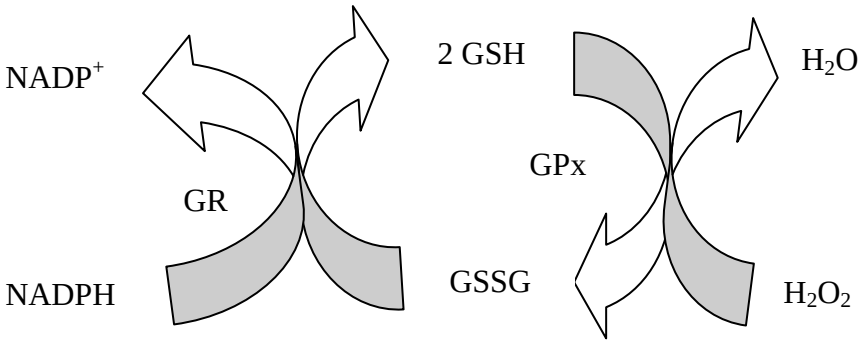

Figure 2. Pathway of ROS clearance (Adopted from Droge, 2002). Oxidants such as H_2O_2 is converted to H_2O by the action of GPX (or Catalase) using GSH. Regeneration of GSH requires NADPH and GR enzyme.

GR=glutathione reductase

GPx=glutathione peroxidase

DNA accumulates oxidative damage induced by ROS generated by endogenous and exogenous sources. This damage is a major contributor to diseases such as cancer, heart disease, cataracts, brain dysfunction, and aging (Ames, 1989; Ames, Shigenaga *et al*., 1993). It is estimated that the number of oxidative hits to DNA per cell per day is around 100,000 in the rat and 10,000 in the human (Ames, Shigenaga *et al*., 1993). It is possible that oxidative lesions in endogenous mammalian DNA exceeds 100 different types, of which 8-hydroxyguanine (8-oxoG) is one of the most abundant (Ames, 1989; Ames, Shigenaga *et al.*, 1993). In normal functional cells, DNA repair enzymes efficiently remove most of the lesions formed by ROS. Several different methods are used to remove any mutation or mismatch (Demple and Harrison, 1994). In 2008, Petta *et al*., have shown the role of human DNA polymerase iota in protecting cells against oxidative stress (Petta *et al.*, 2008). However, increased ROS generation in cancer cells leads to the accumulation of oxidative products of DNA, proteins, and lipids in tissues, and their release into the blood and urine. DNA oxidative products (8-oxoG), and lipid peroxidation have been detected in many cancer tissues, such as colorectal adenocarcinomas, mammary ductal carcinomas, renal cell carcinoma, and blood samples from leukemia patients (Olinski *et al*., 1992; Okamoto *et al*., 1994; Devi *et al*., 2000; Wu *et al*., 2004).

3. GSH and Cancer

Many types of cancer cell have increased levels of free radicals and ROS compared with their normal counterparts (Toyokuni, Okamoto *et al.*, 1995; Kawanishi, Hiraku *et al.*, 2006). However, several studies using primary cancer tissues have revealed increased levels of ROS-scavenging enzymes and antioxidant compounds (Goodwin and Baylin, 1982; Oltra *et al*., 2001). This increase could be a result of an adaptive response to intrinsic ROS stress.

While GSH is important in the detoxification of carcinogens, its elevated state in many types of tumors may also increase resistance or alters the cytotoxicity of many chemotherapy drugs or radiation (Clark *et al*., 1984; Vojislav *et al*., 2001; Ganesaratnam *et al*., 2004). One example is human fibroblast tumor cell lines which has higher levels of cellular GSH than did normal human fibroblasts (Goodwin and Baylin, 1982; Carney *et al*., 1983). This increased GSH may be an important factor in chemo- or radiotherapy resistance seen in these cells (Yu and Brown, 1984; Guichard *et al.*, 1986).

Manipulation of intracellular GSH using drugs such as 2-oxothiazolidine-4-carboxylate (OTZ) (a compound that stimulates GSH synthesis (Williamson *et al.*, 1982) or L-buthionine-(S,R)-sulfoximine (BSO) (a compound that inhibits GSH synthesis (Griffith *et al*., 1979)) has been used to increase the sensitivity of different tumor cell lines to therapy and showed that selective differential

chemotherapy responses of normal versus tumor cells is possible (Griffith *et al.*, 1979; Williamson *et al.*, 1982).

It has been shown that manipulating intracellular oxidant status of tumor cells can be of clinical value. Increasing ROS or decreasing free radical scavengers such as GSH was shown to be toxic to tumor cells. Weydert *et. al*.(2008) have shown that combining GSH depletion using 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) chemo-therapy with superoxide dismutase (SOD) gene therapy could be extremely successful in the treatment of breast cancer. Simon *et al*. (2007) have also shown that BSO sensitizes cancer cells to chemotherapy agents. Combining agents that induce mitochondrial dysfunction, such as AZT, and GSH depletion with BSO causes significant toxicity in head and neck cancer. However, it's important to note that different cells respond differently to oxidative stress inducing therapies (Mattson *et al.*, 2009).

Other factors might play an important role in the GSH therapy mechanism and should be considered when using GSH manipulation drugs. One important factor is a group of transferases enzymes called glutathione-S-transferases (GSTs). Elevated levels of GST in many tumor cell types have been demonstrated to limit the effectiveness of chemotherapy (Blair *et al*., 1997; Cullen *et al*., 2003). Moreover, GSTs have been associated with multidrug resistance of tumor cells, and over expression of GSTs can increase susceptibility to carcinogenesis and inflammatory disease (Townsend and Tew, 2003; Ganesaratnam *et al.*, 2004; Ken *et al*., 2004; Hayes *et al*., 2005). One mechanism by which chemotherapy resistance may occur is by gene amplification of GST(s). It has been shown that over expression of the gene products of GST-π, can provide a tumor cells with survival advantage relative to normal cells. High GST-π, expression was associated with poor overall survival and may be associated with a more aggressive phenotype in head and neck squamous cell carcinoma (Shiga *et al.*, 1999; Ulrike *et al.*, 2002; Cullen *et al.*, 2003).

4. GSH level, more or less is better?

GSH is involved in a variety of cellular functions such as DNA repair, cell cycle, regulation of cell signaling and transcription factors (Arrigo, 1999). It has been reported that GSH can modulate the activity of multiple stress genes which act to regulate the genes of cell proliferation, differentiation and apoptosis (Wiseman and Halliwell, 1996).

The fact that the changes in the intracellular GSH/GSSG ratio are critical for activation of cell proliferation and cell death makes it a very important to consider when using any treatment that has an effect on intracellular GSH levels. As shown in Figure 3, a higher level of GSH (left side of Figure 3) is important for normal cellular functions, signal transduction and protection against certain carcinogens. However, this high level (whether induced by certain drugs or as normal response to stimulants) can slow down any effective cancer treatment that works by increasing intracellular ROS (Figure 3).

On the other hand, when intracellular GSH levels are low (using certain drugs such as BSO), the cells are more vulnerable to ROS attacks. Increased ROS might activate different intracellular oncogenic pathways or mutate a tumor suppressor gene pathway, which will activate a tumorigenesis process (Irani *et al.*, 1997; Komatsu *et al*., 2008). Because the increase of ROS in cancer cells maybe part of the initiation and progression of cancer, such intrinsic oxidative stress is often viewed as an adverse event. However, as excessive levels of ROS stress can also be toxic to the cancer cells and cells are likely to be more vulnerable to damage by further ROS induced by exogenous drugs and make them more responsive to ROS producing cancer treatments (Figure 3). Therefore, changing ROS levels by GSH modulation is a way to selectively kill cancer cells without causing significant toxicity to normal cells (Trachootham *et al.*, 2009). It is important to take into consideration that under increased levels of ROS, certain cancer cells may acquire some cancerous measures such as: proliferation, immortalization, and metastasis (Behrend *et al.*, 2003; Hu *et al.*, 2005; Makiya 2008).

5. Recommendations

It is clear that different cancer cells respond differently to certain cancer therapies. This difference could be due to inherent features of these cells or could be due to the nature of the action of drugs. In general, future drugs should be able to increase ROS production and used in combination with other drugs that interfere with ROS scavenging at the same time. It is important to find out whether these cells have a drug resisting mechanisms. These mechanisms can reverse the drug effect and implicate the need of higher doses.

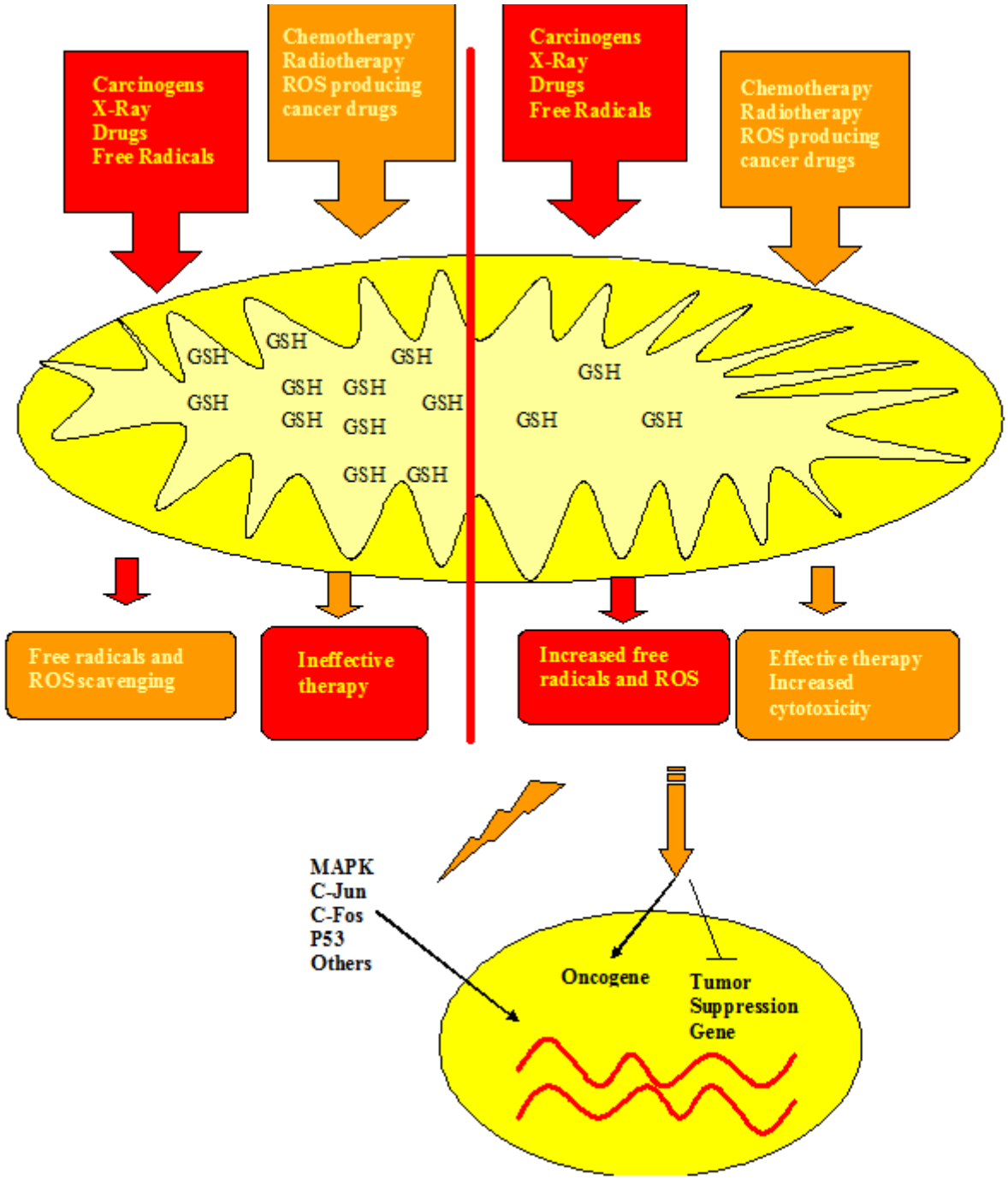

Figure 3. Glutathione level can affect expected outcome. High level of GSH (left side) is needed for cellular functions, signal transduction and protection against certain carcinogens. However, it can slow down any effective cancer treatment that works by increasing intracellular ROS. Low GSH levels (right side) renders cells more vulnerable to ROS attacks. Increased ROS activates intracellular oncogenic pathways or mutate a tumor suppressor gene pathway, which will activate a tumorigenesis process.

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