Modulation of p53 and c-myc in DMBA-Induced Mammary Tumors by Oral Glutamine

Valentina K. Todorova, Yihong Kaufmann, Shaoke Luo, and V. Suzanne Klimberg

Abstract: Previous studies established that oral glutamine (GLN) reduced tumor development in implantable and 7,12-dimethylbenz(a)anthracene (DMBA)-induced breast cancer models. This finding was associated with a decrease in tumor glutathione (GSH) levels, while maintaining normal gut, blood, and breast GSH. Alterations in GSH levels contribute to the control of apoptotic and cell cycle-regulating signaling. The aim of this study was to examine the role of dietary GLN on activation of p53 and c-myc, which play critical roles in cancer development and sensitivity to radiation and chemotherapy. Mammary gland carcinomas were induced in rats by DMBA. The rats were gavaged daily with GLN or water (controls), starting 1 wk prior DMBA-application and throughout the duration of the experiment (11 wk after DMBA). Tumor DNA was examined for mutations in p53 exons 5 and 6. Protein and mRNA levels of p53, p21WAF1/CIP1, PTEN, IGF-IR, mdm2, and c-myc in tumors of GLN-supplemented rats were compared with those of the control rats (received water). The sequencing of p53 showed that it was wild type. Increased phosphorylation of p53, as well as higher mRNA and protein levels of p21WAF1/CIP1, PTEN, and mdm2, and lower levels of IGF-IR were detected in tumors of GLN-supplemented rats vs. controls. Both phosphorylated c-myc and c-myc mRNA levels were reduced by GLN. The up-regulation of tumor p53 signaling and down-regulation of c-myc, in addition to previously established inhibition of Akt signaling in DMBA-breast cancer model, suggest that dietary GLN could be a useful approach for increasing the effectiveness of cancer treatment.

Introduction

Glutamine (GLN) via glutamate is one of the precursors for the synthesis of the natural antioxidant glutathione (GSH). The oxidation of GSH to glutathione disulfide (GSSG) plays a central role in the protection against genotoxic agents and oxidants (1,2), as well as in the control of cellular thiol/disulfide redox state, which is essential for normal redox signaling (3,4). Several studies established a relationship between cellular GSH concentration and proliferation. Higher intracellular GSH levels stimulate the proliferation, whereas GSH depleting agents such as buthionine sulfoximine (BSO) inhibit cell growth in cultured cells (reviewed in 5). The antiproliferative effect of BSO could be reversed by supplemental GSH or GSH precursors (6,7). The GSH/GSSG ratio, which determines intracellular redox state has been implicated in the activation of signaling molecules (8,9), including p53 (10,11) and regulation of cell proliferation, differentiation and apoptosis (12–15).

Resistance of tumor cells to anti-cancer treatment is often associated with increased GSH and GSH/GSSG in transformed cells, suggesting that tumor GSH depletion would increase the sensitivity of tumor cells to radiation and chemotherapy (16–18). There is no direct evidence that GLN availability affects cellular redox state (1), however several reports suggest that supplementation with GSH constituent amino acids inhibit tumor promotion, at least in part, by their interference with the GSH metabolism (19,20). Our previous studies found that dietary GLN caused a significant decrease in tumor GSH levels with a resulting reduction in GSH/GSSG (21,22) in 7,12-dimethylbenz(a)anthracene (DMBA)-breast cancer model. In the normal, non-tumorous mammary tissues, serum and gut mucosa, however, GLN caused a several-fold increase in GSH content (23). Moreover, the differential effect of GLN supplementation on GSH synthesis in tumors and normal tissues was detected in clinical studies (24,25).

In the present study, we investigated the hypothesis that oxidative stress resulted from the reduction of tumor GSH by GLN supplement, stimulates p53-apoptotic signaling in DMBA-induced mammary tumors.

Materials and Methods

Experimental Animals and Treatment

All studies were approved by the Animal Care and Use Committee at the Central Arkansas Veterans Healthcare System. Thirty-two age-matched female Sprague-Dawley rats...
(36 days old), purchased from Harlan Sprague-Dawley, Inc (Indianapolis, IN) were housed in polycarbonated rat shoeboxes with bedding, maintained on a 12:12 light/dark cycle and given 1 wk to acclimate to the animal care facility. During that time, the rats were allowed ad libitum intake of standard rat chow (Purina Rodent Chow 5001, Purina Inc., St Louis, MO) and water. During the whole experiment, all rats were pair-fed the purified research diet TD 96163 (Teklad, Madison, WI) and given water ad libitum. Pair-feeding is necessary because the tumor and/or the various treatments may depress food intake. TD96163 diet is soy-free casein-based diet, which omits artificial anti-oxidants and supplies the recommended concentration of mineral elements and vitamins. This diet contains 1.84% of its protein as GLN; thus, no group is GLN-deprived. At the age of 43 days, the rats were divided randomly into 2 treatment groups, GLN-supplemented (n = 16) and controls (n = 16), and were gavaged GLN (1g/kg body weight) or water daily for the duration of the entire experiment. GLN suspension formulation AES-14 was kindly provided by Aesgen Inc. (MG Pharma, Princeton, NJ). The dose of GLN has been determined previously and has no side effects (25). Pair-feeding is necessary because the tumor and/or the various treatments may depress food intake. At the age 50 days, the rats were gavaged with a single dose of 100 mg/kg body weight DMBA in sesame oil or with sesame oil alone (vehicle control). At this age (50–60 days) in Sprague-Dawley rats, this dose of DMBA has been shown to be 100% effective in inducing tumors by 60–90 days post-gavage (26). DMBA is a polycyclic aromatic hydrocarbon (PAH), widely used as a model chemical carcinogen in experimental rat mammary cancer. Administration of DMBA to rats, in which the mammary glands are undifferentiated and exhibit a high rate of cellular proliferation, results in development of carcinoma (26). Histological features, biological behavior, and carcinogen dose schedules have been described by many laboratories and yield similar results: DMBA causes development of well differentiated mammary adenocarcinomas, which are hormone-responsive and share common morphological similarities with human breast carcinomas (27,28).

The animals were sacrificed 11 wk after the DMBA-application. All tumors were excised. Tumor number, volume, and biological behavior, and carcinogen dose schedules have been described by many laboratories and yield similar results: DMBA causes development of well differentiated mammary adenocarcinomas, which are hormone-responsive and share common morphological similarities with human breast carcinomas (27,28).

Lymph nodes were taken only when palpable. Lymphnode disease in this model is rare and only occurs when tumors are allowed to reach large size.

DNA Extraction and Direct Sequencing of p53

Tumor DNAs were extracted using DNeasy tissue kit (Qiagen, Valencia, CA). The DNAs were subjected to polymerase chain reaction (PCR) for p53 exons 5 and 6 that code for areas containing published mutational hot-spots in DMBA-induced cancer (29,30) using the oligonucleotides presented on Table 1. The PCR conditions were 35 cycles of 94°C for 1min, 55°C for 1 min, and 72°C for 2 min, followed by 10 min at 72°C. The amplifications were carried out in Perkin-Elmer 2400 thermal cycler (Applied Biosystems, Foster City, CA). The PCR amplicons were excised and eluted from 2% low-melting agarose (SeaKem LE agarose, FMC BioProducts, Rockland, ME) in tris-acetate-EDTA (TAE) buffer using Qiagen purification kit (Qiagen, Valencia, CA). The DNAs were subjected to polymerase chain reaction (PCR) for p53 exons 5 and 6 that code for areas containing published mutational hot-spots in DMBA-induced cancer (29,30) using the oligonucleotides presented on Table 1. The PCR conditions were 35 cycles of 94°C for 1min, 55°C for 1 min, and 72°C for 2 min, followed by 10 min at 72°C. The amplifications were carried out in Perkin-Elmer 2400 thermal cycler (Applied Biosystems, Foster City, CA). The PCR amplicons were excised and eluted from 2% low-melting agarose (SeaKem LE agarose, FMC BioProducts, Rockland, ME) in tris-acetate-EDTA (TAE) buffer using Qiagen purification kit (Qiagen, Valencia, CA) and sequenced in DNA Sequencing Core Facility of University of Arkansas for Medical Sciences (UAMS), Little Rock, AR. The sequences were analyzed using GCG Wisconsin Package provided by UAMS, Little Rock, AR.

Protein Extracts and Western Bloting

Total protein extracts were prepared from 1) eight randomly selected tumors of each GLN-supplemented- and control groups and 2) non-tumorous mammary tissue of 8 rats from the control group and 16 rats from the GLN-supplemented group, including 8 rats with tumors and 8 rats without tumors, as described previously (22). All tumors and normal mammary tissue were evaluated independently. Briefly, 40μg total protein from each sample were fractionated on 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membrane using a Mini Vertical Gel System (BioRad Laboratories, Hercules, CA). Membranes were stained in 0.2% Ponzo S after the transfer in order to control the equal protein loading. Following blocking with 5% non-fat milk in TBS-T buffer (100mMTris,pH7.5;150mMNaCl;0.1% Tween20) for 1h at room temperature, the membranes were incubated overnight at 4°C in primary antibody and 1 h at room temperature with HRP-labeled secondary antibodies (Santa Cruz Biotech Inc., Santa Cruz, CA). The following primary antibodies were used as recommended by the manufacturers: anti-phospho p53, anti-p21Waf1/Cip1, anti-PTEN, anti-phospho mdm2, anti-(p85) PI3K, anti-phospho-c-myc, and anti-c-myc (Cell Signaling Technology, Beverly, MA). The effect of GLN on

<table>
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<th>Exon</th>
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<th>Reverse Primer</th>
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<tr>
<td>5a</td>
<td>TCATCGACTCAACTCTGACCTCTCTG</td>
<td>CAGCTGCAAGGGGATGTTTTCG</td>
</tr>
<tr>
<td>5b</td>
<td>TACCTCACCTCTGCTGACCTCTCTG</td>
<td>AAGAGCAATCAAGAAACATCACGG</td>
</tr>
<tr>
<td>6</td>
<td>CAATTAGAAATGCTTGCCGCGG</td>
<td>AGTCTGGGGTGAAGCAGAAACTAAC</td>
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Table 1. Oligonucleotides Used for Direct Sequencing of Exons 5 and 6 of p53
The protein levels of IGF-IR was reported previously (31). The equal protein loading was verified by re-probing the membranes with anti-β actin antibody. Proteins were visualized on a STORM phosphoimager (Amersham Pharmacia Biotech) using the ECL Plus detection system (Amersham Biosci., Piscataway, NJ). The area and density of the bands resulted from Western blotting were measured using Scion Image Program for IBM (Scion Corporation, Frederic, MD). The results, expressed as relative densitometric units and calculated as ratio between the densitometric units of each protein band divided by the densitometric units of β-actin for the same sample, were analyzed statistically by a one-way analysis of variance (ANOVA) using statistical software StatView 5.0.1. Data was expressed as mean ± standard error of the mean (SEM) and results with \( P < 0.05 \) were considered statistically significant.

### Results

**Effect of Dietary GLN on Carcinogenicity of DMBA**

The results published elsewhere (22,31,34), showed that tumor development was significantly (\( P = 0.007 \), GLN vs. control, Fisher Exact Test) reduced by GLN supplementation at the end of the study (11 wk after DMBA application). The GLN-supplemented group had 50% less tumors in comparison with the control group (Fig. 1). The reduced tumor incidence in GLN-supplemented rats was associated with a reduction in the mean tumor weight and volume (summarized in Table 3). Histopathological evaluation of mammary tumors from each group (GLN-supplemented and controls), performed in three different experiments with the same model, consistently showed that 50% of the tumors in the GLN group were malignant, classified as ductal carcinoma in situ (DCIS); the others were classified as intraductal proliferation (IDP). In the control group, all of the tumors (100%) were classified as malignant, either DCIS (30%) or invasive carcinoma (70%) (not shown).

### DNA Sequencing

A mutation within the p53 gene can result in the synthesis of a nonfunctional p53 protein. In contrast to frequent p53 mutations in human breast cancer, DMBA-induced breast cancer is less likely to contain p53 mutations. Alterations in p53 expression are associated with malignant transformation of mouse mammary gland epithelial cells (22). In our study, there were no detectable alterations in the p53, p21Waf1/Cip1, PTEN, mdm2, IGF-IR, and c-myc transcripts in tumors from the GLN-supplemented group compared to the control group. The results are consistent with the hypothesis that the protective effect of dietary GLN on the development of DMBA-induced tumors is due to modulation of other signaling pathways.

### Figure 1

Effect of dietary glutamine (GLN) on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumor development. Tumor appearance was determined in sixteen GLN-supplemented rats and sixteen control rats for a period of 11 wk after DMBA application (\( P = 0.007 \), GLN vs. control, Fisher Exact Test).
cancer is rarely associated with p53 alterations, although such have been well documented (29,30). We have performed a direct sequencing of p53 exons 5–6 and compared the sequences with those published in PubMed rat p53 nucleotide sequences L07910, L07907, and L07781. The results showed that p53 in the examined 16 tumors (8/experimental group) were wild-type (not shown).

### Effect of Dietary GLN on Phosphorylation of p53

One of the main post-translational mechanisms of p53 activation is through its phosphorylation. The p53 protein is phosphorylated on several serine residues within the N- and C-terminal regions by cellular kinases (reviewed in 35). Recent reports suggested that Ser20 plays a critical role in p53 stabilization and p53 apoptotic activity in the response to environmental stress (36,37). Therefore, in order to examine the effect of dietary GLN on p53 activation, we compared the levels of phosphorylated p53 (Ser20) in tumors and normal (tumor-surrounding) mammary tissue of GLN-supplemented and control rats with DMBA-induced breast cancer. Higher levels of phosphorylated p53 were found in the tumors of rats gavaged with GLN in comparison with those of the controls. In the GLN-supplemented group without tumors, the western blot assay detected higher levels of phosphorylated p53 in non-tumorous mammary tissue in comparison with tumors of the controls (Fig. 3, p53 PTEN-a). Higher PTEN protein levels were established also in the mammary tissue of GLN-supplemented rats, which did not develop tumors (2,105 ± 206; Fig. 3, p58 PTEN-c) vs. tumor-surrounding mammary tissue of GLN-supplemented rats (1,599 ± 192; Fig. 3, p58 PTEN-d) and controls (2,195 ± 258; Fig. 3, p58 PTEN-e). These results indicate that the carcinogenic substance DMBA causes accumulation of PTEN in the mammary tissue, which however is reduced by GLN supplementation in the presence of tumors. Because PTEN antagonizes PI3K function and consequently inhibits the downstream signaling through Akt, we next examined the effect of GLN on the protein expression of PI3K.

PI3K is a heterodimer made up of a regulatory (p85) and catalytic (p110) subunits. The p85 subunit protects p110 from degradation and inhibits its enzymatic activity in quiescent cells. When cells are stimulated by receptors, p85 mediates p110 translocation to the cell membrane where PI3K substrates are found (reviewed in 38). We determined the effect of dietary GLN on protein levels of p85 PI3K in tumors and non-tumorous mammary tissue. Tumors of GLN-supplemented rats (Fig. 3, p58 PI3K-a) had lower p85 PI3K protein levels in comparison with tumors of the controls (Fig. 3, p85 PI3K-b; mean value ± SEM, in relative densitometric units, 2,298 ± 129 vs. 2,298 ± 129) and normal mammary tissue of control rats (2,105 ± 206; Fig. 3, p85 PI3K-c) in comparison with the normal mammary tissue of GLN-supplemented rats with tumors (Fig. 3, p85 PI3K-d; 805 ± 88 vs. 954 ± 105 vs. 1,218 ± 252, P < 0.05). Lower levels of p85 PI3K were detected also in the normal mammary tissue of GLN-supplemented rats without tumors (Fig. 3, p85 PI3K-c) in comparison with normal mammary tissue of GLN-supplemented rats with tumors (Fig. 3, p85 PI3K-d; 805 ± 88 vs. 2,298 ± 129) and normal mammary tissue of control rats (Fig. 3, p85 PI3K-e; 805 ± 88 vs. 2,503 ± 193; P < 0.05).

### Effect of GLN on Protein Expression of p21<sup>Waf1/Cip1</sup>, PTEN, phospho-mdm2, and PI3K(p85)

We next determined the effect of dietary GLN on p53-targets p21<sup>Waf1/Cip1</sup>, a key regulator of cell cycle progression; PTEN, a potent natural inhibitor of PI3K and regulator of PI3K; and phospho-mdm2, a regulator of p53 transcriptional activity.

The western blot analysis showed up-regulation of p21<sup>Waf1/Cip1</sup> in tumors collected from the GLN-supplemented group in comparison with the tumors from controls (mean value ± SEM, in relative densitometric units, 2,300 ± 105 for tumors from GLN-supplemented rats vs. 1,600 ± 110 for tumors of control rats, P < 0.05; Fig. 3, p21-a; Fig. 3, p21-b). Elevation of p21 protein was detected also in tumor-surrounding mammary tissue of some of the rats that received GLN (Fig. 3, p21-c), but the alterations were not statistically significant.

Significantly higher levels of PTEN were established in tumors of rats, which received dietary GLN (mean value ± SEM, in relative densitometric units, 850 ± 65 for tumors from GLN-supplemented rats vs. 450 ± 52 for tumors of control rats, P < 0.05; Fig. 3, p85 PTEN-a). Higher PTEN protein levels were established also in the mammary tissue of GLN-supplemented rats, which did not develop tumors (2,105 ± 206; Fig. 3, p85 PTEN-c) vs. tumor-surrounding mammary tissue of GLN-supplemented rats (1,599 ± 192; Fig. 3, p85 PTEN-d) and controls (2,195 ± 258; Fig. 3, p85 PTEN-e). These results indicate that the carcinogenic substance DMBA causes accumulation of PTEN in the mammary tissue, which however is reduced by GLN supplementation in the presence of tumors. Because PTEN antagonizes PI3K function and consequently inhibits the downstream signaling through Akt, we next examined the effect of GLN on the protein expression of PI3K.

**Table 3. Effect of GLN on Tumor Number, Weight, and Volume**

<table>
<thead>
<tr>
<th>Tumor-Bearing Rats</th>
<th>Total Number of Tumors/Group</th>
<th>Average Number of Tumors/Rat</th>
<th>Average Tumor Weight (g) P = 0.5&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor volume (cm&lt;sup&gt;3&lt;/sup&gt;) P = 0.6&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLN-fed rats (n = 16)</td>
<td>8</td>
<td>1.5</td>
<td>2.4 ± 0.73</td>
<td>3.83 ± 1.08</td>
</tr>
<tr>
<td>Controls (n = 16)</td>
<td>15</td>
<td>1.7</td>
<td>4.4 ± 2.08</td>
<td>5.9 ± 2.9</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Abbreviation is as follows: GLN, glutamine.

<sup>b</sup> Values are mean ± SEM.
mdm2 in tumors of GLN-supplemented animals vs. controls (983 ± 106 vs. 502 ± 98, P < 0.05; Fig. 3, ph-mdm2-a). Lower levels of phosphorylated mdm2 were established also in mammary tissue of GLN-supplemented rats, which did not develop tumors (c), tumor-surrounding mammary tissue from GLN-supplemented rats with tumors by Week 11 (d) and tumor-surrounding mammary tissue from control rats (water-fed) (e) were analyzed by western bloting. The equal loading was controlled by staining the membranes with 0.2% Pronso S. The membranes probed for phospho p53 were reprobed for p53. Forty micrograms of the same samples were assayed for β-actin. Values are means ± standard error of the mean in relative densitometric units (P < 0.05, ANOVA, a vs. b; P < 0.05, ANOVA, c vs. e or d). Representative immunoblots are shown on Fig. 2B.

Small but statistically significant increase in the mRNA levels of p21^Waf1/Cip1^, PTEN, and mdm2, and decrease of IGF-IR were established in the tumors of GLN-fed rats (mean value ± SEM in relative densitometric units, 1.14 ± 0.07 in GLN group vs. 0.9 ± 0.07, P < 0.05 for p21^Waf1/Cip1^; 0.418 ± 0.07 vs. 0.330 ± 0.06, P < 0.05 for PTEN; 1.7 ± 0.08 vs. 2.1 ± 0.08, P < 0.05 for IGF-IR; and 1.227 ± 0.19 vs 1.04 ± 0.16, P = 0.4 for mdm2; Fig. 4). GLN had no effect on p53 gene expression (not shown).

**Effect of GLN on Protein and Gene Expression of c-myc**

We next determined the effect of GLN on phosphorylation of c-myc in the tumors. The results showed a significant decrease in the levels of phosphorylated c-myc in tumors of GLN-supplemented rats vs. tumors of the control rats (598 ± 103 vs 829 ± 189, in relative densitometric units, P < 0.05; Fig. 5). The concen-
trations of c-myc protein were also decreased in the tumors of GLN-supplemented rats (812 ± 129 vs. 1,185 ± 215 in relative densitometric units, \( P < 0.05 \)).

The c-myc gene transcription was significantly decreased by 40% as a result from GLN supplementation (mean value ± SEM in relative densitometric units, 0.278 ± 0.04 in GLN group vs. 0.504 ± 0.08 in water group, \( P < 0.05 \); Fig.6).

Discussion

The aim of this study was to determine whether the previously established significant reduction of tumor GSH content and a decreased GSH/GSSG ratio by dietary GLN would activate p53 signaling. Activated p53 mediates a variety of stress responses in eukaryotic cells and is activated by factors such as oxidative stress, UV irradiation, hypoxia, DNA-dam-
aging agents (13,14,39), as well as by alterations in GSH/GSSG, which has been suggested to be the most sensitive index of ongoing oxidative stress (40). Activated p53 induces a number of downstream targets, which lead to either cell cycle arrest or to induction of apoptosis (41). In addition, it has been established that p53 is required for apoptosis and enhancement of tumor regression in response to radiation and chemotherapy (14,15). Resistance to a variety of anticancer agents is often associated with increased GSH levels of tumor cells, suggesting that lowering tumor GSH would increase the sensitivity of transformed cells to chemotherapy and radiotherapy (19,42). In agreement with the previous findings, our present results showed increased presence of phosphorylated p53 in tumors and tumor-surrounding mammary tissue of GLN-supplemented rats with DMBA-induced breast cancer. Accordingly, the protein and gene expression of p53 targets p21Waf1/Cip1, PTEN, mdm2 and IGF-IR were affected in GLN-supplemented rats.

The p21^{Waf1/Cip1} protein mediates p53-induced arrest of the cell cycle (43), which gives time for DNA repair before replication or mitosis. The p21 gene is transcriptionally up-regulated by wild-type p53 (44). PTEN (phosphatase and tensin homolog) tumor suppressor is a negative regulator of PI3K/Akt-dependent cellular survival. p53 has been found to activate PTEN transcription and increase PTEN protein levels in response to stimuli that result in p53 induction (45). Studies on formalin-fixed and paraffin embedded tissues from patients with breast carcinomas revealed that 32% of the invasive ductal carcinoma did not express PTEN (46). The protein levels and transcriptional activity of p53 are regulated by the mdm2 protooncogene. The mdm2 gene is induced by p53, and the encoded protein binds the transcriptional activation domain of p53, thereby blocking recruitment of additional factors necessary for induction of gene expression (47). Once formed, the mdm2-p53 complex shuttles from the nucleus to the cytoplasm where mdm2 targets p53 for degradation by acting as an ubiquitin ligase (48). Inhibition of the interaction of p53 with mdm2 prevents p53 degradation and leads to stabilization of p53 (49). Recent studies showed that phosphorylation of p53 at several regions interfere with the binding of mdm2 to p53, thus making p53 resistant to inhibition by mdm2 and enhancing its transcriptional activity (50). In addition to its role as transcriptional activator, p53 can also function as a transcriptional repressor. Activated p53 can suppress several growth-related genes, such as IGF-IR (51). IGF is considered as a paracrine growth factor for breast cancer, which acts mainly through its receptor IGF-IR, one of the prime substrates of which is PI3K (52). IGF-IR is a transmembrane protein which mediates the autocrine/paracrine effects of the IGF-I (53). Our previous studies showed reduction in circu-

![Graph showing relative density units for different gene expressions](image-url)
lating- and breast tissue levels of IGF-I by GLN supplement in rats with DMBA-induced breast cancer (21,31). The decreased protein levels of IGF-I, together with a reduction in IGF-IR protein were associated with down-regulation of Akt and Bcl-2, and up-regulation of Bad, Bax, and caspase-3. Moreover, higher IGF-IR levels have been detected in cancer cells versus normal breast tissue or benign mammary tumors (reviewed in 54).

This study established also that dietary GLN significantly reduced tumor c-myc. The transcription factor c-myc promotes cell proliferation by regulating the expression of numerous target genes. It has been found that c-myc is overexpressed in 20–60% of human primary breast tumors and in 60% of benign biopsies from patients who subsequently developed breast carcinomas (55,56). Amplification of c-myc gene was associated with poor prognostic features and poor relapse-free survival (reviewed in 57). Myc collaborates with a variety of signaling pathways, including Ras, Bcl-2, and factors that inactivate p53 (reviewed in 58). Experimental studies established that c-myc overexpression could override p53-associated growth arrest following expression of G1 cyclin-dependent kinase inhibitors (59) and could inhibit the transcription of some of the p53 targets, such as p21 and gadd45 (60). A direct correlation between c-myc expression and GSH levels has been suggested, moreover GSH depletion had been associated with down-regulation of c-myc in melanoma cells (61).

Previous studies in our laboratory showed that in the early stages of DMBA-induced breast cancer model GLN caused an initial two- to threefold increase in breast GSH levels, followed by a rapid decrease in GSH with tumor appearances (21). The levels of GSH, however, showed a gradual return toward normal levels in GLN-supplemented rats and remained three- to sixfold higher than the controls. The differential effect of GLN on GSH of normal and transformed cells in a tumor-bearing host, which we and others have established (62,63), would determine increased sensitivity of tumor cells to chemotherapy-related injury while protecting normal tissues (63,64,65).

In conclusion, the results from this study and our previous data indicate that dietary GLN supplementation could modulate the apoptotic- and cell cycle-regulating mechanisms in a tumor-bearing organism and could be used as an adjunct to increase the therapeutic index of radiation and chemotherapy.
Acknowledgments and Notes

This study was supported by VA Merit Review Award to V. S. Klimberg. Address correspondence to V. K. Todorova, Division of Breast Surgical Oncology, Department of Surgery, UAMS, 4301 W. Markham St., Mail Slot 725, Little Rock, AR 72205. Phone: 501–257–4884. FAX: 501–257–4789. E-mail: toodorovavalentinak@uams.edu.

Submitted 29 April 2005; accepted in final form 13 January 2006.

References


Figure 6. Effect of glutamine (GLN) supplementation on c-myc mRNA levels in 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors of rats as established by relative reverse transcriptase-polymerase chain reaction (RT-PCR). The relative expression level was calculated as a ratio between the size of the c-myc band and 18S. Values are means ± standard error of the mean in relative densitometric units (P < 0.05, ANOVA, GLN vs. water). Representative gels are shown under the columns.


