Glutathione S-Transferase π in Squamous Cell Carcinoma of the Head and Neck

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**Objectives/Hypothesis:** Oxidative/reductive (redox) DNA damage from radical species such as nitric oxide (NO•) are increasingly being implicated in the development of cancer. Moreover, redox-protective cellular mechanisms, such as glutathione S-transferase, may determine cellular susceptibility to this redox-mediated damage. **Methods:** Formalin-fixed, paraffin-embedded tissue samples of 11 normal oral mucosa, 15 reactive/dysplastic lesions, and 131 head and neck squamous cell carcinomas (HNSCCs) were immunohistochemically stained using a polyclonal antibody against glutathione S-transferase π (GST-π). Slides were reviewed in a blinded fashion by the study pathologist (G.K.H.) and intensity was graded, noting the pattern of immunostaining. These staining characteristics were compared with those obtained using monoclonal antibodies against endothelial constitutive nitric oxide synthase (ecNOS) and nitrotyrosine, a marker of NO•'s pathological nitrosylation of proteins on serial sections of the same tissue. **Results:** The expression of GST-π was significantly increased in reactive/dysplastic and HNSCC samples compared with normal squamous mucosa (P < .001 for both). Furthermore, among the carcinomas the expression of GST-π was significantly increased in higher-grade lesions (P < .02). The expression of GST-π correlated highly with the expression of ecNOS and nitrotyrosine (P < .0001 for both). **Conclusions:** These findings demonstrate that GST-π is upregulated in conjunction with the NO-generating ecNOS isoform and implicate GST-π in protecting HNSCC from the cytotoxic effects of high concentrations of NO• found in the tumor bed. **Key Words:** Cancer, squamous cell carcinoma, glutathione, nitric oxide.

_Laryngoscope, 110:1642–1647, 2000_

**INTRODUCTION**

It is increasingly apparent that oxidative/reductive (redox) DNA damage, such as that noted in chronic inflammation, is a key factor in carcinogenesis. Over the past decade, nitric oxide (NO•) has been shown to have broad and complex roles in physiological and pathophysiological processes. While demonstrating both antimicrobial and antitumor effects, this redox molecular species has also been implicated in a number of pathological consequences of chronic inflammation. Under well-controlled conditions, NO• or a number of its metabolic byproducts, including peroxyxynitrite, can either be protective against or promote carcinoma formation. With prolonged inflammation, NO• can induce mutational events potentially leading to cancer.

Recently, the individual isozenzymes of nitric oxide synthase (NOS) have been identified within the microenvironment of head and neck squamous cell carcinoma (HNSCC), and it is becoming evident that certain isoforms may be upregulated with progression of this disease. In fact, we have demonstrated increased expression and activity of the endothelial constitutive isoform of NOS along the multistep process of HNSCC carcinogenesis. Furthermore, markers of aberrant NO• production, as signified by the nitrosylation of proteins, have also been shown to increase along the multistep process of HNSCC carcinogenesis.

Glutathione S-transferases (GSTs) represent a family of isoenzymes (α, μ, π, and τ isoforms) that play an important role in the protection of cells from the cytotoxic and carcinogenic effect of redox molecular species. These GST enzymes catalyze the nucleophilic addition of glutathione to lipophilic electrophiles of a wide variety of compounds leading to the formation of mercapturic acids, which is the first step toward the elimination of such xenobiotics as tobacco, betel nut, and chemotherapeutics. Studies of the π isoenzyme of GST in normal human tissues have demonstrated that this isoenzyme is expressed in normal epithelial tissues of the urinary, digestive, and respiratory tracts, which suggests that this isofrom may play a role in the detoxification of toxic substances. In fact, GST-π expression in oral mucosa has been linked to betel nut and tobacco use. The expression of GST-π has been hypothesized to be a marker of increased carcinogen exposure.
Overexpression of GST-\(\pi\) has also been associated with malignant transformation or drug resistance to chemotherapy agents, or both.\(^9,10\) Furthermore, various studies have implicated the upregulation of this protective enzyme in preneoplastic and neoplastic changes of the upper aerodigestive tract.\(^11\) No studies to date have examined the expression patterns of these protective enzymes and how these patterns correlate with the expression of enzymes responsible for the production of these redox molecular species. The purpose of this study was to examine whether a cellular protective mechanism against redox damage correlates with the expression of these redox species-generating enzymes or the development of HNSCC, or both.

**MATERIALS AND METHODS**

**Patient Data**

One hundred thirty-one consecutive specimens of HNSCC from a total of 75 patients were selected from the archival pathology files of the Veterans Affairs Chicago Health Care System and Northwestern University Medical Center. Criteria for exclusion included the lack of pathology records, inadequate formalin-fixed, paraffin-embedded tissue, or lack of available patient data, or a combination of these. Patient charts were reviewed and data were recorded on size of the primary tumor, TNM stage, grade of disease, and follow-up data when available. In addition, a series of 11 normal oral mucosal specimens obtained from uvulopalatoplasty and tonsillectomy procedures, three samples of reactive oral lesions, and 12 samples of various stages or degrees of squamous dysplasia were stained with anti-ecNOS and anti-nitrotyrosine monoclonal antibodies. This well-characterized panel of human tissues has been previously immunostained with these antibodies, unique staining patterns have been demonstrated. No immunostaining was noted in this study on omission of the primary antibody.

**Immunohistochemistry**

A well-characterized, commercially available polyclonal antibody against GST-\(\pi\) (Vector Laboratories, Burlingame, CA) was used for immunoperoxidase analysis as previously described.\(^5,6\) Slides were reviewed in a blinded fashion by the study pathologist (G.K.H.). The overall intensity of the staining reaction was scored as follows: 0 indicating no staining; 1+, weak staining; 2+, mild staining; 3+, moderate staining; 4+, strong staining intensity; and 5+, the highest staining intensity for GST-\(\pi\). The staining patterns and intensity were compared with those previously published for ecNOS and nitrotyrosine.\(^5,6\)

**Statistical Analysis**

Immunoperoxidase staining intensity was subjected to statistical evaluation performed using the SigmaStat statistical program (Jandel Scientific Software, San Rafael, CA). Kruskall-Wallis ANOVA on ranks, Mann-Whitney rank sums, and Spearman’s correlation coefficient tests were performed where appropriate. A \(P\) value of less than .05 was considered significant. Values given in the present study represent mean ± SEM unless otherwise indicated.

**RESULTS**

First, the immunoperoxidase pattern and intensity of expression for GST-\(\pi\) were examined in a group of normal oral mucosal samples (\(n = 11\)). The mean age of this group of patients was 32.2 ± 4.0 years. Five of the 11 patients reported that they were smokers at present. No data were collected on the degree or extent of smoking. The mean staining intensity for patients who admitted to smoking at present (\(n = 5\)) was 2.0 ± 0.6, whereas the mean staining intensity for those without a smoking history (\(n = 6\)) was 1.2 ± 0.5. This was not found to be significantly different between the two groups.

Very little staining for GST-\(\pi\) was seen in the 11 samples with a mean GST-\(\pi\) staining intensity of 1.5 ± 0.4 (Table I). In the two cases that demonstrated histological evidence of chronic inflammation, the mean intensity for GST-\(\pi\) was found to be 0.5 ± 0.5, whereas samples that lacked inflammation (\(n = 9\)) demonstrated a staining intensity of 1.7 ± 0.4.

We also examined samples of reactive lesions (\(n = 3\)) and dysplastic squamous epithelium (\(n = 11\)) obtained from diagnostic biopsies of oral lesions. The mean age for patients in this group was 53.5 ± 4.6 years. Reactive lesions included one sample of fibroepithelial hyperplasia and two of keratoses without atypia. Dysplastic samples included five samples of moderate dysplasia, six samples of severe dysplasia/carcinoma in situ, and one microinvasive squamous cell carcinoma. The sites of origin included six sites that were not specified in the pathological records, four tongue lesions, two samples from the floor of mouth, and one specimen each from the buccal mucosa, soft palate, and labial gingiva. The mean staining intensity for GST-\(\pi\) in reactive lesions was found to be 3.7 ± 1.3; the mean GST-\(\pi\) expression in dysplastic lesions was found to be 3.4 ± 0.4 (Table I). Using Dunn’s method of multiple comparisons, both categories examined were found to be significantly different from normal mucosal staining intensity (\(P < .05\)) (Table I). Regional variation in staining characteristics for GST-\(\pi\) was found in these samples of reactive and dysplastic lesions. No data were available regarding whether HNSCC later developed in these patients.

These data were compared with areas of dysplastic mucosal changes found within a sample of HNSCC. Mucosa that were histologically normal and adjacent to dysplasia demonstrated GST-\(\pi\) expression similar to “normal” mucosal samples (Fig. I). Intense focal staining for GST-\(\pi\) was found in the basal and parabasal areas, which was lost in the lower and middle layers of the stratum spinosum. The expression of GST-\(\pi\) was found to either decrease or significantly increase in the upper spinous and stratum corneum layers. Dysplastic mucosa surrounding these tumor samples demonstrated intense global staining for GST-\(\pi\), which lost regional variation (Fig. 1C).

These staining characteristics mirrored the characteristics found in serial sections of ecNOS- and nitrotyrosine-stained tissues (Fig. 1).

Our panel of HNSCC represented primary lesions from a variety of sites including oropharyngeal (\(n = 27\)), oral cavity (\(n = 16\)), hypopharyngeal (\(n = 10\)), nasopharyngeal (\(n = 2\)), and laryngeal (\(n = 5\)) samples. Most of our specimens were from advanced-stage lesions, with the vast majority being stage IV disease (\(n = 42\)); fewer were stage III disease (\(n = 15\)), and much fewer were stage II samples (\(n = 3\)). Neoplastic lesions had a wide range of
GST-\(\pi\) expression without regional variation, with 0.7% having a staining score of 0; 9.3% a score of 1+; 17.8% a score of 2+; 27.1% a score of 3+; 27.1% a score of 4+; and 17.8% having a score of 5+ staining intensity. The overall staining intensity of the samples of HNSCC demonstrated significantly increased staining intensity for GST-\(\pi\) (\(P < .001\)) when compared with normal mucosal samples (Table I). An example of the staining characteristics in HNSCC is shown in Figure 1D. Very little staining for GST-\(\pi\) was found in inflammatory cells within the microenvironment of a developing focus of tumor. Staining characteristics did not significantly correlate with TNM stage, grade, location of the primary, recurrence, final patient status, disease-free survival, or total survival.

Among the staining characteristics of the various samples, all samples demonstrated cytoplasmic localization of GST-\(\pi\). Within the tissue samples, regional localization differed between normal oral mucosa and dysplastic/reactive lesions when compared with HNSCC. In general, GST-\(\pi\) staining was localized to the basal and spinous layers of normal mucosa and dysplastic or reactive lesions with very little staining being localized to the cornified layers. In contrast, HNSCC demonstrated global localization of GST-\(\pi\) throughout the tumor foci without regional variations.

Using Spearman’s rank-order correlation to determine whether immunoperoxidase staining intensities for each antigen correlated, it was found that the staining intensities for GST-\(\pi\) correlated highly with ecNOS and nitrotyrosine (coefficient = 0.410 and coefficient = 0.465, respectively; \(P < .001\) for both) (Fig. 2).

No immunostaining was noted when the primary antibody was omitted in this procedure. These same cases (serial sections) have been stained with other monoclonal antibodies that resulted in unique staining patterns.\(^{5,6}\)

**DISCUSSION**

It is well known that certain factors increase a patient’s risk for HNSCC. Yet, the exact molecular mechanisms of carcinogenesis have not yet been defined. Squamous cell carcinomas develop within an environment of high microbial colonization, in association with smoking and alcohol abuse, viral infection, lichen planus, and gastroesophageal reflux. These conditions make inflammation-associated redox insult a potential final common pathway leading to this disease.\(^1\) It is well known that early lesions demonstrate a significant perilesional inflammatory response. Conversely, patients with late-stage disease demonstrate variable degrees of immunosuppression. From these facts, inflammation, and its various inflammatory mediators including redox species, may be implicated in the induction and promotion of these tumors.

Within a focus of chronic inflammation, a number of redox species are produced by the host immune system to kill tumor cells through apoptotic and/or other mechanisms. Conversely, these species may also lead to a series of mutational events leading to carcinogenesis or escape from immunosurveillance. Therefore squamous cell carcinoma of the head and neck offers a unique opportunity to explore the role of redox molecular species in the development of HNSCC.

One such redox molecular species that has demonstrated a mutagenic potential and has been implicated in both tumor cell killing and carcinogenesis is NO·.\(^{3,12}\) Furthermore, NO· is interrelated with other redox molecular species, expanding its scope of pathological potential. It is

| Table I. Normal Mucosa, Reactive/Dysplastic, and HNSCC Lesion Staining. |
|---------------------------------------------------|
| Staining Intensity                           | GST-\(\pi\)| ecNOS* | Nitrotyrosine* |
|---------------------------------------------------|
| Normal mucosa (n = 11)                        | 1.5 ± 0.4†‡ | 0.9 ± 0.2‡§ | 1.5 ± 0.2‡§ |
| No inflammation (n = 9)                       | 1.7 ± 0.4 | 0.6 ± 0.2 | 1.5 ± 0.5 |
| Inflammation (n = 2)                          | 0.5 ± 0.5 | 1.5 ± 0.5 | 1.5 ± 0.7 |
| Hyperplasia or dysplasia (n = 14,15)          | 3.4 ± 0.4†‡ | 2.4 ± 0.2‡§ | 2.6 ± 0.4‡§ |
| Reactive mucosa (n = 3)                        | 3.7 ± 1.3 | 2.7 ± 0.3‡§ | 2.7 ± 1.2 |
| Fibroepithelial hyperplasia (n = 1)            | 1.0 ± 0.0 | 2.0 ± 0.0 | 1.0 ± 0.0 |
| Keratosis without atypia (n = 2)              | 5.0 ± 0.0 | 3.0 ± 0.0‡§ | 3.5 ± 2.1 |
| Dysplastic mucosa (n = 11,12,12)§             | 3.4 ± 0.4 | 2.3 ± 0.3‡§ | 2.6 ± 0.4 |
| Moderate dysplasia (n = 4,5,5)§               | 3.2 ± 0.9 | 2.4 ± 0.5‡§ | 2.6 ± 0.7 |
| Severe dysplasia or carcinoma in situ (n = 6)  | 3.5 ± 0.6 | 2.3 ± 0.3‡§ | 2.7 ± 0.6 |
| Microinvasive squamous cell carcinoma (n = 1)  | 3.0 ± 0.0 | 2.0 ± 0.0 | 2.0 ± 0.0 |
| Squamous cell carcinoma (n = 125,127,131)¶    | 3.3 ± 0.1†‡ | 2.6 ± 0.1‡§ | 2.7 ± 1.1‡§ |
| Grade 1 [n = 11,11,12]$†‡                    | 2.3 ± 0.3| 2.5 ± 0.3 | 2.2 ± 0.8 |
| Grade 2 [n = 41,42,43]$†                    | 3.4 ± 0.2‡| 2.4 ± 0.2 | 2.6 ± 1.1 |
| Grade 3 [n = 11,10,11]$†                 | 3.6 ± 0.3* | 2.7 ± 0.3 | 2.9 ± 1.3 |

*From previously published references (Bentz et al.\(^{5,6}\)).
†\(P = .001\) by Kruskall-Wallis one-way ANOVA.
‡\(P < .05\) by Dunn’s method of multiple comparisons versus the control group (normal).
§\(P < .001\) by Kruskall-Wallis one-way ANOVA.
¶Number of samples in GST-\(\pi\), ecNOS group, and nitrotyrosine group, respectively.
**GST-\(\pi\) = glutathione S-transferase; ecNOS = endothelial constitutive nitric oxide synthase.
known that the interaction of NO· and superoxide (O$_2^-$) yields a highly reactive redox species, peroxynitrite (ONOO$^-$), which can cause nitrosation of tyrosine. These and various other redox molecular species have the potential for causing mutations leading to carcinogenesis. It is currently known that NO· can induce structural changes in the p53 protein that may account for the alteration in the function of this tumor-suppressor gene.$^{13}$ Furthermore, in other models, NO· has demonstrated the capability of inducing double-stranded DNA breaks, which is a hallmark of mutational insult.$^{12}$ Whether mutational events are mediated through the inflammatory production of NO· and its numerous potentially carcinogenic metabolites has not been definitively proven.

We have put forth the hypothesis that carcinomas may use the same molecular mechanisms that are used by the antitumor immune response to elicit immunosuppression, thus negating immunological attack and destruction. Perhaps, upregulation of NOS expression in tumor cells uses the very NO·-mediated mechanisms of the antitumor response to suppress the immune system. A chronic inflammatory response to a developing focus of cancer would appear to potentially provide the right cytokine stimuli to induce iNOS in tumor-adherent macrophages or tumor tissues themselves, thus downregulating the antitumor immune response. In fact, various investigations are beginning to reveal the complex and central role of redox species, including NO·, in HNSCC carcinogenesis.$^{4-6}$ Interestingly, Bentz et al.$^{5,6}$ have demonstrated that premalignant lesions and tumors contribute to this reactive environment by overexpression of NOS.

Individual isoforms of the family of enzymes responsible for the generation of NO· have been identified within the microenvironment of HNSCC.$^4$ One report demonstrated that the calcium-independent isoform (iNOS) activity was high during early stages of disease and decreased as the disease progressed. Conversely, the calcium-dependent isoform’s activity (ecNOS) progressively increased as the disease progressed.$^{14}$

Our data support the central role of various redox molecular species including NO· in the induction and promotion of HNSCC. We have found that a significant upregulation of the ecNOS isoform has been documented during the transition from normal oral mucosa to premalignant lesions and HNSCC.$^5$ Furthermore, markers of pathological nitrosylation of proteins by NO· appear to parallel the increased expression of NOS III in HNSCC,$^6$ thus demonstrating the

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**Fig. 1.** A. Normal squamous mucosa staining very little with diaminobenzidine against glutathione S-transferase π (GST-π). B. Staining of squamous dysplasia demonstrating an increased staining intensity against GST-π. C. Squamous cell carcinoma demonstrating global, intense staining for GST-π. D. Perilesional inflammatory cells staining very little for GST-π when compared with adjacent squamous cell carcinoma. Original magnification × 200.

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functional NOS III and the pathological potential within tumors.

The production of redox species must be kept in balance with protective mechanisms to prevent nonspecific cellular damage. Redox DNA damage reflects an imbalance of protective and destructive mechanisms. One such intracellular protective mechanism is the glutathione/GST enzyme system. Glutathione is a tripeptide, γ-glutamylcysteinyl glycine, which is found in all mammalian tissues and acts to detoxify exogenous or endogenous compounds such as electrophiles and redox species generated during oxidative metabolism and stress. The GST superfamily of enzymes acts to conjugate or reduce many of these toxic molecular species to reduced glutathione. The GST superfamily consists of seven classes of soluble GSTs and numerous polymorphisms. Expression regulation of the π class of GST is currently being discovered, but the half-life of this class is not known. It probably is dependent on the cellular population under study, as well as the level of redox stress. High redox stress causing increased conjugation of toxic species to glutathione may shorten the half-life of GST-π (Thomas D. Boyer, MD, personal communication). Chronic inflammation, as well as overexpression of redox generating enzymes, may shorten the half-life of these enzymes. Yet, whether the expression intensity found in dysplastic and neoplastic lesions in this study represents spuriously high levels secondary to long enzyme half-lives cannot be determined at present.

The glutathione/GST enzyme system has been documented in normal mucosa of the urinary, digestive, and respiratory systems. Furthermore, its expression has been implicated in the detoxification of such carcinogenic agents as betel nut quid and tobacco. We did demonstrate an increase in GST-π staining intensity in normal oral mucosa samples from patients with an admitted history of smoking and/or alcohol intake. Although this did not reach statistical significance, the power of analysis was limited, because of the small numbers of samples evaluated.

It has been shown that the overexpression of the GST-π isoenzyme is associated with the transformation from normal oral mucosa to dysplastic mucosa and HNSCC. More recently, glutathione has been implicated in protecting tumor cells from the antiproliferative/apoptotic effects of high levels of NO−, as opposed to low, continuous levels of NO− that would be seen with the expression of ecNOS. In the same study, depletion of tumor cell lines of glutathione augmented the antiproliferative effects of high levels of NO−.

In light of these reports, we undertook a study to examine the expression pattern of GST-π in relationship to the multistep process of HNSCC carcinogenesis and the expression of a redox species-generating enzyme ecNOS. It was found that these two correlated very highly in expression, and that they both were upregulated along the path to HNSCC. Furthermore, GST-π was also found to be highly correlated with the expression of a marker of aberrant nitrosylation of proteins by NO−, nitrotyrosine. These data seem to indicate that GST-π may not completely detoxify all NOS-generated redox molecular species. In light of the previously reported data, it appears that GST-π may act to protect HNSCC from the antiproliferative effects of high levels of NO− produced both by the tumor themselves and by immune cells. Furthermore, once the ecNOS levels of expression within the tumor are increased, the GST-π may protect the tumor cells from self-induced apoptotic cell death. Bystander immune cells exposed to lethal levels of NO− may be induced to undergo apoptosis. In effect, this would make the tumor bed a negative sink for inflammatory cells, which ultimately results in the decline of immune system function that is noted in these patients.

CONCLUSION

We have demonstrated a significant increase in the expression of GST-π during the progression from normal oral mucosa to reactive, premalignant lesions and on to HNSCC. The expression of this enzyme was found to be significantly correlated with the expression of ecNOS and nitrotyrosine. These data implicate GST-π in protecting a developing focus of HNSCC against immune cell–generated NO−.

BIBLIOGRAPHY

1. Koufman JA, Burke AJ. The etiology and pathogenesis of laryngeal carcinoma. Otol Clin North Am 1997;30:1–19.
2. Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res 1994;305:253–264.
3. Hibbs JB, Vavrin Z, Taintor RR. L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. J Immunol 1987;138:550–565.
4. Rosse KW, Prazma J, Petrusz P, Mims W, Ball SS, Weisler MC. Immunohistochemical characterization of nitric oxide synthase activity in squamous cell carcinoma of the head and neck. Otol Head Neck Surg 1995;113:541–549.
5. Bentz BG, Haines GK, Hanson DG, Radosевич JA. Nitric oxide synthase type 3 is increased in squamous hyperplasia, dysplasia, and squamous cell carcinoma of the head and neck. Ann Otol Rhinol Laryngol 1999;108:781–787.
6. Bentz BG, Haines GK, Radosевич JA. Increased protein nitrosylation in head and neck squamous cell carcinoma. Head Neck 2000;22:84–70.
7. Terrier P, Townsend AJ, Coindre JM, Triche TJ, Cowan KH.
An immunohistochemical study of pi class glutathione S-transferase expression in normal human tissue. Am J Pathol 1990;137:845–853.
8. Sarkar G, Nath N, Shukla NK, Ralhan R. Glutathione S-transferase π expression in matched human normal and malignant oral mucosa. Oral Oncol 1977;33:74–81.
9. Chen YK, Lin LM. Immunohistochemical demonstration of epithelial glutathione S-transferase isoenzymes in normal, benign, and premalignant and malignant human oral mucosa. J Oral Pathol Med 1995;24:316–321.
10. Chasseaud LF. The role of glutathione and glutathione S-transferase in the metabolism of chemical carcinogens and other electrophilic agents. Adv Cancer Res 1979;29:175–274.
11. Mulder TPJ, Mann JJ, Roelofs HMJ, Peter WHM, Wiersma A. Glutathione S-transferases and glutathione in human head and neck cancer. Carcinogenesis 1995;16:619–624.
12. Delaney CA, Green MHL, Lowe JE, Green IC. Endogenous nitric oxide induced by interleukin-1β in rat islets of Langerhans and HIT-T15 cells causes significant DNA damage as measured by the "comet" assay. FEBS Lett 1993;333:291–295.
13. Calmels S, Hainaut P, Ohshima H. Nitric oxide induces conformational and functional modifications of wild-type p53 tumor suppressor protein. Cancer Res 1997;57:3365–3369.
14. Galvilanes J, Moro MA, Lizasoin I, Lorenzo P, Pérez A, Leza JC, Alvarez-Vieent JJ. Nitric oxide synthase activity in human squamous cell carcinoma of the head and neck. Laryngoscope 1999;109:148–152.
15. Petit JF, Nicaise M, Lepoivre M, Guissani A, Lemaire G. Protection by glutathione against the antiproliferative effects of nitric oxide. Biochem Pharm 1996;52:205–212.

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