Protein Phosphorylation Is a Regulatory Mechanism for $O^6$-Alkylguanine-DNA Alkyltransferase in Human Brain Tumor Cells


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Abstract

The biochemical regulation of human $O^6$-alkylguanine-DNA alkyltransferase (AGT), which determines the susceptibility of normal tissues to methylating carcinogens and resistance of tumor cells to many alkylating agents, is poorly understood. We investigated the regulation of AGT by protein phosphorylation in a human medulloblastoma cell line. Incubation of cell extracts with [γ-32P]ATP resulted in Mg$^{2+}$-dependent phosphorylation of the endogenous AGT. Immunoprecipitation after exposure of the cells to 32P-labeled inorganic phosphate showed that AGT exists as a phosphoprotein under physiological conditions. Western analysis and chemical stability studies showed the AGT protein to be phosphorylated at tyrosine, threonine, and serine residues. Purified protein kinase A (PKA), casein kinase II (CK II), and protein kinase C (PKC) phosphorylated the recombinant AGT protein with a stoichiometry of 0.15, 0.28, and 0.44 (mol phosphate incorporated/mol protein), respectively. Residual phosphorylation of the endogenous AGT by the PKs present in cell homogenates and phosphorylation of the recombinant AGT by purified serine/threonine kinases, PKA, PKC, and CK II reduced AGT activity by 30–65%. Conversely, dephosphorylation of cell extracts by alkaline phosphatases stimulated AGT activity. We also identified consensus phosphorylation motifs for many cellular kinases, including PKA and CK II in the AGT protein. These data provide the first and conclusive evidence of AGT phosphorylation and suggest that reversible phosphorylation may control the activity of this therapeutically important DNA repair protein in human normal and cancer cells.

Introduction

AGT, also called $O^6$-methylguanine-DNA methyltransferase (EC 2.1.1.63), is a ubiquitous DNA repair protein involved in protecting the cellular genome from the mutagenic and toxic actions of a variety of alkylating agents. AGT functions by a unique mechanism in which the alkyl groups at the $O^6$ position of guanine or $O^4$ position of thymine in DNA are transferred to an internal cysteine (Cys 145) in its active site (1, 2). This reaction is stoichiometric, and the irreversible, covalent binding of the alkyl group to the active site results in the functional inactivation of one molecule of the AGT protein after each reaction cycle (1–3). AGT is overexpressed in a significant proportion of human brain tumors and other cancers (4) and plays a central role in conferring resistance to many clinically active methylating and chloroethylnitrosoureas (5, 6). AGT removes the $O^6$-methyl groups introduced in guanine by the monofunctional agents, thereby restoring intact guanine, and preventing GC→AT transitions. With regard to bifunctional alkylators like the chloroethylnitrosoureas, AGT prevents the production of cytoxic DNA interstrand cross-links by repairing the $O^6$-chloroethylnitrosourea cross-link precursors induced by these agents (1, 5). Among the pseudosubstrates developed for AGT is BG, which effectively depletes cellular AGT and enables significant potentiation of drug-induced cytotoxicity (7). BG is currently undergoing clinical trials to improve the efficacy of chloroethylnitrosoureas against brain tumors and other human cancers (8). In contrast to its overexpression in neoplasms, AGT is expressed at very low levels in normal tissues such as brain and bone marrow (4, 9), which increases its susceptibility to the toxic and mutagenic effects exerted by the environmental carcinogens and anticancer drugs.

Currently, the expression and biochemical regulation of AGT in human cells are poorly understood. DNA hypermethylation has been shown to repress the transcriptional activity of the AGT gene in some human tumor cell lines (10). However, in a significant majority of human cancers, AGT is expressed at various levels, and the biochemical mechanisms that affect the AGT protein or its activity are largely unknown. The dealkylation reaction of AGT, which comprises its binding to DNA, recognition of the $O^6$-alkylated guanine, and self-transfer of the alkyl group is a direct process that does not involve other proteins or cofactors. However, the existence of intracellular pathways that can alter AGT activity, its stability, and/or its subcellular distribution will have a significant effect on AGT function and the extent of DNA repair. Clarification of such mechanisms in cells of both normal tissues and tumors is important not only for understanding the physiological regulation of AGT but also for developing new strategies to enhance the efficacy of AGT-targeted chemotherapy. We have shown previously that the AGT protein after being inactivated by BG or 1,3-bis(2-chloroethyl)-1-nitrosourea, is targeted for polyubiquitination and subsequent degradation by the proteasome complex (11). Because ubiquitin conjugation of many target proteins is often interlinked with phosphorylation (12, 13) and because phosphorylation affects the DNA binding and function of many enzymes in nucleic acid metabolism including DNA repair (14–16), we investigated protein phosphorylation as a potential regulatory mechanism for human AGT.

Materials and Methods

Cell Line, Antibodies, and Chemicals. The UW228 human medulloblastoma cell line was established in our laboratory from a primary specimen as described previously (17) and is routinely maintained in DMEM supplemented with 10% FCS. These cells are AGT proficient with activity levels comparable to that in the HT-29 colon carcinoma cell line (18). Monoclonal and polyclonal antibodies against human AGT (19) were kindly provided by Dr. Darrel Bigner of Duke University (Durham, NC). Monoclonal antibodies specific to phosphotheorene and phosphoserine were obtained from Sigma Chemical Co. (St. Louis, MO), and monoclonal phosphothreonine antibodies (PY20) were from Santa Cruz Biotechnology (Santa Cruz, CA). Purified PKs [PKA (the catalytic subunit), PKC from rat brain, and recombinant CK II]...
holoenzyme) were obtained from Calbiochem (San Diego, CA). 32P-Labeled inorganic phosphate (H632P04; 6000 Ci/mmole) and [γ-32P]ATP (3000 Ci/mmole) were purchased from ICN Radiochemicals (Costa Mesa, CA). Bacterial and calf intestine alkaline phosphatases were procured from Sigma.

**AGT Activity Assay.** Exponentially growing UW228 cells were trypsinized and washed with Tis-buffered saline (TBS; pH 8.0). Cell extracts were prepared by sonication in TGED buffer (40 mM Tris-HCl (pH 8.0), 10% glycerol, 1 mM EDTA, and 0.5 mM DTT), followed by centrifugation at 10,000 g for 10 min. AGT activity was determined by quantitating the transfer of the [3H]-labeled methyl group from the ω position of guanine in the DNA to the AGT protein (20). Briefly, cell extracts (50–200 μg of protein) were supplemented with 2 μg of DNA substrate enriched with ω-32P-methylguanine (~10,000 cpm) and incubated at 37°C for 30 min, after which the DNA was hydrolyzed in trichloroacetic acid at 80°C for 30 min. The protein precipitates were collected on glass fiber filters and solubilized, and the radioactivity was counted (20).

**Combined Immunoprecipitation/Immunoblotting Analysis of AGT Protein.** This procedure has been described elsewhere (11). Briefly, cell extracts containing AGT protein (>150 μg/assay) were precleaned with protein A-agarose beads and incubated with 2 μg of polyclonal antibodies to human AGT. After 6 h at 4°C, immune complexes were collected by adding 10 ml of ice-cold TBS containing 10 mM EDTA, and the AGT protein present in the extracts was immunoprecipitated as described above. After electrophoretic transfer of the proteins to Immobilon-P membranes (Millipore Co.) and blocking in 4% BSA solution, the blots were probed with the appropriate antibodies. Positive bands were visualized by enhanced chemiluminescence (ECL, Amershams Co.).

**Phosphorylation of Endogenous AGT in Cell Extracts and Its Influence on AGT Activity.** To study 32P incorporation, cell extracts (150 μg of protein in a volume of 100 μl) prepared in TGED buffer were supplemented with [γ-32P]ATP (10 μM, 0.1 μCi in the presence or absence of MgCl2 (0–10 mM). After incubation at 30°C for 20 min, the reactions were terminated by adding 300 μl of ice-cold TBS containing 10 mM EDTA, and the AGT protein present in the extracts was immunoprecipitated as described above. After electrophoretic transfer of the proteins to Immobilon-P membranes (Millipore Co.) and blocking in 4% BSA solution, the blots were probed with the appropriate antibodies. Positive bands were visualized by enhanced chemiluminescence (ECL, Amershams Co.).

**Quantitation of the effect of phosphorylation on AGT activity was performed in two steps, i.e., in vitro phosphorylation followed by analysis of AGT activity in the phosphorylated extracts. Cell extracts in TGED buffer (50–200 μg of protein) were treated with 0.5 mM ATP and 2.5–10 mM MgCl2. Extracts with no supplements, Mg2+ alone, or with ATP alone served as controls. After incubation at 30°C for 1 h, EDTA was added at 10 mM to stop the kinase reactions. To avoid any inhibitory effects that Mg2+ had on AGT activity, the assay sample volumes were adjusted to 75 μl in TGED buffer and desalted by microgel filtration by using 1-ml spin columns packed with Biogel p6 beads (Bio-Rad), according to the manufacturer’s instructions. After applying the samples, the columns were centrifuged at 3500 × g into tubes containing 50 μl of TGED buffer. Protein recovery from the spin columns was >90%. All sample volumes were adjusted to 100 μl, [3H] DNA substrate was added, and AGT activity was quantified as described above.

**Dephosphorylation and AGT Activity in Cell Extracts.** UW228 cell-free extracts (50–150 μg protein) were treated with 20 units of alkaline phosphatase from Escherichia coli or calf intestine in 30 mM Tris-HCl (pH 8.0) at room temperature for 1 h. The phosphatases were highly active under these conditions. Controls included cell extracts, and all reaction components except the alkaline phosphatase. EDTA (to 1 mM) and EGTA (to 0.5 mM) were added to inactivate the phosphatases. The samples were then filtered on Biogel p6 columns, DNA substrate was added, and AGT activity was quantified as described above.

**Intracellular Phosphorylation of AGT.** UW228 cells cultured in 25-cm2 flasks were washed with phosphate-deficient DMEM (Life Technologies, Inc.) and incubated for 6 h with the same medium containing [γ-32P]Pi (10 μCi/ml) for 6 h. The radioactivity was determined by washing the cells twice with ice-cold TBS. After trypsinization, cells were lysed in 0.5 ml of TBS containing 0.5 mM phenylmethylsulfonyl fluoride, 50 mM sodium fluoride, 0.5 mM sodium vanadate, and 1% NP40. The supernatants recovered by centrifugation were immunoprecipitated with AGT antibodies as described earlier. The immunocomplexes were resolved on 12% SDS-polyacrylamide gels, and the gels were dried and subjected to autoradiography.

**Phosphorylation of Recombinant AGT by PKA, PKC, and CK II: Reaction Stoichiometry and Effect on AGT Activity.** Full-length human AGT protein was expressed in E. coli BL21 (DE3, pLys) by using a T7 promoter-based expression vector, and the protein was purified to homogeneity as described previously (21). Phosphorylation of this substrate by the purified kinases was performed according to published protocols (22). To determine the stoichiometry of phosphorylation, we suspended AGT protein (1 μg/assay) in phosphorylation buffer containing 20 mM Tris-HCl (pH 7.4) and 2.5–10 mM MgCl2. For reaction with PKA, 0.05% Triton X-100, 1 mM EDTA, and 2 units of PKA were added. For reaction with PKC, 1 mM CaCl2, 0.5 mg/ml phosphatidylinerse, 10 μg/ml diolene, and 0.5 units of PKC were added. For reaction with CK II, 25 mM KCl and 4 units of CK II were added. The kinase amounts were chosen to maximize AGT phosphorylation. The reactions were started by adding [γ-32P]ATP (3 μCi, 0.1 mM) and terminated after 1 h incubation at 30°C by adding the SDS-PAGE sample buffer. The samples were electrophoresed on 15% polyacrylamide gels, the gels were stained with Coomassie blue, and the AGT protein bands were cut and added to 5 ml of scintillation fluid for counting. A duplicate gel was dried and autoradiographed to visualize the phosphorylated AGT.

To study the effect of the phosphorylation by PKA, PKC, and CK II on AGT activity, kinase reactions were performed under conditions similar to those described above except for the use of 0.25 mM unlabeled ATP and 4 μg of recombinant AGT protein as the substrate, and incubation time was decreased to 30 min. Controls for these reactions included all components except the PKs. The reactions were terminated by adding 5 mM EDTA, the samples were filtered on Biogel P6 spin columns, and AGT activity was assayed in the eluates as described above.

**Identification of Phosphorylated Residues in the AGT Protein.** Immunoprecipitation/immunoblotting analysis (11) and chemical cleavage (23) were applied to identify which amino acids in the AGT protein are phosphorylated. In the first method, the AGT protein was immunoprecipitated from UW228 extracts (300 μg protein) in quadruplicate as described earlier. Solubilized immunoprecipitates were electrophoresed and Western blotted. The membrane strips were probed separately with monoclonal antibodies specific to AGT (2 μg/ml), phosphotyrosine (1 μg/ml), phosphoserine (3 μg/ml), and phosphothreonine (0.5 μg/ml). We also examined the differential stability of phosphate groups linked to aliphatic and aromatic amino acids under acidic or alkaline conditions (23). This procedure involved immunoprecipitating the AGT protein from 32P-labeled UW228 cells, solubilizing the immunocomplexes, and dividing into three equal portions, one of which was untreated (control), another treated with 0.2 M HCl at 60°C, and the third treated with 1 M NaOH at 75°C for 30 min. All samples were neutralized to pH 6.5 and electrophoresed on 12% SDS gels, which were then dried and subjected to autoradiography.

**AGT Migration after Phosphorylation and Dephosphorylation.** Under the reaction conditions described in the previous sections, recombinant AGT protein (1 μg) was treated with CK II, and UW228 cell extract (60 μg) which contains constitutively phosphorylated AGT was dephosphorylated with alkaline phosphatase. These samples were subjected to SDS-PAGE and Western blotting to detect differences in the mobility of the AGT protein.

**Statistical Analysis.** All experiments including the effect of phosphorylation and dephosphorylation on AGT activity were performed at least five separate times unless otherwise indicated. Results were assessed by Student’s t test. Significance was defined as P < 0.05.

**Results and Discussion**

**Potential Phosphorylation Sites Present in the Human AGT Protein.** The present study was prompted by our initial observations that the human AGT protein harbors consensus recognition motifs for several PKs (Fig. 1). Computer-assisted motif analyses (24, 25) revealed the presence of multiple, often overlapping, phosphorylation sites for the serine/threonine PKs, PKA, CK I, CK II, and glycogen
Phosphorylation of AGT Protein in Cell-free Extracts. As a first step in characterizing AGT phosphorylation, we examined label incorporation from $[^\gamma-32\text{P}]\text{ATP}$ in UW228 cell-free extracts to the endogenous AGT protein. In the absence of Mg$^{2+}$ ions, no $32\text{P}$ label was transferred to the AGT polypeptide; however, this incorporation was greatly enhanced as Mg$^{2+}$ concentration increased in the reaction (Fig. 2A). A Western blot performed in parallel and probed with AGT antibodies showed that equal amounts of AGT protein were present in all samples (Fig. 2B). These data indicate that the AGT protein undergoes phosphorylation in vitro, and that the PK or kinases that mediate this reaction are present intracellularly. The low level of $32\text{P}$ incorporation observed in this experiment may reflect substrate limitation attributable to constitutive AGT phosphorylation in UW228 cells.

Phosphorylation of AGT Protein in Human Brain Tumor Cells. To demonstrate that phosphorylation of a given protein is physiologically relevant, it is important to show that the modification occurs in intact cells. To this end, the UW228 and MGR1, another human glioma cell line, were metabolically labeled with [$^{32}\text{P}]\text{Pi}$, followed by immunoprecipitation of the AGT protein, SDS-PAGE, and autoradiography. The major band corresponding to the AGT protein ($M_r$ 22,000) was phosphorylated in both cell lines under these conditions (Fig. 2C). On the basis of our previous studies (11), we believe that the higher molecular weight $^{32}\text{P}$-labeled bands observed in Fig. 2C represent the polyubiquitinated forms of AGT. Therefore, it is tempting to speculate that AGT phosphorylation may precede its conjugation with ubiquitin; such a relationship between these two posttranslational events has been demonstrated for other proteins (12, 13). The addition of purified AGT protein to the cell extract before immunoprecipitation quenched the signal and confirmed the identity of the radioactive immunoprecipitated protein as AGT. Furthermore, ~50% of the radioactivity associated with the AGT was released by treatment with alkaline phosphatase. These data provide clear evidence that AGT exists as a phosphoprotein in human glioma cells and suggest that phosphorylation is an important biochemical modification for this protein.

Identification of Amino Acids Phosphorylated in the AGT Protein. We used an immunological and chemical approach to identify the phosphogroup acceptors in the DNA repair protein. In the first procedure, the AGT protein was purified from UW228 cell extracts by immunoprecipitation and subjected to Western blotting in quadruplicate. The blots were probed independently with antibodies specific to

Fig. 2. Phosphorylation of AGT in UW228 cell extracts and cells. A, in vitro incorporation of $^{32}\text{P}$ into endogenous AGT protein. Cell extracts were incubated with $[^\gamma-32\text{P}]\text{ATP}$ in the absence or presence of MgCl$_2$, followed by SDS-PAGE and autoradiography. Film was exposed for 15 days. B, Western analysis of AGT under reaction conditions used for $^{32}\text{P}$ incorporation shown in A. Unlabeled ATP (10 $\mu$M) was used instead of $[^\gamma-32\text{P}]\text{ATP}$. C, identification of AGT as a phosphoprotein in brain tumor cells. UW228 cells and MGR2 (a human glioma cell line) were metabolically labeled with $^{32}\text{P}$-labeled inorganic phosphate, and AGT was immunoprecipitated from cell extracts, followed by SDS-PAGE and autoradiography. The protein bands higher than $M_r$ 22,000 represent the ubiquitinated species of AGT, which are also recognized by the AGT antibodies used for immunoprecipitation (11).
AGT, phosphotyrosine, phosphoserine, and phosphothreonine. The specificity of antiphosphoamino acid antibodies was verified by competition with 1 mM free phosphoamino acids. All three phosphoamino acid antibodies reacted strongly with the AGT protein bands, indicating the presence of serine, threonine, and tyrosine phosphorylations in the cellular AGT protein (Fig. 3A). These results were further confirmed by exploiting the differential lability of the phosphate groups bound to aliphatic and aromatic amino acids; whereas the phosphate linked to serine and threonine is acid stable but alkali labile, O-tyrosyl phosphate groups remain stable in both acid and alkali (23). The autoradiographic patterns after the acid or alkali treatment of AGT immunoprecipitates prepared from 32P-labeled UW228 cells are shown in Fig. 3B. The intensity of the 32P-labeled AGT bands after acid treatment (Fig. 3B, Lane 1) was similar to that of the untreated control (Fig. 3B, Lane 2). However, alkali treatment generated faint radioactive bands of AGT (Fig. 3B, Lane 3), reflecting the loss of phosphates associated with serine and threonine and retention of the tyrosine-linked phosphate groups. Collectively, these results demonstrate that AGT is phosphorylated at multiple sites, and that serine, threonine, and tyrosine are the modified residues.

Effect of Phosphorylation and Dephosphorylation on AGT Activity in Cell Extracts. To determine whether phosphorylation of the AGT protein influences its function, we phosphorylated and dephosphorylated the endogenous AGT protein. Encouraged by the finding that residual sites on the AGT protein could be phosphorylated in vitro (Fig. 2A), we performed similar experiments by incubating cell extracts in the presence or absence of unlabeled ATP and Mg2+ and then desalting them and quantitating AGT activity. When extracts were incubated with either ATP or Mg2+ alone, no significant changes in AGT activity were observed. However, when ATP-Mg2+ were both present, AGT activity was consistently inhibited (Fig. 4A). The extent of inhibition ranged from 40 to 65% in different experiments, regardless of the protein concentration used. Significantly, Mg2+ at the physiologically relevant concentration of 1 mM could promote AGT phosphorylation and therefore its inhibition.

To dephosphorylate AGT protein, UW228 cell extracts were exposed to alkaline phosphatase from *E. coli* or calf intestine at neutral pH. Dephosphorylated extracts showed a significant increase in AGT activity, ~60% more than that of controls (Fig. 4B). Lower protein concentrations (in the cell extracts) and longer incubations with the phosphatases were necessary to observe the increases in AGT activity. The calf intestinal enzyme was more efficient in the dephosphorylation reaction. Treatment of the cell extracts with agarose-immobilized alkaline phosphatase also resulted in increased AGT activity. Taken together, the findings of decreased AGT activity after *in vitro* phosphorylation (Fig. 4A) and enhanced activity after dephosphoryl-
Phosphorylation of Recombinant AGT Protein by Purified Serine/Threonine PKs. Human AGT expressed by E. coli lacks major posttranslational modifications and is thus an appropriate substrate for phosphorylation by exogenous kinases. Encouraged by finding consensus recognition motifs for different PKs in the AGT protein (Fig. 1), we used PKA, PKC, and CK II to phosphorylate purified recombinant AGT protein. All three kinases phosphorylated the AGT protein in a Mg²⁺ concentration-dependent manner (Fig. 6A). No ³²P was incorporated in the absence of Mg²⁺ with all kinases (Fig. 6A, Lane 1), and the rate of phosphorylation increased with increasing Mg²⁺ levels. Phosphate incorporation remained linear for up to 30 min for all kinases. The stoichiometry (mol phosphate/mol AGT) was 0.15 for PKA, 0.44 for PKC, and 0.28 for CK II. These results, the means of two separate experiments, demonstrate that PKC caused efficient phosphorylation of AGT, a finding that came as a surprise because no strong PKC consensus motifs were detected in the AGT protein. Because substrate recognition by PKs does not follow strict patterns (28), PKC may have recognized a weak motif in the NH₂-terminal cluster of serine/threonine phosphorylation sites of the AGT protein (Fig. 1). A recent study has also implicated PKC in AGT transcription (29). Although our in vitro studies showed that all well-characterized serine/threonine kinases, PKA, PKC, and CK II, can use the AGT protein as a substrate, the question of whether these kinases phosphorylate AGT under physiological conditions awaits further study.

Inhibition of AGT Activity after Its Phosphorylation by Serine/Threonine PKs. To determine whether phosphorylation by purified PKs affects AGT activity, we incubated recombinant AGT protein with PKA, CK II, and PKC under appropriate reaction conditions, removed the kinase reaction components by gel filtration, and quantitated the AGT activity. All three kinases inhibited AGT activity to some extent (Fig. 6B), with the degree of inhibition correlating with that of phosphorylation by the individual kinases. For example, the PKA, which phosphorylated AGT to the least extent, caused a 15% inhibition compared with 35 and 55% inhibition by CK II and PKC, respectively. Prolonged incubation of AGT with the PKs did not result in complete inhibition of AGT activity. Although the reasons for this are not clear, it is possible that a partial modification of the protein and/or the inability of these kinases to phosphorylate the tyrosine may account for the incomplete inhibition. Taken together, the inhibition of AGT activity by the purified kinases (Fig. 6B) and the data pertaining to the phosphorylation and dephosphorylation of endogenous AGT (Figs. 4 and 5) confirm that phosphorylation serves as a negative regulatory mechanism for AGT.

Conclusions. Reversible protein phosphorylation catalyzed by the opposing activities of PKs and phosphatases is a fundamental regulatory mechanism that controls numerous biological processes including enzyme regulation (30). This article is the first report of the phosphorylation of human AGT protein. We have provided evidence to suggest that phosphorylation and dephosphorylation may regulate AGT activity under physiological conditions. The existence of cellular AGT as a phosphoprotein under basal conditions is clearly supportive of this notion. Therefore, a reversible phosphorylation of AGT occurring in response to various signals may serve as an acute cellular means of altering the activity levels of this repair protein. Such a regulatory mode assumes greater significance for AGT, because of its stoichiometric reaction mechanism and the strict need for de novo protein synthesis to restore AGT activity after its inactivation. The circumstances and extent to which AGT is regulated by phosphorylation in cells is presently unclear; however, the presence of multiple phosphorylation sites (serine, threonine, and tyrosine) and consensus motifs for established cellular kinases in the AGT protein seem to suggest that regulation of this posttranslational modification is complex and tight.

Tyrosine phosphorylation of AGT is of particular interest because such a modification is relatively infrequent in cytosolic proteins, occurring only once in every 100 phosphorylation events (31). Of the three tyrosines at positions 69, 114, and 158 in the human AGT protein, the latter two are located in the putative DNA binding region and in the vicinity of active site, respectively (32). Tyrosine 114 and tyrosine 158 are highly conserved in mammalian alkyltransferases,
12. Chen, Z., Hagler, J., Palombella, V. J., Melandri, F., Scherer, D., Ballard, D., and Pieper, R. O. Understanding and manipulating O-phosphorylation is associated with down-regulation of AGT activity. Money doesn’t buy love. The basis for the previous studies is on the AGT protein, which may in turn account for the variations in AGT activity among human gliomas and other cancers. Further clarification of this posttranslational modification may provide important clues for elucidating the cellular regulation of AGT and for devising new therapeutic approaches targeting this protein, because phosphorylation is associated with down-regulation of AGT activity.

References

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