Silence Is Golden: Gene Hypermethylation and Survival in Large-Cell Lymphoma

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Much effort has been directed at identifying molecular markers in human tumors that predict response and/or survival after treatment with chemotherapeutic agents. Molecular predictors help to identify patients most likely to benefit from a particular therapy, with the ultimate goal of selecting optimal treatment for each patient. Gene microarray analysis has already identified genes that may be useful for predicting the clinical behavior of certain tumors *(1,2)*. In malignant lymphoma, microarray analysis has clearly identified subgroups of tumors within the category of diffuse large B-cell lymphoma (B-DLCL) that are histologically indistinguishable but differ considerably in outcome after treatment with standard therapies *(3)*. The specific genes responsible for the different outcomes have not yet been identified. In this issue of the Journal, Esteller et al. *(4)* describe a new predictive marker of survival in patients with B-cell lymphoma that is a DNA repair protein, O^6-methylguanine DNA methyltransferase (MGMT).

MGMT is a unique protein that removes O^6-guanine adducts from DNA, thereby restoring the original DNA in a single step *(5,6)*. There are no other proteins or cofactors involved in this reaction, and the MGMT protein is inactivated in the repair process. Because production of adducts at the O^6 position of guanine is the primary mechanism of cytotoxicity of some alkylating agents, silencing or inactivating MGMT results in an increase in the number of toxic and/or mutagenic lesions in DNA. In particular, methylating agents (i.e., temozolomide, dacarbazine, and procarbazine) and chloroethylating agents (i.e., carmustine) are known to produce toxic lesions at the O^6 position of guanine. There is compelling evidence demonstrating the importance of MGMT expression in mediating resistance to carmustine *(5,6)*. Consistent with this evidence, Esteller et al. *(7)* previously established that MGMT promoter region methylation in brain tumors was a strong predictor of response, overall survival, and time to disease progression in patients treated with carmustine. MGMT promoter methylation is associated with loss of messenger RNA and lack of protein *(8,9)*. There is an inverse association between MGMT activity and the number of O^6-chloroethylguanine lesions that eventually form cytotoxic interstrand cross-links *(5)*; thus, silencing of the gene would result in a higher number of cross-links and in greater antitumor activity.

In this issue, Esteller et al. *(4)* have evaluated the relationship between MGMT promoter methylation and the clinical outcome in patients with B-DLCL who were treated with multiagent chemotherapy including the alkylating agent cyclophosphamide. Bioactivation of cyclophosphamide yields two reactive species toward DNA: acrolein and phosphoramide mustard *(10)*. Adducts formed from acrolein are cyclic adducts between the N1 position and exocyclic amino nitrogen of guanylic acid in DNA. Didechlorocyclophosphamide, which releases acrolein and a nontoxic analogue of phosphoramide mustard, was not found to have antitumor activity; therefore, acrolein is not thought to play a major role in the antitumor activity of this agent *(11)*. However, acrolein likely contributes to the urothelial toxicity of cyclophosphamide *(12)* and is known to be mutagenic *(13)*. Covalent DNA adducts formed from phosphoramide mustard are intrastrand or interstrand cross-link DNA adducts and mono adducts at the N7 position of guanine. The antitumor effect of cyclophosphamide is thought to be associated with phosphoramide mustard-induced interstrand N7–N7 cross-links involving the two guanines in GNC • GNC ($5' \rightarrow 3'/5' \rightarrow 3'$) sequences *(14)*.

One hypothesis suggested by Esteller et al. *(4)* to explain the observation of improved survival in lymphoma patients with MGMT hypermethylation is greater sensitivity to cyclophosphamide. Evidence has emerged recently suggesting a role for MGMT in the repair of certain cyclophosphamide-induced lesions. Friedman et al. *(15)* demonstrated that MGMT-expressing Chinese hamster ovary (CHO) cells were less sensitive to the toxic effects of both 4-HC (an activated form of cyclophosphamide) and 4-HDC (a generator of acrolein and a nonalkylating form of phosphoramide mustard) than CHO cells without detectable MGMT. Further studies *(16)* demonstrated that MGMT-expressing cells were also less sensitive to the mutagenic effects of 4-HC and 4-HDC. Neither the toxic nor the mutagenic effects of phosphoramide mustard, however, were altered in the presence or absence of MGMT *(16)*. Taken together, these results suggest that MGMT hypermethylation resulting in lack of protein expression is likely to contribute to an increase in acrolein-induced lesions in DNA and unlikely to have an impact on antitumor activity produced by phosphoramide mustard.

Animal and human studies that have attempted to find an association between high MGMT expression and resistance to cyclophosphamide *(17–21)* have produced conflicting results. In a recent study using MGMT knockout mice *(17)*, there was no difference in survival of MGMT (+/+) and MGMT (–/–) mice exposed to cyclophosphamide, suggesting that the effects of cyclophosphamide are not modulated by the action of the MGMT protein. Mattern et al. *(19)* evaluated the response to cisplatin or cyclophosphamide of 14 human lung tumor xenografts expressing a wide range of MGMT activity. They found no association between MGMT activity and cisplatin activity; however, they observed an inverse association between MGMT activity and cyclophosphamide response. In contrast, D'Incalci et al. *(22)* reported no association between MGMT activity in human tumor xenografts and response to cyclophosphamide.

In human clinical trials, no association was observed between MGMT levels in ovarian carcinomas and the survival of patients treated with cyclophosphamide and carboplatin, albeit few pa-

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tients with low MGMT were included in this study *(20).* Another study *(21)* showed a positive association between high tumor MGMT activity and poor initial response of ovarian cancer patients to postoperative combination chemotherapy with cyclophosphamide and cisplatin. A limitation in the design of both studies as well as the current investigation is that patients were treated with multiple chemotherapeutic agents, making it difficult to interpret the contribution of cyclophosphamide alone to response or survival. At this time, one cannot reliably conclude that the favorable outcome of patients with MGMT hypermethylation in their tumor cells is due to increased tumor sensitivity to cyclophosphamide. Another possibility is that MGMT hypermethylation is associated with other biochemical or epigenetic changes resulting in greater sensitivity to the chemotherapeutic regimen or that MGMT hypermethylation is a prognostic marker of natural history that identifies a specific pathogenetic subset of lymphomas with a more favorable outcome.

Esteller et al. *(4)* suggest that an indirect approach to address the relationship between MGMT status and B-DLCL sensitivity to cyclophosphamide may be the use of the MGMT inhibitor O^6-benzylguanine (O^6-BG). However, because O^6-BG is a direct inactivator of MGMT *(23)* and evidence points primarily to a role for MGMT in resistance to acrolein, depleting MGMT could result in more severe side effects after exposure to cyclophosphamide. For example, depletion of MGMT in hematopoietic precursor cells might result in an increase in the mutagenic potential of cyclophosphamide, thereby increasing the risk of therapy-related leukemia. It is interesting that there are some cell lines (CHO and head and neck squamous cell carcinoma SQ20b) devoid of MGMT that are, nevertheless, more sensitive to the cytotoxic effects of 4-HC and phosphoramide mustard in the presence of O^6-BG *(15,24,25).* Although the mechanism of increased sensitivity is unclear, tumors deficient in MGMT might be treated more effectively with the combination of O^6-BG and an alkylating agent. Animal studies to evaluate the combination O^6-BG and cyclophosphamide are ongoing. Combining O^6-BG with other nitrogen mustards that do not generate acrolein should be considered when the mechanism of O^6-BG-induced enhancement is better understood and after human tumor xenograft studies demonstrate efficacy with this combination. Prospective assessment of the methylation status of the MGMT gene could identify patients most likely to benefit from this approach if phase I trials support further testing.

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