Published in final edited form as:

Gynecol Oncol. 2009 January ; 112(1): 224–228. doi:10.1016/j.ygyno.2008.08.038.

Absence of MGMT promoter methylation in endometrial cancer

BJ Rimel^a, Phyllis Huettner^b, Matthew A. Powell^a, David G. Mutch^a, and Paul J. Goodfellow^{a,c}

^aDivision of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine and Siteman Cancer Center, 4911 Barnes Jewish Plaza, Box 8064, St. Louis, MO 63110, USA

^bDepartment of Pathology, Washington University School of Medicine and Siteman Cancer Center, 660 South Euclid Avenue, Box 8067, St. Louis, MO 63110, USA

^cDivision of Endocrine and Oncologic Surgery, Department of Surgery, Washington University School of Medicine and Siteman Cancer Center, 660 South Euclid Avenue, Box 8067, St. Louis, MO 63110, USA

Abstract

Objective—O⁶-methylguanine-DNA methyltransferase (MGMT) acts to repair DNA damaged by alkylation of guanine residues. MGMT promoter methylation and gene silencing is seen in a variety of cancers and pre-cancerous changes [1-3]. The loss of MGMT activity and promoter methylation is associated with increased sensitivity to alkylating agents and is a favorable prognostic indicator in gliomas [4-6]. We sought to determine if MGMT promoter methylation plays a role in endometrial cancer.

Methods—One hundred and twenty primary endometrial cancers were analyzed for MGMT promoter methylation by combined bisulfite restriction analysis (COBRA). The cohort included 77 endometrioid endometrial cancers, 43 endometrial tumors of adverse histologic type, and 6 endometrial cancer cell lines. Twenty-one endometrioid and mixed endometrioid ovarian cancers were also analyzed. A subset of the primary tumors was analyzed for MGMT expression by immunohistochemistry.

Results—No MGMT promoter methylation was seen in the 120 endometrial cancers evaluated or the 6 endometrial cancer cell lines. One of the 21 endometrioid ovarian cancers showed methylation. Immunohistochemistry revealed moderate to high level expression of MGMT in the primary endometrial tumors.

Conclusion—MGMT promoter methylation is an infrequent event in endometrial cancer. MGMT expression and the ability to repair damaged alkylguanine residues could in part explain the limited response of endometrial tumors to alkylating chemotherapy.

Keywords

MGMT; endometrial neoplasms; methylation

Corresponding Author: BJ Rimel, M.D., Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Washington University School of Medicine, 4911 Barnes-Jewish Hospital Plaza, 3rd Floor Maternity, St. Louis, MO 63110, Phone: 314-362-2003, Fax: 314-362-8620, rimelb@wudosis.wustl.edu.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

O⁶-methylguanine-DNA methyltransferase (MGMT) repairs DNA damaged via alkylation by removing a methyl group from the O6 position of guanine, thus preventing DNA mutation [7]. MGMT promoter methylation and gene silencing has been associated with a variety of cancers and pre-cancerous states. Specifically, MGMT promoter methylation is seen in 30-40% of colon cancers [1], oral leukoplakia and early SSC (increased in smokers) [2-3], and up to 90% of gliomas [4]. In gliomas, MGMT promoter methylation is associated with significantly improved survival for patients treated with the alkylating agent temozolomide [5-6]. Improved survival among patients with MGMT promoter methylation may reflect increased temozolomide cytotoxicity because the tumor cells' inability to repair alkylguanine residues.

Endometrial cancer is the most common gynecologic malignancy in women in the US, with over 40,000 new cases in 2008 [8]. Most of these cases will be early stage disease, and most women will be cured, however, recurrent and advanced stage endometrial cancer will still account for an estimated 7,470 deaths in 2008 [8]. Endometrial cancer is likely to become an even greater public health concern with the aging population and rising incidence of obesity in the US.

Alkylating agents have been utilized alone or in combination with other chemotherapeutic agents in patients with advanced stage endometrial cancer. Response rates are variable, but overall alkylating agents are not highly effective in endometrial cancer [9-10]. We hypothesized that MGMT promoter methylation might play a role in endometrial tumorigenesis and as such could in part determine response to therapies. Previous studies examining MGMT promoter methylation in a small series of primary endometrioid endometrial cancers suggested a lack of methylation [11]. Studies of synchronous ovarian and endometrial primary tumors on the other hand demonstrated a high rate of MGMT promoter methylation [12]. We evaluated MGMT promoter methylation in a large collection of primary uterine cancers representative of all grades, stages and histologic types.

Materials and Methods

DNA preparation

All primary endometrial and ovarian tumors, normal endometrium and matched normal tissue specimens used in this study were collected as part of prospective studies to investigate the causes, diagnosis and prevention of endometrial cancer and gynecologic malignancies. All patients consented to molecular analyses and follow-up as part of Washington University Medical Center Human Research Protection Office (HRPO)-approved protocols (HRPO91-507, 93-0828 and 92-242). Tissue specimens and blood were obtained at the time of surgery and stored at -70° C until DNA extraction. Normal DNA was isolated from peripheral blood lymphocytes (PBLs) using the QiaAmp DNA blood Midi kit (Qiagen, Valencia, CA). Genomic DNA from tumor tissues, and normal endometrium and cell lines was extracted using the DNeasy Tissue kit (Qiagen, Valencia, CA). All colorectal specimens were obtained from banked DNA previously studied by Herfarth [13].

Bisulfite Conversion

DNA bisulfite conversion was performed using a commercially available kit (EZ DNA Methylation Gold Kit, Zymo Research, Orange, CA).

Mgmt Cobra Assay

A single round of Touchdown PCR was used for amplification as previously described [14]. Forward Primer: 5' TTGGTAAAATTAAGGTATAGAGTTTT 3' Reverse Primer was made degenerate at an internal CG site to prevent preferential amplification of methylated DNA. Sequences were 5' CTAAAACAATCTACGGCATCCTC 3' and 5' CTAAAACAATCTACAGCATCCTC 3'. PCR products were then digested with Taq I and diagnostic fragments resolved on a 10% polyacrylamide gels, stained with ethidium bromide, and photoimaged with a UV camera (ImageStore 500 Version 7.12, White/UV Transilluminator; UVP, Inc., Upland, CA).

Immunohistochemistry

Immunohistochemistry was performed on a subset of primary tumors. Five micrometer sections of paraffin embedded tissues were obtained of 18 endometrioid tumors. Sections were deparaffinized and stained with anti-MGMT (Mouse anti-human, clone MT3.1 DakoCytomation, Inc, Carpinteria, California) using heat-induced epitope retrieval (ER2, Leica Biosystems, Inc, Bannockburn, IL) and an automatic stainer (Bond System, Leica Biosystems).

Results

No MGMT promoter methylation was seen in the 120 uterine cancers evaluated (Table 1). Forty-three tumors represented aggressive histologies (serous, clear cell and malignant mixed müllerian tumor) and of the endometrioid endometrial cancers 53% were advanced stage disease. The non-endometrioid and high stage endometrial cancer specimens were from women who would typically receive adjuvant therapy, most frequently chemotherapy.

A universally methylated DNA positive control for the COBRA assay demonstrated the expected 97 and 64 bp Taq I restriction fragments whereas none of the uterine cancer amplification products digested with Taq I (Figure 1A). One of 21 primary ovarian tumors showed methylation (Figure 1B). Normal ovarian tissues and omentum were also negative for methylation by COBRA (data not shown). Primary colorectal cancer DNAs (Figure 1C) previously shown to have MGMT methylation [13] demonstrated the expected bands. Normal endometrium and peripheral blood DNA from endometrial cancer patients showed no methylation at the MGMT promoter. No methylation was seen by COBRA in the Ishikawa, HEC-1A, KLE, SKUT- IB, ANCA-3, and RL952 endometrial cancer cell lines (data not shown).

Immunohistochemistry showed abundant nuclear MGMT expression in the uterine epithelium and absent or reduced expression in stroma in both normal and cancer specimens (Fig 2A and B). The ovarian cancer with promoter methylation had high level MGMT staining (Figure 2C and D).

Discussion

This study is the first to assess MGMT promoter methylation in a large number of endometrial cancers. No methylation was seen in our survey of 120 cases representative of all grades, stages and histologic types. The six endometrial cancer cell lines evaluated also showed no evidence of methylation of the MGMT promoter. These results confirm previous findings in a small number (n=17) of endometrial cancers by [15] Jacinto et al. [11] and Esteller et al. The absence of MGMT promoter methylation in the endometrial cancer cell lines is noteworthy given that these cell lines are known to have multiple mutations and that several have been shown to have the so-called CpG island methylator phenotype [16-18]. A recent study by Zigelboim et al.

[19] reported frequent tumor-specific methylation in a series of >350 primary endometrioid endometrial cancers. COBRA evaluation of the TITF1, MLH1 and SESN3 promoters demonstrated nearly a third of cases had two or more methylation events. Our results from a large series of tumors (120) indicate MGMT promoter methylation is rare event in endometrial cancer.

MGMT expression as shown by immunohistochemistry in the endometrioid ovarian cancer with MGMT promoter methylation was unexpected given methylation is correlated with absence of MGMT protein product in gliomas and other cancers [11]. Recent studies from Rodriguez et al., however showed poor correlation between MGMT promoter methylation and MGMT expression by immunohistochemistry in glioblastoma specimens [20]. The authors suggest that since many normal cells are trapped in the infiltrating glioblastoma it may be those normal cells that stain for MGMT complicating interpretation of tumor cells. This however, seems unlikely in the ovarian tumor specimen we investigated.

Previous studies evaluating alkylating agents such as ifosfamide and cyclophosphamide in the treatment of advanced stage or progressive endometrial cancer showed lackluster responses to this class of cytotoxics [21-22]. MGMT activity in endometrial cancers could explain in part the limited response to alkylating agents. MGMT normally repairs methylguanine residues. If MGMT fails to repair this damage, then a mismatch lesion is generated. Normally the DNA mismatch repair complex then engages in futile repair processes which result in strand breakage and ultimately targets the cell for apoptosis [23]. Loss of DNA mismatch repair occurs in 20-25% of endometrial cancers [24-25]. In cancers lacking DNA mismatch repair, DNA damage by alkylating agents may not lead to cell death. Our data suggest MGMT is normally active in the majority of endometrial cancers. Resistance to alkylating agents in these cancers is likely attributable to lesion repair (mediated by MGMT) and loss of DNA mismatch repair.

In neuroblastoma and gliomas, response to alkylating agents has been correlated with methylation of the MGMT promoter [4-6,26]. Wagner et al demonstrated pretreatment of neuroblastoma cells with O6-benzylguanine, an MGMT inactivating agent, reduced the IC₅₀ of temozolomide (an alkylating agent) 10 fold regardless of the level of MGMT expression [26]. Similar experiments have been performed in colon cancer and oral cancer cell lines [27]. Future study of MGMT inhibitors in endometrial cancer may ultimately improve sensitivity to alkylating agents.

Acknowledgments

D. Ashley Hill for helpful advice, Mihaela Onciu and Charlene Henry of St. Jude Children's Research Hospital for immunohistochemical technical support. Financial support for this research is provided by R01CA071754 Defective DNA mismatch repair in endometrial cancers (NCI).

References

1. Ogino S, Meyerhardt JA, Kawasaki T, et al. CpG island methylation, response to combination chemotherapy, and patient survival in advanced microsatellite stable colorectal carcinoma. *Virchows Arch* 2007;450:529-37. [PubMed: 17372756]
2. Rodriguez MJ, Acha A, Ruesga MT, Rodriguez C, Rivera JM, Aguirre JM. Loss of expression of DNA repair enzyme MGMT in oral leukoplakia and early oral squamous cell carcinoma. A prognostic tool? *Cancer Lett* 2007;245:263-8. [PubMed: 16517062]
3. Ishii T, Murakami J, Notohara K, et al. Oesophageal squamous cell carcinoma may develop within a background of accumulating DNA methylation in normal and dysplastic mucosa. *Gut* 2007;56:13-9. [PubMed: 16785283]
4. Weaver KD, Grossman SA, Herman JG. Methylated tumor-specific DNA as a plasma biomarker in patients with glioma. *Cancer Invest* 2006;24:35-40. [PubMed: 16466990]

5. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* 2000;343:1350–4. [PubMed: 11070098]
6. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352:997–1003. [PubMed: 15758010]
7. De Vita, VT.; Hellman, S.; Rosenberg, S. *Cancer: Principles & Practice of Oncology*. 7th. Philadelphia: Lippincott Williams & Wilkins; 2005. Chapter 14: Section 4 “Alkylating Agents”; p. 408-409.
8. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71–96. [PubMed: 18287387]
9. McMeekin DS, Filiaci VL, Thigpen JT, Gallion HH, Fleming GF, Rodgers WH. The relationship between histology and outcome in advanced and recurrent endometrial cancer patients participating in first-line chemotherapy trials: a Gynecologic Oncology Group study. *Gynecol Oncol* 2007;106:16–22. [PubMed: 17574073]
10. Pectasides D, Pectasides E, Economopoulos T. Systemic therapy in metastatic or recurrent endometrial cancer. *Cancer Treat Rev* 2007;33:177–90. [PubMed: 17196749]
11. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res* 1999;59:793–7. [PubMed: 10029064]
12. Furlan D, Carnevali I, Marcomini B, Cerutti R, Dainese E, Capella C, et al. The high frequency of de novo promoter methylation in synchronous primary endometrial and ovarian carcinomas. *Clin Cancer Res* 2006;12:3329–36. [PubMed: 16740754]
13. Herfarth KK, Brent TP, Danam RP, et al. A specific CpG methylation pattern of the MGMT promoter region associated with reduced MGMT expression in primary colorectal cancers. *Mol Carcinog* 1999;24:90–8. [PubMed: 10078936]
14. Kim JR, Lee SR, Chung HJ, Kim S, Baek SH, Kim JH, et al. Identification of amyloid beta-peptide responsive genes by cDNA microarray technology: involvement of RTP801 in amyloid beta-peptide toxicity. *Exp Mol Med* 2003;35:403–11. [PubMed: 14646594]
15. Jacinto F, Esteller M. MGMT hypermethylation: a prognostic foe, a predictive friend. *DNA Repair (Amst)* 2007;6:1155–60. [PubMed: 17482895]
16. Rantanen V, Grenman S, Kurvinen K, Hietanen S, Raitanen M, Syrjanen S. p53 mutations and presence of HPV DNA do not correlate with radiosensitivity of gynecological cancer cell lines. *Gynecol Oncol* 1998;71:352–8. [PubMed: 9887230]
17. Xiong Y, Dowdy SC, Gonzalez Bosquet J, et al. Epigenetic-mediated upregulation of progesterone receptor B gene in endometrial cancer cell lines. *Gynecol Oncol* 2005;99:135–41. [PubMed: 16024066]
18. Whitcomb BP, Mutch DG, Herzog TJ, Rader JS, Gibb RK, Goodfellow PJ. Frequent HOXA11 and THBS2 promoter methylation, and a methylator phenotype in endometrial adenocarcinoma. *Clin Cancer Res* 2003;9:2277–87. [PubMed: 12796396]
19. Zigelboim I, Goodfellow PJ, Schmidt AP, et al. Differential methylation hybridization array of endometrial cancers reveals two novel cancer-specific methylation markers. *Clin Cancer Res* 2007;13:2882–9. [PubMed: 17504987]
20. Rodriguez FJ, Thibodeau SN, Jenkins RB, et al. MGMT immunohistochemical expression and promoter methylation in human glioblastoma. *Appl Immunohistochem Mol Morphol* 2008;16:59–65. [PubMed: 18091318]
21. Sutton GP, Blessing JA, DeMars LR, Moore D, Burke TW, Grendys EC. A phase II Gynecologic Oncology Group trial of ifosfamide and mesna in advanced or recurrent adenocarcinoma of the endometrium. *Gynecol Oncol* 1996;63:25–7. [PubMed: 8898163]
22. Pawinski A, Tumolo S, Hoesel G, et al. Cyclophosphamide or ifosfamide in patients with advanced and/or recurrent endometrial carcinoma: a randomized phase II study of the EORTC Gynecological Cancer Cooperative Group. *Eur J Obstet Gynecol Reprod Biol* 1999;86:179–83. [PubMed: 10509788]
23. O'Brien V, Brown R. Signalling cell cycle arrest and cell death through the MMR System. *Carcinogenesis* 2006;27:682–92. [PubMed: 16332722]

24. Goodfellow PJ, Buttin BM, Herzog TJ, et al. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. *Proc Natl Acad Sci U S A* 2003;100:5908–13. [PubMed: 12732731]
25. Zigelboim I, Goodfellow PJ, Gao F, et al. Microsatellite instability and epigenetic inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. *J Clin Oncol* 2007;25:2042–8. [PubMed: 17513808]
26. Wagner LM, McLendon RE, Yoon KJ, Weiss BD, Billups CA, Danks MK. Targeting Methylguanine-DNA Methyltransferase in the Treatment of Neuroblastoma. *Clin Cancer Res* 2007;13:5418–25. [PubMed: 17875772]
27. Murakami J, Lee YJ, Koikeguchi S, Tsujigiwa H, Asaumi J, Nagatsuka H, et al. Depletion of O6-methylguanine-DNA methyltransferase by O6-benzylguanine enhances 5-FU cytotoxicity in colon and oral cancer cell lines. *Oncol Rep* 2007;17:1461–7. [PubMed: 17487405]

Figure 1. Representative COBRA for three cancer types. A. Endometrial cancers. B. Ovarian cancers. C. Colon cancers. Restriction products were resolved on 10% polyacrylamide gels. UM: universally methylated positive control. +: methylated specimen; - unmethylated specimen. Arrows indicate methylation-specific restriction digestion products.

Figure 2. Representative examples of MGMT immunohistochemistry A. COBRA negative normal endometrium. B. COBRA negative endometrial cancer. C. H&E staining of COBRA positive endometrioid ovarian cancer. D. MGMT staining of COBRA positive endometrioid ovarian cancer

Table 1

Breakdown of tumors analyzed for MGMT promoter methylation

Primary cancers (N)	N (% methylated)
Uterine	120 (0%) Total
<i>Stage</i>	
I (66)	
II (14)	
III (26)	
IV (14)	
<i>Histology</i>	
Endometrioid	
Grade 1 (39)	
Grade 2 (21)	
Grade 3 (17)	
Papillary serous (16)	
Clear cell (8)	
Malignant mixed müllerian tumor (19)	
Ovarian	21 (4.8%) Total
<i>Histology</i>	
Endometrioid	11 (0%)
Mixed (endometrioid and serous or clear cell)	10 (10%)
Colorectal	7 (43%)