COLON CANCER

Human colorectal mucosal O^6-alkylguanine DNA-alkyltransferase activity and DNA-N7-methylguanine levels in colorectal adenoma cases and matched referents

N P Lees, K L Harrison, C N Hall, G P Margison, A C Povey

Gut 2007;**56**:380–384. doi: 10.1136/gut.2006.097899

See end of article for authors' affiliations

Correspondence to:
Dr A C Povey, Centre for Occupational and Environmental Health, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK; apovey@manchester.ac.uk

Revised 22 June 2006
Accepted 4 July 2006
Published Online First 4 August 2006

Background and aims: O^6-alkylguanine DNA-alkyltransferase (MGMT) provides protection against alkylating agent-induced GC→AT transition mutations. Such mutations are frequently seen in the *KRAS* oncogene of large colorectal adenomas, but whether adenoma or mutational risk in humans is influenced by MGMT activity and alkylating agent exposure is unclear. Hence, MGMT activity and, as an indicator of alkylating agent exposure, DNA-N7-methylguanine (N7-MeG) levels were determined in the normal tissue of patients with and without adenomas.
Methods: Biopsy specimens of normal colorectal mucosa were collected during colonoscopy from 85 patients with histologically proved colorectal adenomas (cases) and from 85 patients free of gastrointestinal neoplasia (referents) matched by age, sex and biopsy location. MGMT activity and N7-MeG levels were measured in colorectal tissue extracts and DNA, respectively.
Results: MGMT activity was higher in the normal mucosa of cases than in referents (6.65 ± 3.03 vs 5.61 ± 2.74 fmol/μg DNA, $p = 0.01$). On stratification of cases, MGMT activity was found to be considerably greater in the normal mucosa of cases with large adenomas ($p = 0.003$) and slightly higher in cases with a GC→AT transition mutation in the K-*ras* gene ($p = 0.03$). Elevated MGMT levels were associated with an increased risk of adenoma (OR 1.17, 95% CI 1.03 to 1.33 per unit increase in activity). Detectable levels of N7-MeG were found in DNA from 89% of cases and 93% of referents, with levels ranging from <0.1 to 7.7 μmol/mol dG. Cases and referents had similar DNA-N7-MeG levels.
Conclusions: Human exposure to methylating agents is widespread. MGMT activity is increased in the normal mucosa of patients with adenomas.

Most colorectal cancers (CRCs) develop from colorectal mucosa via a benign adenomatous stage.[1] Acquisition of a *KRAS* mutation is a common event, particularly during the growth of an adenoma: these mutations are seen in only 10% of small adenomas (<10 mm in diameter), but they occur in ~50% of large adenomas (≥ 10 mm in diameter), and in a similar proportion of frank CRCs.[2] Mutations are observed in several regions of the activated *KRAS* oncogene, but the predominant type of mutation is a GC→AT transition.[3] The aetiological factors that cause these mutations are unclear, but they are commonly observed in the *KRAS* oncogene after the experimental induction of tumours by methylating agents such as 1,2-dimethylhydrazine.[4]

Methylating agents induce a number of different DNA base modifications, the most abundant being N7-methylguanine (N7-MeG).[5] This is relatively innocuous to the cell, but it is repaired relatively slowly and has therefore been used as a biomarker of exposure to methylating agents.[6] In contrast, O^6-methylguanine (O^6-MeG) is less abundant, but is the predominant mutagenic and carcinogenic modification resulting in GC→AT transition mutations.[7] O^6-MeG has been detected in human colon DNA and interindividual variation in O^6-MeG levels is large, being at least 10-fold.[8 9] There is some evidence to suggest that the highest O^6-MeG levels occur in the regions of the large bowel where most neoplastic tumours occur—that is, the sigmoid colon and rectum.[9] The levels observed in human colon DNA correspond to tens to hundreds of adducts per cell, but may be much higher in certain cell populations.[10 11] In DNA repair deficient cell lines exposed to methylating agents, it has been calculated that on average, one mutation results from every eight O^6-MeG adducts produced in the coding region of the hypoxanthine phosphoribosyltransferase gene.[12] O^6-MeG levels in human cells may thus be of sufficient magnitude to cause biological effects, particularly in cells that are deficient in the DNA repair protein, O^6-alkylguanine DNA-alkyltransferase (MGMT). MGMT acts in a stoichiometric process that results in the transfer of the methyl group from O^6-MeG to a cysteine acceptor group (position 145) in the human protein, which is inactivated and subsequently degraded through the ubiquitination pathway.[13–15]

Interindividual variation in MGMT activity in normal human colon tissue has been reported to be between 2-fold and 18-fold.[16] In primary human tumours, methylation of CpG dinucleotides in the CpG island of the promoter region of the *MGMT* gene is a common event.[17] Such methylation has been associated with both reduced MGMT expression and an increased frequency of GC→AT transition mutations in *KRAS* in colorectal tumours, and in p53 in colorectal tumours and astrocytomas.[18–23] Reduced MGMT activity in adenomatous tissue has been associated with the presence of these *KRAS* GC→AT transition mutations in adenomas.[24] Low MGMT activity in normal colorectal mucosa has been associated with the occurrence of GC→AT transition mutations in the *KRAS* oncogene of colorectal cancers, and, furthermore, there is evidence to suggest that colorectal tumours occur in regions of low MGMT activity.[25–26]

If alkylating agent exposure is important in the aetiology of colorectal cancers, probably the critical period is during the growth of a small adenoma into a large adenoma, as this is

Abbreviations: CRC, colorectal cancer; MGMT, O^6-alkylguanine DNA-alkyltransferase; N7-MeG, N7-methylguanine

when the key mutational event (ie, a GC→AT transition mutation), often linked to alkylating agent exposure, occurs. Hence, this study was designed to examine whether MGMT activity and methylating agent exposure (as measured by N7-MeG rather than O^6-MeG levels because the latter depend on exposure and MGMT levels) differed in patients without or with adenomas of different sizes and in patients whose adenomas did or did not contain specific *KRAS* mutations.

PATENTS AND METHODS

Study population

All subjects were recruited from patients undergoing a flexible endoscopic examination of the colon and rectum in the Department of Gastrointestinal Surgery, Wythenshawe Hospital, Manchester, UK, for investigation of common colorectal symptoms such as altered bowel habit or rectal bleeding. Cases were patients with polyps in the rectum, sigmoid colon, descending colon, transverse colon and ascending colon/caecum as determined by an experienced endoscopist (NPL). The referents were patients who were age- (within 5 years) and sex-matched to a case individual, had no history (past or present) of gastrointestinal neoplasia, had no adenomas on complete colonoscopy, and whose symptoms were attributed to common simple conditions such as diverticulosis, irritable bowel or piles, conditions that commonly coexist with polyps.

Human tissues

Human tissue samples were obtained with the approval of the local research ethics committee and in accordance with UK ethical guidelines for work with human subjects. During endoscopic examination, the patient received sedation with the short-acting benzodiazepine, midazolam (Antigen Pharmaceuticals, Tipperary, Ireland) and supplemental oxygen, as judged necessary by the endoscopist. Air was used for insufflation.

In patients with polyps, three mucosal pinch biopsy specimens were taken approximately 2 cm away from the base of the polyp, using FB 24U-1 colonoscopy biopsy forceps (Olympus, Southend-On-Sea, Essex, UK). Polyps were treated endoscopically by electrocautery snare or fulguration as determined by the endoscopist on clinical grounds. The maximum diameter of polyps was determined immediately after their removal, and polyps were deemed ''large'' if $\geqslant$10 mm and ''small'' if $<$10 mm. If multiple polyps were encountered, the largest was used as the reference point for biopsy of associated normal mucosa. All polyps were examined histologically by a consultant pathologist, and if confirmed to be adenomas, the source individual was deemed to be a ''case'' in the study.

In referents, three mucosal pinch biopsy specimens were taken from the same large bowel subsite (ie, rectum, sigmoid colon, descending colon, transverse colon or ascending colon/caecum) as the matched case. These individuals served as normal ''referents''. For ease of reference, the terms ''small'' and ''large'' were also applied to matched referents; the term was simply used to indicate the case group (ie, small or large adenoma group) of the case individual to which the neoplasia-free referent was matched. Sex matching and subsite matching were absolute.

For sessile and semisessile adenomas, the entire polyp was required by the histopathologist, whereas a biopsy specimen was taken from the adenoma (away from the junction with the stalk) in patients with large adenomas and a resected stalk of $>$10 mm (n = 34 cases). For each patient, one of the normal mucosa biopsy specimens was fixed in Carson's solution (60% v/v ethanol, 30% v/v chloroform and 10% v/v acetone) and examined histologically. The remaining normal mucosa biopsy specimens and the adenoma biopsy specimens were snap frozen and stored at −80°C until an MGMT assay was performed or DNA extracted. All case–referent matching was performed before samples were assayed.

MGMT activity

Cell-free extracts prepared from between 5 and 15 mg of normal mucosa (two pooled biopsy specimens per patient) were analysed for MGMT activity using calf thymus DNA methylated in vitro with N-nitroso-*N*-[^{3}H]-methylurea (approximately 20 Ci/mmol) as the substrate.[27] MGMT activity was expressed as fmol/µg DNA to avoid the possible effect of variable protein content on apparent MGMT activity expressed per unit protein.[28]

Results are the mean of quadruplicate determinations for each sample. All matched case–control pairs were assayed simultaneously. Cell-free extracts prepared from the human B-lymphoid cell line Raji were assayed for MGMT activity with each batch of mucosal samples as a positive control. The mean coefficient of variation of the quadruplicate MGMT assays for each sample was 7%. The same batch coefficient of variation in MGMT determination based on assays of Raji extracts was 2%, and the different batch coefficient of variation was 11%.

KRAS mutation analysis

Of the 85 cases, adenoma DNA suitable for *KRAS* codon 12 and 13 mutation analysis was available in 63, as no DNA was available from polyps fulgurated by electrocautery. Adenoma DNA was extracted from paraffin tissue blocks and screened for *KRAS* codon 12 and 13 mutations via a restriction site mutation assay.[29] The sequence of all detected mutations was determined using an Amplification Refractory Mutation System (Elucigene, Zeneca Diagnostics, Alderley Edge, Cheshire, UK) following the manufacturer's recommendations.

Determination of N7-MeG in colorectal mucosal DNA

Genomic DNA for N7-MeG measurement was extracted from colorectal mucosal biopsy specimens using the Qiagen genomic DNA extraction kit (Qiagen, Crawley, UK), except that the proteinase K and ribonuclease A digestion was carried out overnight at 4°C, followed by 1 h at 37°C. Levels of N7-MeG in the DNA of colorectal mucosa were measured using an immunoslot blot technique.[30] The mean coefficient of variation of the triplicate immunoslot blot assays was 9.8% for both cases and controls. The limit of detection was 0.1 µmol N7-MeG/mol dG, and those samples with no detectable N7-MeG levels were assumed to have a level equal to half this detection limit (ie, 0.05 µmol/mol dG). Sufficient DNA for analysis was extracted from 73 of the case–control pairs.

Statistical analysis

MGMT activity and N7-MeG levels in matched cases and referents were examined using a paired sample t test and Wilcoxon's signed rank test, respectively: the mean differences (and 95% confidence intervals (CIs)) between the matched subjects are presented with a p value. Transformation of N7-MeG levels into log N7-MeG resulted in a more normal distribution, and results similar to those presented were obtained using parametric procedures. Smoking status in cases and referents was compared using a χ^2 test.

The association between being a case and MGMT activity and N7-MeG levels was then further examined using a conditional logistic regression model. Odds ratios (ORs) and 95% CIs were calculated for MGMT activity and N7-MeG levels alone and together, both with and without adjustment for smoking.

RESULTS

Demographic characteristics

Normal mucosa samples were available for MGMT determination in 85 matched pairs, of which 42 pairs were men and 43 pairs were women. The mean (±SD) age was 67.5 (11.8) and 67.6 (11.5) for the cases and referents, respectively. Cases were more likely to be current smokers than referents (36% *v* 13%; p = 0.001). Of the 85 cases, 24 had small adenomas (7 men and 17 women) and 61 had large adenomas (35 men and 26 women). Cases with large adenomas were significantly older than those with small adenomas (71.5±8.6 *v* 57.5±13.0; p<0.001).

MGMT activity and N7-MeG levels in cases and referents

MGMT activity was detected in the histologically normal mucosa of all 85 cases, with levels ranging from 0.75 to 14.30 fmol/µg DNA, and in 82 of the 85 referents (levels from 1.33 to 13.48 fmol/µg DNA). In three referents there was no detectable MGMT activity. MGMT activity was not associated with sex, patient's age, size of the adenoma or current smoking status (data not shown).

N7-MeG levels were determined in normal colorectal mucosal DNA from 73 of the case–referent pairs. The majority of colorectal mucosal DNA samples contained detectable levels of N7-MeG (89% of cases and 93% of referents). Levels ranged from <0.1 to 7.7 µmol/mol dG in cases and from <0.1 to 4.4 µmol/mol dG DNA in referents. N7-MeG levels were not associated with sex, age, size of the adenoma or current smoking status (data not shown).

KRAS mutations

KRAS codon 12 or 13 mutations were found in 28 (44%) of the adenomas. A GC→AT transition mutation was identified in 19 (30%) of the cases, and a transversion or other mutation in 11 (17%) of the cases, including two in which both a GC→AT transition mutation and a transversion mutation were found.

Case–referent comparison of MGMT activity and N7-MeG levels

Normal mucosal MGMT activity was significantly higher in cases than in matched referents (6.65 (3.03) *v* 5.61(2.74) fmol/µg DNA, p = 0.01, table 1).

In strata defined by adenoma diameter, MGMT activity was significantly higher in the normal mucosa of large adenoma cases (⩾10 mm diameter) than in the mucosa of matched referents (p = 0.003, table 1). There was no significant difference in MGMT activity in the normal mucosa of small adenoma cases and matched referents.

MGMT activity was significantly greater in the normal mucosa of cases with adenomas bearing a GC→AT transition mutation than in the normal mucosa of matched referents (p = 0.03, table 1). In contrast, there was no significant difference between the MGMT activity in the normal mucosa of cases lacking a GC→AT transition mutation (ie, those with no codon 12 or 13 *KRAS* mutation, or those with a transversion mutation) and the normal mucosa of matched referents.

There was no significant difference in N7-MeG levels between cases and referents even after subdivision based on adenoma size or the presence or absence of a GC→AT transition mutation (table 2).

Elevated MGMT levels were associated with an increased adenoma risk (OR 1.17, 95% CI 1.03 to 1.33 per unit increase in activity) but not N7-MeG levels (OR 0.98, 95% CI 0.70 to 1.37 per unit increase in adduct levels). After adjustment for current smoking, the ORs (95% CIs) were 1.15 (1.00 to 1.32) and 0.92 (0.62 to 1.38) for MGMT activity and N7-MeG levels, respectively. Using MGMT activity and N7-MeG levels in the same conditional logistic regression model had little effect on the estimates of risk for MGMT activity (OR 1.15, 95% CI 1.00 to 1.31) or for N7-MeG levels (OR 0.97, 95% CI 0.09 to 1.36). These risk estimates were little altered after adjustment for current smoking (data not shown).

DISCUSSION

This study has shown that MGMT activity in normal colorectal tissue differs between patients with and without adenomas. Our previous work suggested that low MGMT activity in normal tissue was associated with the presence of a CRC.[25] The present study, which focuses on a different stage in the adenoma–carcinoma sequence, possibly 5–10 years earlier in the natural history of polyps, indicates that MGMT activity in normal tissue is higher in cases than in referents. This increase was observed especially in patients with large adenomas and in those with a GC→AT transition mutation in the *KRAS* gene.

MGMT regulation is complex and may depend on both genetic and environmental factors.[31] There is some evidence that intragenic sites may affect MGMT activity, and hence differences in *MGMT* genotype between cases and referents could account for variations in activity.[32] However, given the low frequency of known MGMT polymorphisms in Caucasians, their influence may be limited.[32–34] Although growing tumours may have effects on neighbouring tissues, we have not observed any upregulation in normal tissue adjacent to colonic tumours when examining multiple biopsy specimens from the same patient.[26] Indeed, an elevation of MGMT activity in normal tissue as a result of the presence of an adenoma seems unlikely, as previously colonic cancers themselves tended to have higher MGMT activity than the surrounding non-malignant tissue.[26]

Table 1 O^6-alkylguanine DNA-alkyltransferase (MGMT) activity (fmol MGMT/µg DNA) in normal mucosa from patients with adenomas (cases) and matched referents

Adenoma group	Subgroup	n	Mean (SD) MGMT activity: Case	Mean (SD) MGMT activity: Referent	Difference in MGMT activity between matched cases and referents: Mean (95% CI)	p*
All	–	85	6.65 (3.03)	5.61 (2.74)	1.04 (0.25 to 1.82)	0.01
Large	Polyp ⩾10 mm in diameter	61	6.95 (2.58)	5.60 (2.66)	1.35 (0.49 to 2.21)	0.003
Small	Polyp <10 mm in diameter	24	5.90 (3.92)	5.65 (2.99)	0.24 (−1.53 to 2.01)	0.78
Mut+	Adenoma with *KRAS* GC→AT transition	19	7.23 (2.82)	5.07 (2.18)	2.17 (0.30 to 4.03)	0.03
Mut−	Adenoma without *KRAS* GC→AT transition	44	6.38 (3.45)	5.36 (2.97)	1.02 (−0.15 to 2.19)	0.09

*Paired sample t test.

Table 2 N7-MeG levels (μmol/mol dG) in normal mucosa from patients with adenomas (cases) and matched referents

Adenoma group	Subgroup	n	Mean (SD) N7-MeG levels		Case–referent difference in N7-MeG levels	
			Case	Referent	Mean (95% CI)	p*
All		73	0.93 (1.20)	0.95 (0.88)	−0.02 (−0.35 to 0.30)	0.88
Large	Polyp ⩾10 mm in diameter	54	0.88 (0.96)	0.90 (0.86)	−0.02 (−0.35 to 0.31)	0.92
Small	Polyp <10 mm in diameter	19	1.06 (1.73)	1.11 (0.95)	−0.05 (−0.93 to 0.84)	0.92
Mut +	Adenoma with *KRAS* GC→AT transition	18	0.92 (0.84)	1.06 (0.95)	−0.14 (−0.60 to 0.32)	0.53
Mut −	Adenoma without *KRAS* GC→AT transition	37	0.91 (1.40)	0.88 (0.76)	0.02 (−0.47 to 0.53)	0.92

*Wilcoxon's signed rank test.

Hence, these differences do not seem to result from the process of carcinogenesis in itself.

Chronic exposure to specific alkylating agents can, however, lead to increased MGMT activity, at least in rodent tissues, in particular the rat liver,[35–37] although there has been little evidence to date to show this occurring in the human large bowel. Nevertheless, it is plausible that the relatively higher MGMT activity in normal mucosa in cases may be a consequence of increased exposures to alkylating agents, other mutagens or indeed other factors that may alter MGMT levels. MGMT activity was not associated with smoking status in comparison with MGMT activity in bronchial epithelial cells.[38] Furthermore, as there was no difference in N7-MeG levels in normal tissue DNA from cases and referents, it seems unlikely that the relatively increased repair activity is a consequence of induction by exposure to methylating agents. This may, however, also reflect the nature of the methylating agent as those agents (such as N-methyl-N′-nitro-N-nitrosoguanidine) that react via an S_n1 mechanism induce MGMT activity more than those agents that act via an S_n2 mechanism (eg, methylmethane sulphonate).[39] If exposure was then owing to an S_n2 agent, then no association between MGMT activity and N7-MeG levels would be expected.

N7-MeG is considered a good biomarker of exposure to methylating agents by virtue of the fact that it is more slowly repaired, and is formed in higher amounts than the corresponding mutagenic adduct O^6-methylguanine, and is thus easier to detect.[6] N7-MeG has previously been detected in DNA from a limited range of human tissues, including white blood cells, typically in pilot studies with small numbers of subjects.[40 41] In this study, DNA from a larger number of subjects has been examined and we observed that few patients had undetectable N7-MeG in their DNA. This clearly shows that exposure to methylating agents in the colon is widespread. Levels of N7-MeG in colon DNA are not associated with current smoking. The sources of these agents are thus yet to be established, but they may arise from normal cellular components (eg, S-adenosylmethionine[41]) or from the in situ formation of methylating agents (such as N-nitroso compounds in the colon) from dietary precursors such as meat.[43 44] Red meat has recently been shown to enhance the formation of another alkyl DNA adduct, O^6-carboxylmethyl guanine, which can arise from nitrosated bile acids (that can also methylate DNA[45]) or glycine derivatives.[46] A better understanding of the sources of alkylating agent exposure is therefore important and, with a comprehensive knowledge of other factors that influence mutagenesis, could lead to new measures to reduce cancer risk.

ACKNOWLEDGEMENTS

This work was generously supported by the Association for International Cancer Research, the Christie Hospital Endowment Fund and in part by Cancer Research UK. We also thank Dr P Bishop, consultant histopathologist at Wythenshawe Hospital, for his examination of the adenoma samples.

Authors' affiliations

C N Hall, Department of Gastrointestinal Surgery, Wythenshawe Hospital, Wythenshawe, Manchester, UK
N P Lees*, G P Margison, Cancer Research UK Carcinogenesis Group, Paterson Institute for Cancer Research, Christie Hospital, Manchester, UK
K L Harrison, A C Povey, Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

*Current address: Department of Colorectal Surgery, Hope Hospital, Stott Lane, Salford M6 8HD, UK

Competing interests: None declared.

REFERENCES

1 **Ponz de Leon M**, Di Gregorio C. Pathology of colorectal cancer. *Dig Liver Dis* 2001;**33**:372–88.
2 **Cho KR**, Vogelstein B. Genetic alterations in the adenoma—carcinoma sequence. *Cancer* 1992;**70**(Suppl):1727–31.
3 **Andreyev HJ**, Norman AR, Cunningham D, *et al.* Kirsten ras mutations in patients with colorectal cancer: the ''RASCAL II'' study. *Br J Cancer* 2001;**85**:692–6.
4 **Jacoby RF**, Llor X, Teng BB, *et al.* Mutations in the K-ras oncogene induced by 1,2-dimethylhydrazine in preneoplastic and neoplastic rat colonic mucosa. *J Clin Invest* 1991;**87**:624–30.
5 **Margison GP**, Povey AC. Chemical carcinogenesis. In: Souhami RL, Tannock I, Hohenberger P, Horiot J-C, eds. *Oxford textbook of oncology.*2nd edn. Oxford: Oxford University Press, 2002:129–49.
6 **Bianchini F**, Wild CP. 7-Methyldeoxyguanosine as a marker of exposure to environmental methylating agents. *Toxicol Lett* 1994;**72**:175–84.
7 **Margison GP**, Santibanez-Koref MF, Povey AC. Mechanisms of carcinogenicity/chemotherapy by O^6-methylguanine. *Mutagenesis* 2002;**17**:483–7.
8 **Hall CN**, Badawi AF, O'Connor PJ, *et al.* The detection of alkylation damage in the DNA of human gastrointestinal tissues. *Br J Cancer* 1991;**64**:59–63.
9 **Povey AC**, Hall CN, Badawi AF, *et al.* Elevated levels of the pro-carcinogenic adduct, O^6-methylguanine, in normal DNA from the cancer prone regions of the large bowel. *Gut* 2000;**47**:362–5.
10 **O'Connor PJ**, Manning FC, Gordon AT, *et al.* DNA repair: kinetics and thresholds. *Toxicol Pathol* 2000;**28**:375–81.
11 **Rasouli-Nia A**, Ullah S, Mirzayans R, *et al.* On the quantitative relationship between O^6-methylguanine residues in genomic DNA and production of sister chromatid exchanges, mutations and lethal events in a Mer- human tumor cell line. *Mutat Res* 1994;**314**:99–113.
12 **Pegg AE**. Repair of O^6-alkylguanine by alkyltransferases. *Mutat Res* 2000;**462**:83–100.
13 **Gerson SL**. MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* 2004;**4**:296–307.
14 **Margison GP**, Santibanez-Koref MF. O^6-alkylguanine-DNA alkyltransferase: role in carcinogenesis and chemotherapy. *Bioessays* 2002;**24**:255–66.
15 **Srivenugopal KS**, Yuan XH, Friedman HS, *et al.* Ubiquitination-dependent proteolysis of O^6-methylguanine-DNA methyltransferase in human and murine tumor cells following inactivation with O^6-benzylguanine or 1,3-bis(2-chloroethyl)-1-nitrosourea. *Biochemistry* 1996;**35**:1328–34.
16 **Povey AC**, Hall CN, Cooper DP, *et al.* Determinants of O^6-alkylguanine-DNA alkyltransferase activity in normal and tumour tissue from human colon and rectum. *Int J Cancer* 2000;**85**:68–72.

17 **Esteller M**, Hamilton SR, Burger PC, *et al*. Inactivation of the DNA repair gene *O*⁶-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res* 1999;**59**:793–7.
18 **Costello JF**, Futscher BW, Tano K, *et al*. Graded methylation in the promoter and body of the O⁶-methylguanine DNA methyltransferase (MGMT) gene correlates with MGMT expression in human glioma cells. *J Biol Chem* 1994;**269**:17228–37.
19 **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* 1997;**24**:90–8.
20 **Esteller M**, Toyota M, Sanchez-Cespedes M, *et al*. Inactivation of the DNA repair gene *O*⁶-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. *Cancer Res* 2000;**60**:2368–71.
21 **Whitehall VLJ**, Walsh MD, Young J, *et al*. Methylation of *O*⁶-methylguanine DNA methyltransferase characterizes a subset of colorectal cancer with low-level DNA microsatellite instability. *Cancer Res* 2001;**61**:827–30.
22 **Esteller M**, Risques RA, Toyota M, *et al*. Promoter hypermethylation of the DNA repair gene *O*⁶-methylguanine-DNA methyltransferase is associated with the presence of G:C to A:T transition mutations in p53 in human colorectal tumorigenesis. *Cancer Res* 2001;**61**:4689–92.
23 **Nakamura M**, Watanabe T, Yonekawa Y, *et al*. Promoter methylation of the DNA repair gene MGMT in astrocytomas is frequently associated with G:C→A:T mutations of the TP53 tumor suppressor gene. *Carcinogenesis* 2001;**22**:1715–19.
24 **Lees NP**, Harrison KL, Hall CN, *et al*. Reduced MGMT activity in human colorectal adenomas is associated with K-ras GC→AT transition mutations in a population exposed to methylating agents. *Carcinogenesis* 2004;**25**:1243–7.
25 **Jackson PE**, Hall CN, O'Connor PJ, *et al*. Low *O*⁶-alkylguanine DNA-alkyltransferase activity in normal colorectal tissue is associated with colorectal tumours containing a GC-AT transition in the K-ras oncogene. *Carcinogenesis* 1997;**18**:1299–302.
26 **Lees NP**, Harrison KL, Hill E, *et al*. Longitudinal variation in *O*⁶-alkylguanine DNA-alkyltransferase activity in the human colon and rectum. *Br J Cancer* 2002;**87**:168–70.
27 **Watson AJ**, Margison GP. *O*⁶-alkylguanine-DNA alkyltransferase assay. In: Brown R, Böger-Brown U, eds. *Cytotoxic drug resistance mechanisms*. New Jersey: Humana Press, 1999:167–78.
28 **Gerson SL**, Trey JE, Miller K, *et al*. Comparison of *O*⁶-alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat and mouse tissues. *Carcinogenesis* 1986;**7**:745–9.
29 **Jackson PE**, Hall CN, Badawi AF, *et al*. Frequency of Ki-ras mutations and DNA alkylation in colorectal tissue from individuals living in Manchester. *Mol Carcinog* 1996;**16**:12–19.
30 **Harrison KL**, Wood M, Lees NP, *et al*. Development and application of a sensitive and rapid immunoassay for the quantitation of N7-methyldeoxyguanosine in DNA samples. *Chem Res Toxicol* 2001;**14**:295–301.
31 **Margison GP**, Povey AC, Kaina B, *et al*. Variability and regulation of *O*⁶-alkylguanine-DNA alkyltransferase activity. *Carcinogenesis* 2003;**24**:625–35.
32 **Margison GP**, Heighway J, McGown G, *et al*. Quantitative trait locus analysis reveals two intragenic sites that influence *O*⁶-alkylguanine-DNA alkyltransferase activity in peripheral blood mononuclear cells. *Carcinogenesis* 2005;**26**:1473–80.
33 **Yang M**, Coles BF, Caporaso NE, *et al*. Lack of association between Caucasian lung cancer risk and *O*⁶-methylguanine-DNA methyltransferase-codon 178 genetic polymorphism. *Lung Cancer* 2004;**44**:281–6.
34 **Cohet C**, Borel S, Nyberg F, *et al*. Exon 5 polymorphisms in the *O*⁶-alkylguanine DNA alkyltransferase gene and lung cancer risk in non-smokers exposed to second-hand smoke. *Cancer Epidemiol Biomarkers Prev* 2004;**13**:320–3.
35 **Lindamood C III**, Bedell MA, Billings KC, *et al*. Dose response for DNA alkylation, [³H] thymidine uptake into DNA, and *O*⁶-methylguanine-DNA methyltransferase activity in hepatocytes of rats and mice continuously exposed to dimethylnitrosamine. *Cancer Res* 1984;**44**:196–200.
36 **O'Connor PJ**, Chu Y-H, Cooper DP, *et al*. Species differences in the inducibility of hepatic O⁶-alkylguanine repair rodents. *Biochimie* 1982;**64**:769–73.
37 **Lim IK**, Park TJ, Jee JW, *et al*. Differential expression of *O*⁶-methylguanine-DNA methyltransferase during diethylnitrosamine-induced carcinogenesis and liver regeneration in Sprague-Dawley male rats *J Cancer Res Clin Oncol* 1999;**125**:493–9.
38 **Povey AC**, O'Donnell PNS, Barber P et al. Smoking is associated with a decrease of *O*⁶-alkylguanine-DNA alkyltransferase activity in bronchial epithelial cells. *Int J Cancer* 2006;**119**:463–6.
39 **Frosina G**. Laval F. The *O*⁶-methylguanine-DNA-methyltransferase activity of rat hepatoma cells is increased after a single exposure to alkylating agents. *Carcinogenesis* 1987;**8**:91–5.
40 **Mustonen R**, Schoket B, Hemminki K. Smoking-related DNA adducts: ³²P-postlabeling analysis of 7-methylguanine in human bronchial and lymphocyte DNA. *Carcinogenesis* 1993;**14**:151–4.
41 **Kumar R**, Hemminki K. Separation of 7-methyl- and 7-(2-hydroxyethyl)-guanine adducts in human DNA samples using a combination of TLC and HPLC. *Carcinogenesis* 1996;**17**:485–92.
42 **Rydberg B**, Lindahl T. Nonenzymatic methylation of DNA by the intracellular methyl group donor S-adenosyl-L-methionine is a potentially mutagenic reaction. *EMBO J* 1982;**1**:211–16.
43 **Mirvish SS**, Haorah J, Zhou L, *et al*. N-nitroso compounds in the gastrointestinal tract of rats and in the feces of mice with induced colitis or fed hot dogs or beef. *Carcinogenesis* 2003;**24**:595–603.
44 **Bingham SA**, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. *J Nutr* 2002;**132**(Suppl):3522S–25S.
45 **Shuker DEG**, Margison GP. Nitrosated glycine derivatives as a potential source of *O*⁶-methylguanine in DNA. *Cancer Res* 1997;**57**:366–9.
46 **Lewin MH**, Bailey N, Bandaletova T, *et al*. Red meat enhances the colonic formation of the DNA adduct *O*⁶-carboxymethyl guanine: implications for colorectal cancer risk. *Cancer Res* 2006;**66**:1859–65.

International Forum on Quality & Safety in Health Care

18–20 April 2007
Palau de Congressos, Barcelona

Registration now open

- Book by 23 February 2007 and save up to £60 on the 3-day delegate fee
- Join us at the premier international event for those dedicated to improving quality and safety in health care
- Speakers include: Donald Berwick, Sir John Oldham, Lucien Leape, Richard Smith, Helen Bevan, Lloyd Provost and Rosa Sunol
- For more information and to book online please visit: www.quality.bmjpg.com