DNA Alkylation and Repair in the Large Bowel: Animal and Human Studies\textsuperscript{1,2}

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ABSTRACT \textit{O\textsuperscript{6}}-Methylguanine (\textit{O\textsuperscript{6}}-MeG), a procarcinogenic DNA adduct that arises from exposure to methylating agents, has been detected in human colorectal DNA at levels comparable to those that cause adverse effects in model systems. \textit{O\textsuperscript{6}}-MeG levels vary within the colon, being higher in the cancer-prone regions of the large bowel. In rats and mice, \textit{O\textsuperscript{6}}-MeG persistence in colon DNA is associated with the induction of colon tumors after treatment with methylating agents. These tumors frequently contain K-ras GC→AT transition mutations, which is consistent with the mutagenic properties of \textit{O\textsuperscript{6}}-MeG: such mutations are also commonly found in human colorectal cancers. \textit{O\textsuperscript{6}}-Alkyguanine adducts are removed by the DNA repair protein, \textit{O\textsuperscript{6}}-alkylguanine DNA-alkyltransferase (MGMT). MGMT overexpression in transgenic mice reduces the formation of K-ras GC→AT mutations and tumors induced by methylating agents. Interindividual variations in human colon MGMT activity are large and large bowel tumors can occur in regions of low activity. Low MGMT activity in normal mucosa has been associated with the occurrence of K-ras GC→AT mutations, whereas reduced MGMT expression and an increased frequency of K-ras GC→AT mutations in colorectal cancers have been linked to MGMT promoter methylation. MGMT activity is also lower in adenomas than in adjacent normal tissue but only in those adenomas with this specific mutation. These results are entirely consistent with the hypothesis that GC→AT mutations in the K-ras oncogene result from the formation and persistence of \textit{O\textsuperscript{6}}-alkylguanine lesions in colorectal DNA. Human exposure to endogenous or exogenous alkylating agents may thus be an environmental determinant of colorectal cancer risk. J. Nutr. 132: 3518S–3521S, 2002.

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Despite the major advances in identifying colorectal cancer susceptibility genes (1,2), dietary and environmental factors that are major determinants of sporadic cancer risk remain largely unidentified (3). The identification of these etiological factors is important because this may lead to the development of new approaches to identify high-risk populations or to prevent this cancer.

The variety of molecular changes occurring in human colorectal tumors are not consistent with exposure to a single genotoxic agent but suggest that a number of different agents may increase colorectal cancer risk (4,5). Furthermore, as with most human tissues, colorectal DNA contains damage arising from exposure to a number of different genotoxic agents, including those known collectively as alkylating agents (6–11). The aim of this report was to discuss the role of DNA alkylation damage and the repair of such damage in the etiology of human colorectal cancer.

DNA alkylation damage and DNA repair in human colorectal tissue

Human colorectal DNA has long been known to contain the damaged bases \textit{O\textsuperscript{6}}-methylguanine (\textit{O\textsuperscript{6}}-MeG)\textsuperscript{8} and \textit{N\textsuperscript{7}}-
methylguanine, which arise from exposure to methyla-
lizing agents (9–11). The source of this exposure is unknown but can
toentially occur through dietary, lifestyle and occupational
exposures; from endogenous alkylating agents; and via in situ
formation typically mediated by the bacterial or chemical
nitrosation of amines (12,13). Although there are many po-
tential methylating agents (14), DNA alkylation can also arise
through exposure to other alkylating agents [e.g., ethylating or
carboxymethylating agents (15,16)] and potentially to agents
that are as yet not chemically characterized. The relative
importance of such exposures is currently unknown, but avail-
able evidence shows damage to DNA arising from methyla-
lizing agents (9–11). Interindividual variation in O\(^6\)-MeG levels is
large, being at least 100-fold (9,11), and we reported some evidence
suggesting that the highest O\(^6\)-MeG levels occur in
the regions of the large bowel where most tumors occur—that is,
the sigmoid colon and rectum (11).

O\(^6\)-MeG is a known toxic, mutagenic and carcinogenic
DNA base modification; in the absence of DNA repair, O\(^6\)-
MeG has been shown to induce GC→AT transition muta-
tions (17). In DNA repair–deficient cell lines exposed to
methylating agents, it has been calculated that one mutation
results from every 8 O\(^6\)-MeG adducts produced in the coding
region of the hypoxanthine phosphoribosyltransferase gene
(18). Adduct levels observed in human colon cells correspond
to 10s to 100s of adducts per cell (assuming that 1 μg of DNA
contains 3124 pmol of nucleotides) but may, in certain cell
populations, be much higher (19,20). Human DNA adduct
levels may thus be sufficient to cause biological effects, par-
ticularly in cells that are DNA repair deficient.

Removal of O\(^6\)-MeG by the DNA repair protein O\(^6\)-alky-
lguanine DNA alkyltransferase (MGMT) is 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
mamalian protein (18,21). This process inactivates the pro-
tein, which is subsequently degraded through ubiquitination
pathways (22). Continued protection of the DNA thus re-
quires continuous de novo synthesis of active protein; overex-
pression of MGMT in cells and animals protects against tox-
icty, mutagenicity and carcinogenicity induced by alkylating
agents (23). If O\(^6\)-MeG is not repaired before DNA replica-
tion, then O\(^6\)-MeG-thymidine mispairs can be formed. These
mispairs are recognized by the hMSH2-hMSH6 heterodimer of
the mismatch repair system (24). This results in the generation
of an intermediate structure that, on a further round of DNA
replication, results in a DNA double-strand break, triggering
recombination and p53-mediated apoptotic cell death (25).
Mice lacking both MGMT and functional mismatch repair are
thus more prone to develop cancer in response to alkylation
damage than are MGMT-deficient but mismatch repair-pro-
ficient animals (26).

Interindividual variations in functional colon MGMT ac-
tivity can be large. In different studies, variations between 2-
and 18-fold were reported in normal tissue and variations
between 2- and 33-fold were reported in tumor tissue (27).
Although the complete absence of functional MGMT activity
in human colorectal tissue was reported (28,29), this pheno-
type seems relatively rare, being present in <1% of normal
tissue samples and 3% of tumor samples (27).

Intraindividual variation in MGMT activity has been
poorly characterized. We recently studied MGMT activity in
multiple biopsy samples from the large bowel of patients with
colorectal cancer (30). In five patients, intraindividual vari-
ation was 1.1- to 2.5-fold, but in two patients, intraindividual
variation was 12- and >24-fold: in the latter case there was no
detectable activity in two normal mucosal biopsy samples. A
consistent topographical pattern of MGMT activity in normal
mucosa associated with colorectal cancers was found with
tumors occurring in regions of low MGMT activity. There was
a modest but significant fall in MGMT activity, unrelated to
tumor subsite or stage, upstream of left-sided tumors with a
mean gradient of 0.22 fmol/(μg DNA·cm) (95% confidence
interval, 0.03–0.42; P = 0.02). Over a 10-cm length of tissue,
this would correspond to a 10–80% drop in MGMT activity.
The gradient in activity along the colon may reflect a number
of different mechanisms, including longitudinal changes in
gene expression (and hence be a susceptibility factor) or vary-
ing exposure to alkylating agents (and hence be an exposure
marker).

**DNA alkylation damage and repair in dimethylhydrazine-
induced colon cancer**

To further examine the relevance of DNA alkylation dam-
age and repair in the etiology of colorectal cancer, we used
1,2-dimethylhydrazine (DMH), a methylating agent, to induce
colon tumors in animal models. This system is widely used
because of the pronounced organotropism of the
carcinogen (31), a wide variation in tumor response in differ-
ent rodent strains (32–35), the diet-induced modulation in
tumor incidence (36), a distribution of tumors similar to those
within the human colon (37,38) and the similarity between
animal and human pathology (39). Implicit in such work is
the assumption that DNA alkylation may also be important
in human colon cancer formation.

DMH produces predominantly distal colon tumors but at
widely varying rates depending on the strain of the animal
(32–34,40–42). After DMH administration, susceptible
mouse strains can have higher amounts of DNA strand breaks
and DNA adducts in colonic DNA than do nonsusceptible
strains (43,44), but such observations are not consistently
reported (45,46). DNA alkylation is also detected in tissues
other than the colon (e.g., liver, kidney) and the specific
induction of colon tumors has been ascribed to tissue-specific
differences in the persistence of certain DNA adducts (45,47–
49). Other factors, such as whether damaged stem cells at
positions 1 and 2 within the crypt that are selectively removed
by apoptosis or undergo deleterious mutations, may also be
important in DMH tumor induction (50). Intraclonic dif-
fences in baseline proliferative parameters (crypt length, label-
ing index and proliferative zone) have also been reported and
are associated with the tumor formation (51,52).

Treating female SWR mice with DMH (6.8 mg/kg intraper-
itoneal injection) once weekly for up to 20 wk generated the
site-specific induction of colon tumors with 0%, 43% and
87% of animals having proximal, mid and distal colon tumors,
respectively (53). Separate groups of mice were killed up to 1
wk after the final DMH injection, and the large bowel was
removed and divided into thirds (proximal, mid and distal
colon) (54). O\(^6\)-MeG levels in colonic DNA, colonic MGMT
activity and cell proliferative indexes in the colon were found
to vary in a location-, dose- and time-dependent manner.
O\(^6\)-MeG levels were generally lowest in proximal colon DNA
and highest in distal colon DNA and ranged from <0.1 to 16.6
fmol of O\(^6\)-MeG/μg of DNA. Steady state O\(^6\)-MeG levels
were obtained at the highest cumulative dose (DMH at 136
mg/kg) with levels in the mid and distal colon DNA being 5
and 10 times those in proximal colon DNA. The cumulative
sum of persistent O\(^6\)-MeG was associated with tumor inci-
dence in both the distal and mid colon: the distal colon was
more susceptible in that similar levels of persistent O\(^6\)-MeG
adducts in the mid and distal colon were associated with

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higher tumor yield in the distal colon. Basal MGMT activity varied between 0.97 and 1.22 fmol/μg of DNA within the colon but was not associated with adduct levels, tumor induction or differences in tumor yield within the colon.

Association between DNA repair and molecular changes in human colorectal tumors

Colonic tumors induced by DMH treatment frequently but not always contain GC→AT transition mutations in the K-ras oncogene (53,55). This mutation is consistent with the known mutagenic properties of O6-MeG; because overexpression of MGMT in the colon reduces the formation of DMH-induced K-ras mutations (56), this suggests that the formation and persistence of O6-MeG are important etiological determinants of colon tumors in these models. Because GC→AT transition mutations also account for the majority of the K-ras mutations seen within human colorectal cancers (5), it is not unreasonable to suggest that the persistence of O6-MeG in human tissues plays an important role in cancer formation.

Although we found no association between the presence of O6-MeG in human colorectal tissues and the presence of K-ras GC→AT gene mutations (57), we did find that low MGMT activity in normal colon tissue was associated with K-ras GC→AT transition mutations in colorectal tumors (58). Further evidence of the importance of MGMT in influencing K-ras mutational activation has come from promoter methylation studies. Methylation of CpG islands within the promoter region of the MGMT gene has been associated with both reduced MGMT expression (59) and an increased frequency of GC→AT transition mutations in K-ras colorectal cancers (60,61). More recently we reported that adenomas containing a K-ras GC→AT mutation had lower MGMT levels (relative to adjacent normal tissue) than adenomas without this mutation (62). Because MGMT removes O6-alkylguanine lesions from DNA, these observations strongly support the hypothesis that alkylating agents are involved in the etiology of at least a portion of colorectal cancers.

Evidence from animal models and human studies increasingly implicates exposure to alkylating agents as a key event resulting in K-ras mutational activation of at least a subset of colorectal tumors. The role of alkylating agents in colorectal tumorigenesis is potentially larger than this because alkylating agent exposure has been linked to changes in other key targets such as mismatch repair genes (63). Furthermore the recombination effects of O6-MeG may make an important contribution to genetic instability and other steps associated with the multiple events involved in malignant transformation. The precise nature and origin of the alkylating agents are still uncertain; thus it will be difficult to identify not only individual and populations at increased risk (through exposure or susceptibility) but also ways of reducing this risk. Further research is required to better characterize these agents so as to identify exposure sources and appropriate ways to reduce this exposure.

LITERATURE CITED

Distribution of intestine-associated lymphoid tissue, aberrant crypt foci and tumors in the large bowel of 1,2-dimethylhydrazine-treated mice. Cancer Res. 54: 4304–4307.


