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The “mutator hypothesis” asserts that gene alterations that increase the rate of spontaneous mutation (mutators) underlie tumorigenesis (1, 2). However, the lack of a clear initial selection for the mutation of a mutator gene has been a contentious issue in cancer biology (3). Several recent functional studies have demonstrated a direct link between the human MMR genes and signaling to the DNA damage-induced apoptotic response. The results also implied a selective growth advantage associated with mutations in a subset of the MMR genes that lead to tumorigenesis. These observations appear to explain both the allele frequencies of the mutant MMR genes in HNPCC as well as the lack of identifiable tumor mutations in other DNA repair genes thought to control genomic stability.

One of the defining features of any single human tumor is the large number of altered oncogenes, tumor suppressor genes, and modifier genes (4–6). However, the rate of spontaneous mutation in human cells appears to account for only a few of these alterations (1, 2). This dilemma led Loeb (1, 2) to suggest that an elevated spontaneous mutation rate might account for this discrepancy and that genes that control the mutation rate (mutators) might be the ultimate foundation for the tumorigenic process. A genetic basis for an increase in spontaneous mutation rate is grounded in bacterial studies in which mutator (Mut) genes began to be identified in 1954 (7). The Mut gene products have subsequently been found to function in the repair of physical or chemical damage to the DNA and/or its nucleotide precursors as well as misincorporation errors associated with copying the DNA (reviewed in Ref. 8).

Homologues of several of these Mut genes have been identified in eukaryotes, including humans, and have provided support for the notion that altered Mut genes lead to cancer susceptibility (reviewed in Ref. 9). In 1993, hMSH2 was proposed to be associated with the common cancer predisposition syndrome HNPCC (10). This was confirmed by large-scale genetic analysis of HNPCC kindreds (11). Since the identification of hMSH2 and hMLH1 with HNPCC (12), there have been a paucity of DNA genes known to control genomic stability in bacteria or yeast that have been found to be associated with human tumors (13). In contrast, a number of gene alterations have been identified in human tumors that regulate the cell cycle, cell cycle checkpoints, or apoptosis (14). The selective growth advantage (clonal selection; Ref. 15) attained by neoplastic cells containing mutations in these cellular signaling genes is readily apparent. Yet, the selective advantage acquired during the initial establishment of a mutator phenotype (i.e., by mutation of the MMR or similar genes) is not immediately obvious. In theory, mutation of a mutator establishes a condition in which the acquisition of subsequent multiple mutations required for tumorigenesis is enhanced. Such a condition would not a priori provide any immediate selective advantage that distinguishes these mutator cells from their neighbors. Although clonal selection may not be an important issue in the pathogenesis of HNPCC, in which a single-hit would result in a mutator phenotype, there is a substantial fraction of sporadic tumors in which both alleles of a MMR gene have been inactivated. Independent biallelic inactivation appears to underscore some selection for the inactivation of MMR.

The mutant allele frequencies in HNPCC are also confounding. The vast majority of germ-line mutations have been found in hMSH2 and hMLH1, rare but significant numbers of mutations have been found in hMSH6 (with which patients display atypical characteristics, such as late onset; Ref. 16), and few, if any, mutations have been found in the remaining MMR genes (hMSH3, hPMS1, and hPMS2). This allele distribution has been explained by overlapping and redundant MMR activities in the human MSH and MLH heterodimers, in which the common denominators are hMSH2 and hMLH1 (see Fig. 1). For example, the germ-line mutations in hMSH6, as well as the complete lack of germ-line mutations in hMSH3, have been rationalized by the pathway contributing to tumorigenesis (17). Replication errors within mononucleotide repeats (microsatellite instability or MSI) are presumed to generate single-nucleotide IDL mismatches. Thus, one frequently observes alterations in tumor suppressor genes containing mononucleotide repeats, such as TGF-βRII, IGF-II, BAX, Tcf4, and β-catenin (18). Single-nucleotide IDL mismatches are suggested to be a unique substrate of the hMSH2-hMSH6 heterodimer. Unfortunately, this proposition is not consistent with the observation that the hMSH2-hMSH3 heterodimer also recognizes nearly all of the single nucleotide IDL mismatches (19, 20). Such caveats suggest that the mismatch binding distinctions between hMSH3 and hMSH6 in MMR are unlikely to be sufficiently to explain the mutant allele distribution in HNPCC.

The answers to these issues may lie in a different model for MMR that has resulted from accumulating data over the last 4 years (19, 21–25). This new paradigm, the “hydrolysis-independent SC” model, proposes that the MMR proteins are signaling molecules. These MMR proteins function as “direct sensors” that link signaling pathways that may either incite DNA repair or provoke apoptosis.

Signaling MMR

The SC model is similar to G protein molecular switch systems, such as the Ras oncogene, in which the signal is OFF when the G protein is bound by GDP and ON when the G protein is bound by GTP. Binding of GTP results in a conformational transition that encourages the interaction of the G protein with downstream effectors and the transduction of the signal. It is interesting to note that the vast majority of oncogenic mutations in the Ras gene affect its ability to hydrolyze the bound GTP, leaving it effectively in the ON position.
and continuously signaling cellular proliferation. In the G protein system, nucleotide exchange (GDP → GTP) and hydrolysis (GTP → GDP + P_i) control the time-of-occupancy in the GTP signaling form. G protein systems may also control signaling via additional protein factors that provoke GDP → GTP exchange (GEFs) as well as GTP → GDP exchange (GAPs).

ADP → ATP nucleotide exchange (resulting in an ATP-bound MSH) induces a large conformational transition that links the MSH heterodimer (and implicitly the MutS dimer) to DNA as a SC capable of diffusion along the DNA helix (Fig. 1; Refs. 21, 23). The bacterial MutS dimer bound to a mismatch has been crystallized (24, 25). This structure appears to capture the initial mismatch recognition and ADP → ATP exchange intermediate, which can easily be interpreted as undergoing the transition to a SC. Once the first MSH heterodimer has diffused away from the mismatch, a second (and subsequent) MSH heterodimer may become linked to the DNA in a similar fashion producing a locally high concentration of MSH SCs near the mismatch (Fig. 1; Ref. 23). As in G protein signaling, a “threshold” number of MSH heterodimers in the ATP-bound signaling form is maintained regionally until the mismatch is repaired and the MSH clamps disperse (Fig. 2). The concept of multiple signaling MSH clamps suggested by the SC model also provides a functional redundancy to the repair process that is absent in two other proposed models for MMR: the “hydrolysis-dependent translocation” (HDT) model.
which suggests the formation of a singular motor protein complex at
the mismatch (28, 29), and the “induced-fit” (IF) model, which
proposes the assembly of a protein complex of MutS bound to a
mismatch, MutH bound to hemimethylated GATC site, and MutL
stabilizing the ternary complex (Ref. 27; reviewed in Ref. 30).

The SC model using an A protein molecular switch, similar to the
well-known G protein molecular switches, suggests that there might
be an adenosine nucleotide exchange factor (AEF) as well as an AAP
that may regulate the signal. Mismatched nucleotides appear func-
tionally equivalent to adenosine nucleotide exchange factors because
they control the ADP\textsuperscript{3}ATP exchange process(es) by MSH het-
erodimers. In addition, mismatched nucleotides appear to stabilize the
nucleotide-free state of MSH heterodimers that would be identical to
the role of GEFs with G proteins. However, based on the crystal
structures of bacterial MutS (26, 27), the mechanism of mismatch-
provoked ADP\textsuperscript{3}ATP exchange is likely to be more complicated than
the simple direct interaction mechanism exhibited by a GEF and a G
protein (31). Perhaps the binding of the MSH to a mismatch induces
a modest conformational transition that stimulates an internal peptide
sequence to provoke either cis- or trans-ADP release within/between
protomers. Such a mechanism could be similar to the interactions of
G proteins and GEFs (31).

An AAP activity for MSH proteins has not been identified. The most likely candidates for AAP activity are the MLH proteins that
contain an ATP binding domain and an exceedingly inefficient ATP
hydrolysis (ATP\textsuperscript{ase}) activity (31). Recent results suggest that the
MLH proteins will form a stable association with the MSH proteins
only in the presence of the poorly hydrolyzable ATP analogue,
ATP\textsuperscript{yS}. This observation appears similar to the transient association
of G proteins and GAPs, which can be stabilized with the poorly
hydrolyzable analogue of GTP, GTP\textsuperscript{yS} (see Ref. 33). Furthermore,
reactions containing both the MLH and the MSH proteins display
ATP\textsuperscript{ase} activity above what would be additive. In humans, MLH
heterodimers appear to interact uniquely with MSH heterodimers (Fig.
1), similar to the unique interactions between specific G proteins and
their GAPs (33). Although the role of ATP binding and hydrolysis by
MLH proteins remains enigmatic, it is clear that these proteins per-
form essential function(s) in the transduction of the MSH mismatch
recognition signal.

The Role of MMR in DNA Damage-induced Apoptosis

Marinus and colleagues (34, 35) were the first to recognize a role
for the MMR system in processing DNA damage in bacteria. Mutation
of the bacterial MMR genes resulted in resistance to several DNA
damaging agents including MNNG, cisplatin, and UV lesions. The
bacterial, yeast, and human MSHs have also been shown to bind to
these DNA lesions (36–38). Furthermore, O\textsuperscript{6}-methylguanine has been
shown to provoke ADP\textsuperscript{3}ATP exchange by the hMSH2-hMSH6
heterodimers. In the event of overwhelming lesion-specific genome-wide damage an apoptotic response may be induced that depends uniquely on hMSH2-hMSH6 and hMLH1 (presumably paired with hPMS2), along with an apoptotic pathway that includes cAbl and p73 (46).

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\textsuperscript{4} T. Wilson, M. Beradini, C. Heinen, and R. Fishel. Adenosine nucleotide processing
by hMSH2-hMSH3 and hMSH2-hMSH6, submitted for publication.

\textsuperscript{5} S. Acharya and R. Fishel, unpublished observations.
heterodimer as well as stimulate its intrinsic mismatch/lesion-dependent ATPase (20). These studies suggest that the MMR machinery is activated by more than just mismatched nucleotides.

The resistance of MMR-deficient bacteria to alkylating agents led to the idea that the wild-type MMR machinery may initiate “futile cycles” of repair after recognition of a template strand containing DNA damage (33, 34). In this model, multiple rounds of MMR-directed repair resynthesize the unmodified (lesion-free) nascent strand while the damaged nucleotide persists. Such a cyclic process is proposed to malfunction with some frequency, leading to a lethal DSB. In the absence of MMR-directed initiation, the lesions are “tolerated” allowing cellular survival.

A similar tolerance to DNA damaging agents has been observed in MMR-defective human cells (39). Unlike bacteria, human cells appear to manifest damage tolerance and attendant growth changes by altering the apoptotic response (40–42). For example, Zhang et al. (41) clearly demonstrate that there is an increasing population of apoptotic cells when early-passage wild-type senescence MEFs are subjected to increasing concentrations of the alkylating agent MNNG. In contrast, Msh2−/− MEFs display no increase in apoptotic cells at identical concentrations of MNNG. Several groups have reported that tolerance is the result of a defect in the G2-M damage response checkpoint (43, 44, 48).

Both the hydrolysis-dependent translocation and the induced-fit models propose mismatch/lesion recognition and repair as the singular function of the MMR machinery, inexorably linking them to the futile cycle of damage processing. A futile-cycle mechanism for the alkylation tolerance invokes the formation of DSBs that then provide the ultimate signal for apoptosis. On the basis of a number of studies, one would expect the DSBs to signal apoptosis via activation of a well-defined p53 pathway (45). However, MNNG-induced apoptosis has been shown to be independent of p53 (42, 44). It is certainly possible that the DSBs associated with a futile cycle could signal apoptosis independently of p53. However, the SC model provides an alternative mechanism in which the MMR proteins act as direct sensors capable of signaling to downstream effectors such as the apoptotic machinery. A direct-sensor signaling mechanism would a priori be independent of DSBs, eliminating the need for a p53 response, and would be consistent with the observations of Zhang et al. (41). A direct-sensor model embraces both MMR and apoptotic pathways.

Tracing the pathway of the MMR-dependent DNA damage-induced apoptotic response has been led primarily by Gong et al. (46), who have shown that the c-Abl tyrosine kinase is likely to play a central role via activation of p73, a homologue of p53. Evidence that the MMR machinery may signal directly to the apoptotic process has been underlined by microinjection studies (41). Injection/overexpression of hMSH2 and hMLH1 induced apoptosis within 24 h, whereas injection/overexpression of hMSH3, hMSH6, and hPMS2 did not (41). In addition, stable transfectants expressing hMSH3, hMSH6, and hPMS2 could be isolated, whereas no stable transfectants expressing hMSH2 or hMLH1 have been identified despite numerous attempts after the identification of these genes in 1993 and 1994. These observations may be tempered by the apparent isolation of cells that appear to express “low levels” of hMLH1, although the isolation of cells expressing even low levels of hMSH2 have been refractory.

Studies of cell lines derived from knockout mice have demonstrated that only Msh2−/− and Msh6−/− MEFs display resistance to MNNG, whereas Msh3−/− MEFs remain sensitive and identical to wild-type MEFs (47). Again, the resistance/tolerance in the Msh2−/− MEFs can be traced to a lack of MNNG-induced apoptosis (41). These cellular results are consistent with biophysical studies that suggest the hMSH2-hMSH6 heterodimer is significantly activated to form a signaling SC by methylation DNA damage, whereas the hMSH2-hMSH3 heterodimer is not (20). Similar resistance/tolerance studies with the MLH1 knockout MEFs have not been reported, although hMLH1-deficient human tumor cell lines are indeed resistant to MNNG-induced apoptosis (41, 48). Taken together, these observations support the notion that hMSH2 and hMLH1 (and to a lesser extent, hMSH6) via its functional association in the recognition of alkylation damage as a heterodimer with hMSH2) are unique in signaling an apoptotic response after certain types of DNA damage (Fig. 2). It is interesting to note that a major issue confronting the clinical management of tumors with MMR defects is that they are resistant to several of the common treatment regimes (cisplatin and/or other alkylating agents; Refs. 49, 50).

Selection for MMR Defects and the Recompense Acceleration of Carcinogenesis

The connection of the same subset of MMR genes both to allele frequencies in HNPCC and to the damage-induced apoptotic response provides a rationalization for the selection of MMR gene mutations in human tumors. When presented with DNA damage that is recognized by the MMR machinery, a selective advantage is gained by mutation to apoptotic resistance. Moreover, the unique tissue selectivity of HNPCC-related tumors (colorectal, endometrial, ovarian, and so forth; Ref. 51) might be traced to exposure to certain types of DNA damaging processes (alkylation or oxidative; see Ref. 29) that are specifically recognized by the MMR machinery.

So where does the mutator hypothesis fit? Part of the answer lies in the recorded history of HNPCC that dates to 1913 (52) and was revisited by Lynch et al. in 1966 (53). Importantly, one of the defining observations was made in the 1970s when it was suggested that polyps in HNPCC patients arise at the same frequency as in the normal population; however, these polyps appear to develop into tumors more rapidly (54). This accelerated adenoma-to-carcinoma transition is now generally regarded as a hallmark of HNPCC (50). Such an accelerated tumorigenesis is entirely consistent with the increase in the spontaneous mutation rates observed in MMR-defective human tumor cells (55) and in the decreased latency in mouse xenograft tumor models (56). Once an MMR-defective cell arises, then the condition to acquire multiple mutations has been established as a consequence rather than a cause. Clearly, the missing link in the HNPCC tumorigenic process was the initial selection for MMR mutations, which we now believe to be resistance to DNA damage-induced apoptosis.

Are There Other DNA Repair Proteins Involved in Both Damage Signaling and Control of Mutation Rates?

On the basis of the hypothesis that mutations must provide a selective advantage in the process of tumorigenesis (3, 15), it is possible to understand the lack of mutations in human genes that have known or suspected roles in maintaining genomic stability. The overriding question is: are there other DNA replication, recombination, repair, or chromosomal segregation genes that are similar to the MMR genes and that play direct roles in both genome maintenance and cellular signaling? Some of the obvious candidates involved in DNA damage processing are likely to be downstream of the primary damage recognition and processing activities and include ATM/ATR, cAbl, p53, p21 (waf/cip), p73, and Bub1 (57). Although the functions of BRCA1 and BRCA2 are unknown, their interaction with consensus

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*J. Hinz, G. Phear, R. Fishel, and M. Meuth. Low levels of hMLH1 are sufficient to suppress the mutator phenotype of an hMLH1-deficient colon cancer cell line, submitted for publication.
DNA repair proteins may place them in a category similar to hMLH2 and hMLH1 (58).

One candidate that may play a direct role in damage recognition and cellular signaling is the human RAD50/MRE11/XRS2(NBS1) complex (59). This complex is involved in the recognition, processing and repair of DSBs resulting from physical damage, such as γ-irradiation, or hematopoietic development. Mutations in both XRS2(NBS1) and MRE11 have been found to be associated with increased cancer susceptibility and ataxia telangiectasia-like disorders (60, 61). Whereas the functional role of XRS2(NBS1) is unknown, XRS2(NBS1) and hMRE11 have been found to be associated with repair of DSBs resulting from physical damage, such as (59). This complex is involved in the recognition, processing and repair of DSBs.

This modest revision to the mutator hypothesis appears to explain: (a) the MMR allele frequencies in HPNCC; (b) the lack of mutations in DNA metabolic genes with known or suspected roles in maintaining genomic stability; and (c) perhaps the tissue distribution of HPNCC tumors. One wonders whether most, if not all, “early” mutations found in human tumors (e.g., APC, p53, Rb, and so forth) might both provide a selective advantage and act as mutators.

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