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Mismatch repair defects in human carcinogenesis

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Mismatch repair defects are carcinogenic. This conclusion comes some 80 years after the original description of a type of familial colorectal cancer in which mismatch repair defects are involved, and from decades of dedicated basic science research into fundamental mechanisms cells use to repair their DNA. Mismatch repair (MMR) was described first in bacteria, later in yeast and finally in higher eukaryotes. In bacteria, one of its roles is the rapid repair of replicative errors thereby providing the genome with a 100–1000-fold level of protection against mutation. It also guards the genome by preventing recombination between non-homologous regions of DNA. The information gained from bacteria suddenly became relevant to human neoplasia in 1993 when the RER phenotype of microsatellite instability was discovered in human cancers and was rapidly shown to be due to defects in mismatch repair. Evidence supporting the role of MMR defects in carcinogenesis comes from a variety of independent sources including: (i) theoretical considerations of the requirement for a mutator phenotype as a step in multistage carcinogenesis; (ii) discovering that MMR defects cause a 'mutator phenotype' destabilizing endogenous expressed genes including those integral to carcinogenesis; (iii) finding MMR defects in the germline of HNPCC kindred members; (iv) finding that such defects behave as classic tumor suppressor genes in both familial and sporadic colorectal cancers; (v) discovering that MMR 'knockout' mice have an increased incidence of tumors; and (vi) discovering that genetic complementation of MMR defective cells stabilizes the MMR deficiency-associated microsatellite instability. Models of carcinogenesis now must integrate the concepts of a MMR defect induced mutator phenotype (Loeb) with the concepts of multistep colon carcinogenesis (Fearon and Vogelstein) and clonal heterogeneity/selection (Nowell).

INTRODUCTION

One of the most exciting recent advances in the field of cancer is the discovery that mismatch repair (MMR) defects are carcinogenic (1–8). As DNA repair systems are designed to maintain the integrity of the genome and a fundamental feature of cancer is genomic instability, it is perhaps not so surprising that defects in DNA repair should be carcinogenic. In fact precedence is provided by the well described excision repair defects in xeroderma pigmentosum (9–12). What is new is the discovery of how significantly involved the MMR system is in human carcinogenesis.

CHARACTERIZATION OF MISMATCH REPAIR OCCURRED FIRST IN BACTERIA

Long before the discovery of its involvement in human cancer, MMR was extensively studied in bacteria and yeast. Mismatch repair was originally described in bacteria which are able to repair mispair containing bacteriophages (13,14). One role of MMR is to recognize and repair mistakes made by the DNA polymerases during replication (15,16). The bacterial system determines which base(s) is incorrect by cueing on the fact that the error-containing nascent DNA strand is transiently unmethylated. While the system requires 10 independent components, three critical ones are MutS, MutL and MutH, named after their corresponding bacterial mutator strains. It is the role of MutS to recognize and bind to the mispair or loop. It then recruits MutL and MutH to form a complex which scans the duplex for the nearest hemimethylated site which MutH then nicks on the unmethylated strand. An exonuclease then excises the nascent strand from the nick back past the mismatch. This patch is then resynthesized and ligated. Much of what is known about the bacterial and more recently the human system is derived from the behavior of cell extracts when challenged with mismatched DNA templates containing base–base mispairs and small loops (17–19).

Comparison of the human MMR system to the bacterial system reveals extensive similarities. Most importantly, both provide the genome with a 100–1000-fold level of protection against mutations arising during DNA replication (20,21). Both systems direct repair to the newly replicated DNA strand, require multiple components and can excise the nascent strand in either direction to the mismatch. A major difference between the two systems is that the human system has multiple homologues for each bacterial component (Table 1). Homologues of *MutS* include *hMSH2* (human *mutS* homologue 2) (1,2,22), *GTBP*(G·T mismatch binding protein) (6,7), and *hMSH3* (23). The gene products of *hMSH2* and *GTBP* bind the mismatch as a heteroduplex (6). It is

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not yet fully clear how the new *MutS* homologue, *hMSH3*, participates in the reaction. Multiple homologues of *MutL* also exist including *hMLH1* (human MutL homologue 1) (3,4), *PMS1* (post-meiotic segregation 1) (5), and *PMS2* (5). The *MutL* homologues *hMLH1* and *hPMS2* also bind as a heteroduplex (24) after the initial binding to the mismatch by the *MutS* heterodimer.

Table 1. Human MMR genes

Bacterial MMR homologue	Human gene	Chromosomal localization[a]	Germline mutations[b]	Reference
MutS	*hMSH2*	2p16	Yes	1,2
	GTBP	2p16	–	7
	hMSH3	–	–	23
MutL	*hMLH1*	3p21	Yes	3,4
	hPMS1	2q31–33	Yes	5
	hPMS2	7p22	Yes	5

[a]Chromosomal localization in humans.
[b]Report of germline mutations in humans. Dash signifies not yet reported.

DISCOVERY OF MICROSATELLITE INSTABILITY CHARACTERISTIC OF THE RER PHENOTYPE

The first clue that defective MMR is important in carcinogenesis was the discovery of the RER (Replication Errors) phenotype of microsatellite instability in sporadic and inherited colon cancer (25–27). Microsatellites are normally stable repetitive genetic elements where the repeating unit is only one to six bases (28,29). Because of their repeating nature they are particularly prone to slippage (30–32) during replication which results in a small loop in either the template or nascent DNA strand. Despite these replicative mistakes which occur in all cells, microsatellites are normally stable in length because of the efficiency of the MMR system. However, RER cancers have lost the strict maintenance of microsatellite length, and this phenotype appears to be quite common (33). Very fundamental unresolved issues remain in the diagnosis of a tumor as RER. These include the degree to which the microsatellite has shifted relative to the normal allele (26), whether just one or more than one locus needs to be shifted to diagnose the tumor as RER, what the optimal set of most informative loci are (34), and whether that set is only optimally sensitive for diagnosing RER in specific tumor types. The pathologic characteristics of RER colorectal cancer (CRC) relative to non-RER CRC has been well described (35).

Having recognized that instability exists in microsatellites, one unresolved problem was why instability of microsatellites which are almost always non-coding should contribute to carcinogenesis. This was explained through data generated by us and others which show that RER colorectal cancer cells also exhibit an increased mutation rate in endogenous expressed genes, a so-called 'mutator phenotype' (21,36). Sequence analysis of the mutations in a selectable reporter gene (37,38) shows that the mutator phenotype is not just the predictable frameshift mutations consistent with known microsatellite instability, but rather includes substantial numbers of base substitutions equal in number to the frameshifts. Interestingly, a novel hotspot was discovered which may reflect a spontaneous 'signature' sequence of the underlying defect (37). Also, among the frameshift mutations which did occur, they occurred three orders of magnitude more frequently at homopolymeric runs of 6 bases than at runs of 2 bases (37), a finding previously noted by Kunkel (31). In addition to an increased mutation rate at the selectable *hprt* reporter gene, the mutator phenotype also results in increased mutations in a receptor for TGF-β (RII), which functions as a tumor suppressor gene in RER colon cancers (39,40). TGF-β RII is a gene at risk in this genetic background as its coding region contains a 10 base polyadenine repeat, which is the site of frameshift mutations in over 90% of RER colon cancer (39,40).

DEFECTS IN MMR UNDERLIE THE MUTATOR PHENOTYPE AND MICROSATELLITE INSTABILITY

It is now known that defective MMR is the underlying defect responsible for both the microsatellite instability and the increased mutation rate in at least some RER cancers. As discussed, one role of MMR is immediately to recognize and repair residual loops (and base–base mispairs) following replication so that microsatellites are maintained in essentially all cells at the germline length. Clearly, the microsatellite instability in some RER cancers can be explained by the presence of underlying MMR defects (41,42) because the microsatellites stabilize in length when genetically complemented with a chromosome bearing a wild-type copy of the defective MMR gene.

The critical unanswered question is whether microsatellite instability is an absolute surrogate marker for defective MMR such that all RER cancers will ultimately prove to be mutant in some component of MMR. This remains a possibility because while many RER cancers are known not to carry a defect in one of the four originally identified MMR genes, only a subset of genes involved in human MMR has been examined or is even known (43–45). Alternatively, it is conceivable that these cancers may ultimately be found to harbor underlying defects in other DNA repair systems.

INHERITANCE OF MISMATCH REPAIR DEFICIENCY AND THE PROPENSITY TO CANCER DEVELOPMENT

Hereditary non-polyposis colorectal cancer (HNPCC or Lynch syndrome) (46), is a familial colon cancer syndrome which is now being ascribed to defects in MMR (25) some 80 years after the original description of the first affected family (47). HNPCC is inherited as an autosomal dominant trait, and at the cell level the associated MMR deficiency is generally that of a classic tumor suppressor gene (48). Individuals in MMR-defect-associated HNPCC kindreds inherit one mutant MMR allele and tumors arising in such individuals generally have acquired a 'second hit' in that gene (2,3,49). HNPCC family members' adenomas generally (57%) show microsatellite instability consistent with an MMR deficient state (50,51) suggesting that MMR deficiency is indeed an early event in colon carcinogenesis in these kindreds. In addition to their involvement in HNPCC, MMR defects have also been demonstrated in a substantial portion of sporadic CRC and adenomas as well (2,7,52,53). In fact, microsatellite instability may even be present in aberrant crypts (54), the earliest putative adenoma precursor lesion. The majority of HNPCC cases are due to underlying defects in either *MSH2* or *MLH1*. However, many cases have not been associated with defects in any of the four implicated genes. The relative contributions of the human MMR genes to HNPCC and sporadic CRC is shown in Figure 1.

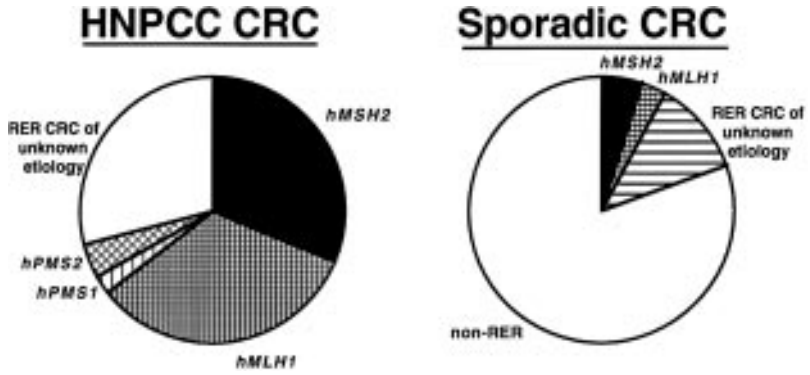

Figure 1. MMR defects in HNPCC (56) and Sporadic CRC (34). The contribution of the MMR genes to sporadic CRC is derived from their incidence in individuals who are less than 35 years of age and hence may be an overestimate of their overall contribution to sporadic CRC.

Table 2. Cancers with documented mismatch repair defects

Cancer site	Genes involved	Reference
Colon, HNPCC	*MSH2*	1,2,55,56
	MLH1	3,4,49,56,57
	PMS1, PMS2	5,56
Colon, sporadic	*MSH2*	2,34,52,55
	MLH1	34,52
	GTBP	6,7
Endometrial, sporadic	*MSH2*	58
	MSH3	23
Turcot's syndrome[a]	*MLH1, PMS2*	59
Muir-Torre	*MSH2*	60
Uterine sarcoma[b]	*MSH2*	61
Ovarian serous cystadenocarcinoma[b]	*MSH2*	62

[a]The majority of underlying mutations identified were in the adenomatous polyposis coli (APC) gene.
[b]Only one case described.

In addition to the involvement of MMR deficiency in colorectal carcinoma, mutations in colorectal and other tumors are summarized in Table 2.

One major impact of the discovery of MMR defects in HNPCC kindreds is the ability to perform molecular diagnosis of family members. This is of tremendous value because clinical screening efforts can be focused on those individuals who carry the disease propensity mutant MMR allele rather than on the whole family. Those without the defect should be at population risk and can be spared screening efforts and concerns. However, technical and ethical concerns remain. Technically, there is some difficulty in identifying those at risk because the entire set of genes causing the disease is not yet known, and while there is some clustering of mutations, they are not focused on one hotspot thereby complicating the molecular identification of mutant alleles (63). In addition to these technical concerns, some patients are reluctant to have a potential genetic deficiency become a permanent part of their medical record.

THERAPEUTIC IMPLICATIONS OF MMR DEFICIENCY

Perhaps the most important implication of the involvement of MMR deficiency in cancer is for therapy. Early reports commented on the slightly better prognosis of RER cancers treated conventionally (26,64). Specifically, those with *MLH1* defective cancers show a modest 20% improved survival rate relative to sporadic CRC (65). As the heterozygous state is functionally MMR competent and the tumor is MMR incompetent, in theory an ideal selective clinical agent would be one to which MMR deficient cells are uniquely sensitive. Some of the earliest discovered MMR deficient cell lines were isolated by their significant resistance to the alkylating agent, MNNG (66–68). The resistance to MNNG appears to be a common feature of MMR deficiency (41). In addition to their resistance to MNNG, they appear to be slightly more resistant to cisplatin (69). While highly preliminary, one agent to which the MMR deficient cells may be more sensitive is camptothecin, a topoisomerase I inhibitor (70). It was found that an *hMLH1*-deficient line was three- to fourfold more sensitive to this agent than its sister cell line, which was complemented with chromosome 3 bearing a wild-type version of the *hMLH1* gene. It will of course be imperative to show that such results can be extended to additional cell lines and that the *in vitro* findings will translate into tumor sensitivity. Moreover, it is unfortunately possible that a given chemotherapy-based approach will be specific to only one type of MMR gene defect. In this vein, analysis of recently constructed knockout mice for both *MSH2* (71) and *PMS2* (72) will be most useful. In addition to the potential for a therapeutic window between MMR-deficient tumor cells and MMR competent normal cells for conventional chemotherapy, gene therapy could theoretically be applied in this setting.

INTERSECTION OF MMR AND OTHER CELL SYSTEMS

Stability of the genome is an essential aspect of survival of the individual; however, this is balanced against mutations in the genome which are required for evolutionary survival of the species [for a discussion, see Radman *et al.* (73)]. It is becoming clear that the MMR system performs much more than the simple function of the repair of errors made during replication. In bacteria, MMR prevents recombination between non-homologous regions of DNA (74), probably by inhibiting the branch migration step (75). Additional evidence for the involvement of MMR in

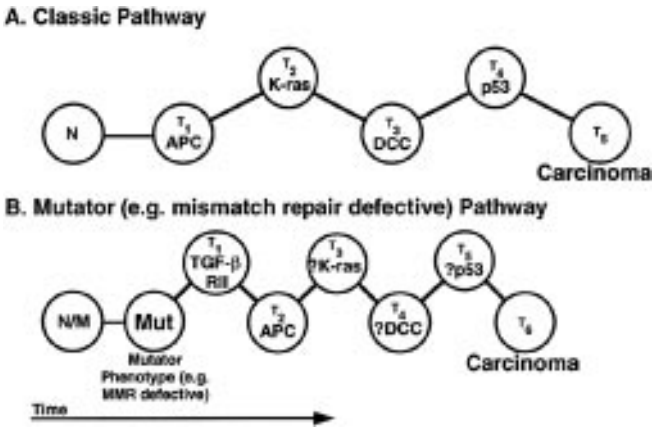

Figure 2. Classic versus mutator cancer pathways. Schematic incorporating the concepts of Nowell and Fearon and Vogelstein to generate the classic pathway to colon carcinogenesis (**A**) where each circle (T_1...) represents a subclone during tumorigenesis. In (**B**), the addition of a mutator phenotype as described by Loeb (designated MUT) is now integrally involved in the pathway and arises from an HNPCC heterozygous MMR mutant background (N/M, normal/mutant). Note that the mutator pathway occurs more quickly because of the contribution of the mutator phenotype. In the mutator pathway, the precise timing of TGF-β-RII and the involvement of other colon cancer genes are areas of current investigation (designated by '?').

recombination is that in *MSH2*-deficient cells, which were used to build the *MSH2* defective transgenic mice, inappropriate recombination is dramatically increased between non-homologous vectors (71). In addition to these effects, the MMR system is also involved in mediating the G_2 checkpoint (76). Moreover, recent evidence suggests that defects in MMR result in defects in transcription-coupled excision repair (77).

SUMMARY

MMR defects are integrally involved in carcinogenesis. Determining the exact contribution of MMR will depend first on the complete identification of all of the human homologues involved in the process. Whether MMR defects will underlie all RER tumors or whether defects in other DNA repair systems will explain them remains to be seen. The discovery of yet another DNA repair system's involvement in carcinogenesis provides credence to the Loeb hypothesis (78) which postulates the requirement of a mutator phenotype so that malignant cells can acquire the requisite number of changes described during multistep carcinogenesis (79). Incorporating the concept of a mutator phenotype (80) as an early event in the Fearon/Vogelstein multistep colon carcinogenesis (79), and integrating these concepts into the Nowell clonal heterogeneity/selection model (81), results in the proposed pathway shown in Figure 2.

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