Making Sense of Missense in Lynch Syndrome:
The Clinical Perspective

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Abstract

The DNA mismatch repair (MMR) system provides critical genetic housekeeping, and its failure is associated with tumorigenesis. Through distinct domains on the DNA MMR proteins, the system recognizes and repairs errors occurring during DNA synthesis, but signals apoptosis when the DNA damage cannot be repaired. Certain missense mutations in the MMR genes can selectively alter just one of these functions. This affects the clinical features of tumors associated with defective DNA MMR activity. New work reported by Xie et al. in this issue of the journal (beginning on page 1409) adds to the understanding of DNA MMR. Cancer Prev Res; 3(11); 1371–4. ©2010 AACR.

Introduction

It would be ideal if we knew so much about each patient’s tumor that we could precisely individualize the approach to the care of that patient. Unfortunately, there seems to be an enormous amount of diversity among tumors, and it is not unreasonable to feel a bit overwhelmed by this challenge. Nonetheless, it is possible to understand the details of some tumors, making them candidates for a personalized therapeutic approach. We may have to take these advances one by one until we gain a fuller understanding of the majority of tumors. Fortunately, our understanding of the clinical, molecular, and pathologic diversity in Lynch syndrome has emerged with great speed during the past two decades. However, more progress is needed (1–7).

The discovery of cancer-causing germline mutations has proved to be highly advantageous in determining patients’ lifetime risk status (1). For example, Watson et al. (8) have shown that mutation testing increases the accuracy of cancer risk assessments in relatives, thereby producing a large decrease in the number of persons who need to be worried about whether they are a carrier. This study involved cohort members from 75 hereditary breast-ovarian cancer syndrome families and 47 Lynch syndrome families that comprised 10,910 cohort members, of whom 1,408 underwent testing for a mutation and then found out their results. Findings disclosed a change in carrier risk status “in 2906 subjects…. The most common type of carrier risk change, from at risk to noncarrier status, accounted for 77% of the risk changes; 12% were a change to known carrier status from a lower risk. Sixty percent of persons with a carrier risk status change were not themselves tested; their risk status changed because of a relative’s test result.” Therefore, carrier risk status changes from risk uncertainty to certainty, namely to carrier or noncarrier status, accounted for 89% of all risk changes resulting from testing. Clearly, these results affect decision making by patients and their physicians regarding surveillance and management.

DNA Mismatch Repair in Colorectal Cancer

The discovery of the role of defective DNA mismatch repair (MMR) in the genesis of about 15% of colorectal cancers (CRC) represented the first major step in the personalization of the care of this disease. Defective MMR activity results in microsatellite instability (MSI) in the DNA of the neoplastic cells, and tumors with MSI are biologically different from the rest. These tumors are mostly nonanaploid, tend to occur in the proximal colon, have a different natural history and response to adjuvant chemotherapy (9), and have a unique mutational signature in the tumor DNA (10). This subset of tumors includes nearly all Lynch syndrome tumors (~3% of all CRCs) and a group of tumors in which the Mut L homologue (MLH1) gene has been silenced by promoter methylation (~12% of all CRCs). It is now possible to test all CRCs for MSI, which would permit screening simultaneously for Lynch syndrome while providing prognostic and predictive information to the patient (11). However, we are just beginning to peel the onion of the personalization of care for these patients, and as we do, the story becomes more complex and fascinating.

We define Lynch syndrome as the genetic disease caused by a germline mutation in a DNA MMR gene (12). This definition implies that the tumors will have MSI, which is the basis of the Bethesda Recommendations on how
to screen for this disease (13, 14). However, it has been appreciated that not all Lynch syndrome tumors, and not all DNA MMR-defective tumors, have MSI (at least as we currently measure and define it), which challenges the conceptual basis of this disease (15). About 5% of DNA MMR–defective tumors show either low-level MSI (MSI-L) or no MSI, called microsatellite stable (MSS). How does this happen?

Some MMR-defective CRCs seem to be MSS or MSI-L because of the markers used to measure MSI. For example, nearly all of the CRCs associated with Lynch syndrome MSH2 (Mut S homologue 2) type and MLH1 type, and all CRCs with acquired methylation of MLH1, have high-level MSI (MSI-H). However, CRCs from patients with Lynch syndrome MSH6 type often have a MSI-L or MSS phenotype (16). This happens because the absence of MSH6 activity is partially compensated by the presence of the MSH3 protein, which can correct some of the mutations that would be used in the identification of MSI (17). This problem can be mitigated by selecting the appropriate microsatellite markers (18). In this issue of the journal, however, Xie et al. describe a MLH1 mutation that is associated with an MSS tumor phenotype (19). This finding is exceptional because it is a deviation from the dogma that mutations in MMR proteins abolish MMR activity and lead to MSI cancers.

It is important to appreciate that there are important differences between the different forms of Lynch syndrome. For example, Lynch syndrome MLH1 type seems to be associated with a deficit of extracolonic cancers (such as endometrial cancers) and an excess of CRCs, when compared with Lynch syndrome MSH2 type, which is prominently associated with extracolonic cancers. On the other hand, Lynch syndrome MSH6 type is associated with later-onset CRCs and a greater number of endometrial carcinomas. Lynch syndrome PMS2 type seems to have a later age of onset of CRC; however, we do not know enough about these families to provide a complete description of how they differ from the phenotypes mentioned above. Certain alterations in the EPCAM gene add a whole new dimension to Lynch syndrome because they can inactivate the MSH2 gene (20); families with deletions in the 3′ terminus of EPCAM seem to have a strong proclivity toward site-specific familial CRC (unpublished data of HTL).

Xie et al. describe a specific MLH1 mutation (L607H) that disrupts the interaction of MLH1 protein with the BRCA1-associated COOH-terminal helicase (BACH1), also called Fanconi anemia complementation group J (FANCJ) protein, thus predisposing cells to CRC (19). A crucial next step will be the investigation of the effect of this MLH1 mutation on the development of cancer, both clinically and mechanistically, as seen in Lynch syndrome, or Lynch-syndrome-like, families.

**Dual Functions for the DNA MMR System and Their Selective Loss**

The MSH proteins MSH2+MSH6 and MSH2+MSH3 recognize mismatch lesions in newly synthesized DNA during the S phase, and the MLH proteins MLH1+PMS2 function as molecular matchmakers to recruit DNA excision proteins to the site of the mismatch or DNA adduct (10). In the presence of certain types of DNA damage that cannot be repaired, the MMR system signals cell cycle arrest and apoptosis because cell death is a preferred option to the replication of mismatched DNA (which would create a permanent mutation). Therefore, the dual functions of the DNA MMR system are to fix the damage when it can, or signal apoptosis when it cannot. Interestingly, in mice, the absence of Msh2 leads to a complete loss of DNA MMR activity, whereas the missense mutation Msh2L607H abrogates the DNA-repair function but not the cell cycle–checkpoint function (21). Many germline mutations in DNA MMR genes are either nonsense mutations (i.e., they create a stop codon) or are complete deletions. These mutations are unambiguous and easy to interpret. However, missense mutations (i.e., DNA sequence variations that change the coding sequence from one amino acid to another) do not always have predictable consequences and create a challenge in their interpretation, as is the case with the missense mutation of MLH1 discussed here.

Xie et al. highlight an interesting aspect of DNA MMR (19). Normal cells experience toxicity and cell death in response to the genotoxin methylnitrosourea (MNU), a response that is mediated by DNA MMr (22). MNU is an alkylating agent, and the ensuing DNA damage can be corrected either by MMR or by the enzyme methylguanine methyltransferase (MGMT). Mitomycin C (MMC), on the other hand, is a DNA cross-linking agent. Correction of the cross-linking lesion requires an interaction between the MMR protein MLH1 and FANCJ. Cells without this helicase activity, or with mutated FANCJ that cannot bind MLH1, respond to MMC by entering a prolonged cell cycle arrest. Responses to either MNU or MMC require the presence of the DNA MMR system; however, the events downstream of DNA MMR signaling are quite different, involving cell cycle arrest in one case but not in the other.

Continuing and extending previous research (23), Xie et al. now report that the MLH1 missense mutation L607H ablates the protein-interaction site that mediates binding of MLH1 to FANCJ without disrupting DNA MMR repair activity. Cells that express only this mutant form of MLH1 are still sensitive to MMC (i.e., they cannot pass through the cell cycle and suffer growth arrest), but they become resistant to MNU (i.e., they continue through the cell cycle) and can conceivably repair the alkylation of DNA as long as MGMT is active. Moreover, these researchers speculate that the failure of FANCJ to bind to MLH1L607H prolongs the period required to move through the cell cycle, providing more time for MGMT to remove the adducts produced by MNU, as illustrated in their Fig. 5 (1).

Studying the functionality of a missense mutant like MLH1L607H beyond simple in vitro MMR assays is important because, as Xie et al. show, its effect may stem not from impairment of MMR but from loss of binding to another protein. This issue also raises questions of how many additional protein interactions with MLH1 are lost...
because of this mutation and of how critical the role of FANCJ really is. Cantor’s laboratory offers strong evidence that FANCJ is the primary culprit; they characterized a FANCJ mutation (K141I/K42A) that is defective in MLH1 binding, and this FANCJ mutant had a phenotype comparable with MLH1L607H, which lacks FANCJ binding. It seems that the critical factor is not just the presence of either the MLH1 or FANCJ protein, but the ability of the two proteins to bind to each other and form a functional complex.

Further research will be required to clarify the role of FANCJ in MLH1-mediated DNA-damage signaling and to ascertain the causality of MLH1L607H in CRC formation. Because this mutation is MMR competent and the associated tumors test as MSS, it might be uniquely suited for mouse models. Thus far, mouse models of Lynch syndrome have had limited success because the disease is based on MMR deficiency and the subsequent accumulation of microsatellite mutations in a specific set of tumor-suppressor genes that control growth in colonic epithelium, which are different in mice than in humans.

**Clinical Implications for Lynch Syndrome**

There are numerous implications of the observation that specific mutations in a single DNA MMR gene might be associated with unique clinical features. First, it is apparent that this is not the only type of sequence variation that will affect the function of MLH1. Interpreting the mutational spectrum of missense mutations in MLH1 has been a particularly active area of research (24, 25). It is even possible that some 3’ truncating mutations or splice-site variations might be associated with variant phenotypes as well. How should we categorize a familial cluster of CRC in which the phenotype is linked to a mutation like MLH1L607H and there is no MSI in the tumor DNA? Is this still Lynch syndrome? Do we refer to a missense mutation that selectively inactivates either the DNA MMR or cell-cycle–checkpoint functions as a defective genotype? Should we start screening MSS CRCs in familial clusters for FANCJ mutations? After all, the mutation FANCJ K141I/K42A might be functionally equivalent to MLH1L607H. A similar issue can be found in the MSH2 gene, in which certain missense mutations can selectively abrogate either the mismatch-recognition function or the critical adenosine triphosphatase activity of the MutS complex (26). The complexity of this problem can be found in both the MutS (MSH2 and MSH6) and MutL (MLH1 and PMS2) genes. We may have to rethink the nomenclature we use for diseases associated with these exceptional mutations.

Perhaps even more important, what if selective losses of DNA MMR or checkpoint function are essential determinants of the response to therapy? For cancer therapy, we use drugs such as platinum drugs that crosslink DNA in a manner similar to MMC and others such as cyclophosphamide that create DNA adducts which trigger apoptosis. MMC and cyclophosphamide are not typically used to treat CRC, but 5-fluorouracil, perhaps the most widely used drug for this disease, is incorporated into DNA and is recognized by DNA MMR proteins, and the absence of DNA MMR activity is associated with tolerance of this drug (9, 27, 28). It may be necessary to have a more complete understanding of all of the genes involved in the response to DNA damage, as well as the type of mutation, to plan rational drug prevention and therapy. The finding of Xie et al. that MLH1L607H confers increased sensitivity to MMC might point the way toward identifying and exploiting the Achilles’ heel of one kind of cancer. This is a promising step, although it raises the intimidating likelihood that much more effort will be required before we truly understand the disease. It may not be enough to do complete sequencing of cancer genomes or to do functional *in vitro* tests for enzyme activity of mutated proteins. Rather, we may also have to probe the interaction of a mutated tumor (suppressor) protein with the rest of the proteome because, as illustrated here, the real defect may be loss of a specific protein interaction. This task may seem daunting now, but so did the idea of having the whole human genome in our hands, and not too long ago.

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