Published in final edited form as:
Cancer Prev Res (Phila). 2010 November ; 3(11): 1371–1374. doi:10.1158/1940-6207.CAPR-10-0204.

Making Sense of Missense in the Lynch Syndrome: The Clinical Perspective

Henry T. Lynch[1], **Thomas Jascur**[2], **Stephen Lanspa**[1], and **C. Richard Boland**[2]

[1] Department of Preventive Medicine and Public Health, Creighton University; Omaha, Nebraska

[2] Baylor University Medical Center, Sammons Cancer Center, Baylor Research Institute; Dallas, Texas

Abstract

The DNA mismatch repair system provides critical genetic housekeeping, and its failure is associated with tumorigenesis. Through distinct domains on the DNA mismatch repair 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 mismatch repair genes can selectively alter just one of these functions. This impacts the clinical features of tumors associated with defective DNA mismatch repair activity. New work reported by Xie et al. in this issue of the journal (beginning on page **XXX**) adds to the understanding of DNA mismatch repair.

Keywords

colorectal cancer; hereditary cancer; cancer genetics; Lynch syndrome; MLH1

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 appears 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. But more progress is needed (1–7).

The discovery of cancer-causing germline mutations has proved to be highly advantageous in determining patients' life-time 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

Corresponding Author: Henry T. Lynch, M.D., Department of Preventive Medicine and Public Health, Creighton University, 2500 California Plaza, Omaha NE 68131. Phone: 402-280-2942; Fax: 402-280-1734; htlynch@creighton.edu.

Disclosure of Potential Conflicts of Interest
[XXXX]

Note: Contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the State of Nebraska or the Nebraska Department of Health and Human Services, which provide funding support for work that is represented herein.

syndrome families and 47 Lynch syndrome families which comprised 10,910 cohort members, of whom 1408 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 non-carrier 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 non-carrier status, accounted for 89% of all risk changes resulting from testing. Clearly, these results impact 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 (CRCs) 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 non-aneuploid, 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 homolog (*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 appear 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 homolog 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 appears 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

appears to have a later age of onset of CRC, but 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* appear 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 C-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 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, since 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. Of interest, in mice the absence of *Msh2* leads to a complete loss of DNA MMR activity, whereas the missense mutation Msh^{G674A} 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, but 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 $MLH1^{L607H}$ 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 *MLH1*^{L607H} beyond simple *in vitro* MMR assays is important because, as Xie et al. demonstrate, 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 lab offers strong evidence that FANCJ is the primary culprit; they characterized a *FANCJ* mutation (*K141/142A*) that is defective in MLH1 binding, and this *FANCJ* mutant had a phenotype comparable with *MLH1*^{L607H}, which lacks FANCJ binding. It appears 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 *MLH1*^{L607H} in CRC formation. Because this mutation is MMR-competent and the associated tumors test as MSS, it might be uniquely suited for mouse models. So 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 *MLH1*^{L607H} 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*$^{K141/142A}$ might be functionally equivalent to *MLH1*^{L607H}. 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 disease 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, in order to plan rational drug prevention and therapy. The finding of Xie et al. that *MLH1*^{L607H} confers increased sensitivity to MMC might point the way towards identifying and exploiting the Achilles' heel of one kind of cancer. This is a promising step, but 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 entire 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.

Acknowledgments

Grant Support

This work was supported in part by revenue from Nebraska cigarette taxes awarded to Creighton University by the Nebraska Department of Health and Human Services (H. T. Lynch) and in part by Grant R01 CA72851 from the National Institutes of Health (C. R. Boland, T. Jascur). Partial funding also was provided by the Charles F. and Mary C. Heider Chair in Cancer Research held by H. T. Lynch at Creighton University.

Reference List

1. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet 2009;76:1–18. [PubMed: 19659756]
2. Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. J Med Genet 1999;36:801–18. [PubMed: 10544223]
3. Lynch HT, de la Chapelle A. Genomic medicine: hereditary colorectal cancer. N Engl J Med 2003;348:919–32. [PubMed: 12621137]
4. Lynch HT, Boland CR, Rodriguez-Bigas MA, Amos C, Lynch JF, Lynch PM. Who should be sent for genetic testing in hereditary colorectal cancer syndromes? J Clin Oncol 2007;25:3534–42. [PubMed: 17687158]
5. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. Science 2010;318:1108–13. [PubMed: 17932254]
6. Lin J, Gan CM, Zhang X, et al. A multidimensional analysis of genes mutated in breast and colorectal cancers. Genome Res 2010;17:1304–18. [PubMed: 17693572]
7. Sjöblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2010;314:268–74.
8. Watson P, Narod SA, Fodde R, et al. Carrier risk status changes resulting from mutation testing in hereditary nonpolyposis colorectal cancer and hereditary breast-ovarian cancer. J Hum Genet 2003;40:591–6.
9. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010;28:3219–26. [PubMed: 20498393]
10. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138:2073–87. [PubMed: 20420947]
11. Boland CR, Shike M. Report from the Jerusalem Workshop on Lynch Syndrome-Hereditary Nonpolyposis Colorectal Cancer. Gastroenterology 2010;138:2197.e1–2197.e7. [PubMed: 20416305]
12. Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer 2005;4:211–8. [PubMed: 16136380]
13. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–57. [PubMed: 9823339]
14. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96:261–8. [PubMed: 14970275]
15. Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med 2009;11:42–65. [PubMed: 19125127]

16. Hendriks YMC, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to *MSH6* mutations: impact on counseling and surveillance. Gastroenterology 2004;127:17–25. [PubMed: 15236168]

17. Fishel R. The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: revising the mutator hypothesis. Cancer Res 2001;61:7369–74. [PubMed: 11606363]

18. Goel A, Nagasaka T, Hamelin R, Boland CR. An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. PLoS ONE 2010;5:e9393. [PubMed: 20195377]

19. Xie, et al. An MLH1 mutation links BACH1/FANCJ to colon cancer, signaling, and insight towards directed therapy (this issue).

20. Ligtenberg MJL, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of *MSH2* in families with Lynch syndrome due to deletion of the 3′ exons of *TACSTD1*. Nat Genet 2009;41:112–7. [PubMed: 19098912]

21. Lin DP, Wang Y, Scherer SJ, et al. An Msh2 point mutation uncouples DNA mismatch repair and apoptosis. Cancer Res 2004;64:517–22. [PubMed: 14744764]

22. Friedman LS, Ostermeyer EA, Szabo CI, et al. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 1994;8:399–404. [PubMed: 7894493]

23. Peng M, Litman R, Xie J, Sharma S, Brosh RM Jr, Cantor SB. The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. EMBO J 2007;26:3238–49. [PubMed: 17581638]

24. Raevaara TE, Korhonen MK, Lohi H, et al. Functional significance and clinical phenotype of nontruncating mismatch repair variants of *MLH1*. Gastroenterology 2005;129:537–49. [PubMed: 16083711]

25. Takahashi M, Shimodaira H, Andreutti-Zaugg C, Iggo R, Kolodner RD, Ishioka C. Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays. Cancer Res 2007;67:4595–604. [PubMed: 17510385]

26. Wilson S, Guerrette S, Fishel R. Dissociation of mismatch recognition and ATPase activity by hMSH2-hMSH3. J Biol Chem 1999;274:21659–64. [PubMed: 10419475]

27. Carethers JM, Chauhan DP, Fink D, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. Gastroenterology 1999;117:123–31. [PubMed: 10381918]

28. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247–57. [PubMed: 12867608]