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The Mystery of Mismatch Repair deficiency: Lynch or Lynch-like?

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Syndromic familial colorectal cancer (CRC) has gone through an evolution of names, and at this time, we refer to Lynch syndrome as the familial syndrome characterized by a germline mutation in a DNA mismatch repair (MMR) gene[1]. This helped distinguish Lynch syndrome from other familial collections of tumors that were not linked to MMR gene mutations, and underscored the unique clinical consequences of tumors with DNA MMR deficiency, which involve differences in the growth rates of the tumors, their location in the colon, the natural history, the risks of cancer in non-colonic organs, and differences in the responses to chemotherapy[2]. The collective agreement to use this nomenclature was in part based upon the fact that one can detect almost all Lynch syndrome CRCs by virtue of the presence of microsatellite instability (MSI) and abnormal MMR protein immunohistochemistry (IHC) in the tumor tissues.

However, there are some patients with CRCs that have MSI and abnormal MMR IHC in the tumor, but no germline mutation can be found in the patient's DNA MMR genes. The largest group of these is caused by acquired hypermethylation of the promoters of both alleles of the *MLH1* gene, and it is thought that this accounts for about 10–12% of all CRCs[2]. After the recognition of this “acquired” form of MSI in CRC, it was thought that all CRCs with MSI were the result of either Lynch syndrome, or the acquired methylation of *MLH1*. A paper in this issue of *Gastroenterology* by Rodriguez-Soler et al, from Spain suggests that there may be more to the story[3].

The EPICOLON consortium has gathered population-based cohorts of CRC cases from Spain, and published several prominent papers from this database. In the current study, they analyzed 1,705 patients with CRC from 2 multicenter studies collected in 2000–01, and in 2006–7. They performed MSI and MMRIHC testing on all of the tumors, selected the cases in which both tests were abnormal, excluded all cases of acquired methylation of *MLH1*, and then looked for germline mutations in the 4 DNA MMR genes (*MSH2, MLH1, MSH6* and *PMS2*), large deletions, and the specific deletions in *EPCAM* that lead to silencing of the *MSH2* gene. This was necessary since deleting the stop codon of EPCAM leads to

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methylation of the next gene downstream (which is *MSH2*), in any tissue that expresses *EPCAM* (such as the colon). There were 135 cases of MMR-deficiency, defined by having *both* MSI and abnormal IHC, which was 8% of the cohort. They excluded 79 MSI CRCs because they found hypermethylation of *MLH1*, leaving 56 as patients with suspected Lynch syndrome, which was 3.2% of the cohort. They then sought germline mutations in the DNA MMR genes, but found convincing mutations in only 16 of them – which is 0.9% of the cohort, and only 29% of the putative Lynch syndrome patients. The group with MMR-deficiency not linked to a germline mutation in a DNA MMR gene was termed “Lynch-like syndrome” or LLS. Also, they excluded 16 CRCs because the MSI and IHC gave discrepant results - the same as the number of confirmed Lynch syndrome cases.

So, what is going on in the group of 40 patients with MMR-deficiency, but no germline mutation in a MMR gene? There are at least 2 possible explanations for these tumors. Either the investigators were unable to find “cryptic” germline mutations in the 4 DNA MMR genes in actual Lynch syndrome patients (i.e., mutations were present, but not detected), or, there is some pathological process other than a germline mutation in, or methylation of, a DNA MMR gene that can produce a CRC with MSI.

Let's consider each possibility. First, how difficult it is to find every possible germline mutation in a gene? As the authors acknowledge, it is very hard. When the genes responsible for Lynch syndrome were first identified, routine sequencing strategies probably identified fewer than half of the mutations that were actually present. It was initially difficult to definitively classify missense mutations and splice site variations, as these can be ambiguous. Much of this has been clarified by painstakingly matching all sequence variants with the risk of CRC in families carrying the variants. There is a lot of DNA sequence variability among individuals, and most of it does not cause a disease. Large deletions and genetic rearrangements are common causes of genetic inactivation, especially in the DNA MMR genes, and it took technical advances to find these[4]. Moreover, it was not discovered until 2009 that alterations of the *EPCAM* gene (which is immediately upstream of *MSH2*) that delete its termination codon lead to methylation-induced silencing of the *MSH2* gene, and Lynch syndrome[5]. Our understanding gene promoter function is still primitive. For example, loss of portions of the *APC* promoter 1B, which is almost 55 kb from the start site of the gene, causes familial adenomatous polyposis[6]. Who knows what might be going on in the promoters of the DNA MMR genes? Moreover, we have yet to explore what intronic sequence variations might alter gene function. We have a long way to go in our understanding of genetic pathology.

What other process might produce a CRC with MSI? Based upon the published literature, it would appear that MMR-deficient tumors do not often arise from biallelic somatic mutations in a MMR gene. However, a recent report indicates that this can occasionally happen[7]. A French group carried out genetic analyses on blood and tumor tissue on 18 CRCs with MSI that had neither Lynch syndrome nor methylation of *MLH1*, and found biallelic MMR gene mutations in 5 tumors. In 3 of these patients, both mutations were somatic, and not present in the germline (i.e., blood). One had a previously overlooked germline mutation, and one was a mosaic – which is essentially a somatic mutation that occurs during embryogenesis, and is only carried by some of the somatic cells. This is presumably how “de novo” mutations occur. So, biallelic somatic mutations remain a possible explanation, but we do not know how common these are.

What other technical challenges might have limited full discovery of Lynch syndrome patients? Most feel that we cannot find all germline mutations using current technologies. One group reported in 2005 (before several diagnostic advances) that Lynch syndrome accounted for at least 2.2% of population-based CRCs[8], and others have suggested that

Lynch syndrome may account for as many as 4.4% of CRCs[9, 10], whereas this diagnosis was reached in only 0.9% in this report. It would seem that there could be undiagnosed Lynch syndrome in this cohort, which would be one obvious explanation for the results.

There were other technical issues that could have affected the results, such as the use of only 2 microsatellite markers, but the authors have previously shown that their approach is valid[11]. Nonetheless, most groups report that the proportion of CRCs with MSI is in the range of 12–15% rather than 8%, as reported here[2, 12, 13]. Additionally, 16 cases had either MSI or abnormal IHC (but not both), and were excluded from consideration. It is possible that the use of tissue microarrays for the IHC suffered from inadequate sampling of the tumor for abnormal IHC[14, 15], or that the use of just 2 microsatellite markers led to an underestimate of the frequency of Lynch syndrome. In any event, the investigators excluded a large number of cases that might have had true Lynch syndrome[12]. However, it is not apparent that either of these possible confounders would have selected for a higher proportion of CRCs with MSI that are not linked to a germline mutation in a DNA MMR gene.

Is LLS clinically identical to Lynch syndrome? The investigators examined family histories of their CRC patients, first by a retrospective review of the history provided by the patients, and then by a prospective updating of the pedigrees in 2011, looking for new, incident cases of Lynch syndrome-related cancers in the first degree relatives of the index case. In the follow-up study of new, incident cancers, the standardized incidence ratio (SIR) for CRC was highest in the Lynch syndrome families (6.04), lowest for those with apparent sporadic CRCs (0.48), and in-between for LLS patients (2.12), suggesting that at least some of the CRCs with MMR-deficiency may have had something other than familial CRC. The SIR for non-CRC Lynch syndrome-associated cancers was slightly (but not significantly) higher for those with Lynch syndrome (2.81), compared with for those with LLS (1.69) or sporadic CRC (1.20). This provides evidence that the LLS cohort may contain a proportion of true Lynch syndrome cases. Interestingly, the mean age of onset for CRC in the Lynch syndrome patients was 48.5±14.13 years, which was statistically similar to the age in LLS (53.7±16.8 years), both of which were significantly younger than that for sporadic CRC patients (68.8±9 years), again highlighting clinical similarities between Lynch syndrome and LLS. Whatever skepticism one might have for the challenge of finding all the germline mutations in Lynch syndrome, when mutations were not found, the relatives were less likely to suffer from cancer; also, those who did, got their tumors slightly later. So, for at least a proportion of the LLS patients, there are important clinical differences that should be taken into account when managing that situation.

The work presented by Rodriguez-Soler and the EPICOLON group raises the novel concept that there are CRCs with MSI that are not Lynch syndrome, and not caused by the acquired hypermethylation of the *MLH1* gene. There are numerous reasons why this may not be the case, principally based on the difficult challenge of finding all of the ways a gene can undergo inactivation. However, the different clinical features in the family members of those with LLS suggest that there may be some other mechanism for generating DNA MMR-deficiency and MSI. I would speculate that the LLS group is heterogeneous, and contains some true Lynch syndrome - and something else. Time will tell.

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