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Molecular analysis of colorectal tumors in Lynch syndrome

Lynch syndrome, most often caused by a germline mutation in *MSH2, MSH6, MLH1,* or *PMS2*, contributes to a number of malignancies, including colorectal and endometrial cancer. A second somatic hit in the respective gene leads to defective DNA mismatch repair, causing microsatellite instability (MSI) or changes in the lengths of repetitive sequences in DNA. MSI can also occur when there is hypermethylation of the *MLH1* promoter region, silencing the gene. Although both mechanisms result in MSI, some studies have shown that Lynch syndrome-associated carcinomas have better clinical outcome than spontaneous colorectal carcinomas that exhibit MSI via gene silencing. Many recent studies have furthered understanding of the molecular basis of colorectal carcinoma. However, many of these studies have either excluded colorectal carcinomas associated with Lynch syndrome or have not been designed to interrogate how they differ from spontaneous colorectal carcinoma. The authors of this study analyzed paired tumor and non-neoplastic mucosa from 11 patients with Lynch syndrome and compared them with samples from microsatellite-stable and MSI spontaneous colorectal carcinomas. They performed whole genome DNA sequencing and gene-expression analysis by RNA sequencing. The authors also determined MSI slippage analysis by comparing lengths of repeated sequences with a reference genome. In the patients with Lynch syndrome, the samples stratified into two groups, which were designated G1 and G2. The G1 tumors demonstrated significantly higher mutation burden, more frameshift mutations, and a higher rate of MSI slippage than the G2 tumors. Furthermore, the types of mutations seen in the G1 tumors more closely paralleled those in spontaneous colorectal carcinoma with MSI, whereas the G2 tumors exhibited mutations that more closely paralleled those in spontaneous microsatellite-stable carcinoma. Gene-expression analysis also demonstrated differences between the G1 and G2 tumors. While both groups of tumors showed upregulation of genes associated with proliferation, the G1 tumors showed altered expression of genes associated with inflammatory processes. The authors then compared the paired non-neoplastic mucosa from patients with G1 tumors with the non-neoplastic mucosa from patients with G2 tumors and to the G1 tumors themselves. Interestingly, the paired non-neoplastic mucosa from patients with G1 tumors showed greater expression of *HLA* and genes associated with immune checkpoints, as well as increased infiltration of CD4 lymphocytes by immunohistochemistry. Therefore, the authors propose that the G1 tumors are initially kept in check by the highly immunogenic microenvironment, which responds to the high number of neoantigens in the tumors. Ultimately, however, the G1 tumors escape the immune system by altering genes related to immunogenicity. In contrast, the G2 tumors do not develop in the highly immunogenic milieu, and, therefore, do not exhibit the same alteration in genes related to immunogenicity. Overall, this study demonstrates significant heterogeneity in colorectal tumors in Lynch syndrome patients and their relationship to the immune microenvironment. The clinical implication of the classification system presented deserves further investigation.

Binder H, Hopp L, Schweiger MR, et al. Genomic and transcriptomic heterogeneity of colorectal tumours arising in Lynch syndrome. *J Pathol.* 2017;243:242–254.

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NGS in multifocal primary lung adenocarcinomas and intrapulmonary metastases

With multifocal lung adenocarcinoma, accurate staging requires determining whether the tumors represent separate concurrent primaries or an intrapulmonary metastasis originating from a single neoplastic focus. Treatment and prognosis vary in each of these cases. For patients with multiple separate primary tumors, surgical resection with curative intent would likely be offered. However, for patients with an intrapulmonary metastasis, prognosis would be poor and treatment would likely include systemic therapy or palliation. Comprehensive histologic evaluation is the traditional means for distinguishing intrapulmonary metastases from separate concurrent primaries. The eighth edition of the American Joint Committee on Cancer (AJCC) cancer staging manual discusses criteria to predict whether tumors of lung adenocarcinoma are independent or related. These criteria include semi-quantitative evaluation of tumor architectural patterns and the presence of extrapulmonary metastases. Selected biomarkers and sequencing of driver mutations can also be applied and are useful in some situations. Targeted next-generation sequencing (NGS) panels are increasingly being used to evaluate lung cancer, identifying not only driver mutations but also passenger mutations. In the study reported herein, the authors used an NGS panel that interrogates 50 genes using Ion Torrent technology to help stage patients with multifocal lung adenocarcinoma. They examined 11 patients with multifocal adenocarcinoma and no evidence of metastatic disease, including no evidence of malignant pleural effusion, pleural nodules, or lymph node stage greater than N1. They compared these patients with a control group of eight patients with known metastatic lung adenocarcinoma. The authors examined 42 specimens, including 31 lung tumors and eight distant metastases. Of the eight patients with known metastatic disease, five had identical mutation status and three shared some but not all mutations. Driver mutations in genes such as *KRAS, EGFR*, and *BRAF* were observed in seven of the eight patients, and these were mutually exclusive and concordant between the paired primary tumor and distant metastasis. In the eighth patient, no mutations were seen in the primary or distant metastasis, which is also a concordant result. In the study group of 11 patients with multifocal adenocarcinoma, three patients had identical mutations in the paired tumor samples, including identical driver mutations. One of these patients also had identical *TP53* mutations. The tumors exhibited completely discordant mutations in the eight remaining patients, and driver and *TP53* mutations were always discordant in this group. In the patients with concurrent separate primary tumors, the application of AJCC histologic criteria was also able to definitively classify the two tumors as separate. However, in the three patients with intrapulmonary metastases, application of the AJCC criteria resulted in an equivocal classification, suggesting an important role for gene panels in determining intrapulmonary metastases. The authors also showed a worse clinical outcome in the patients demonstrated to have intrapulmonary metastases than in those with multiple synchronous primary tumors. One important caveat is that driver events are often conserved between the primary tumor and metastasis. Therefore, while concordance does not necessarily prove relationship, it strongly favors separate primaries. Similarly, concordance for rare mutations or those seen in passenger genes is highly supportive of clonal relatedness.

Patel SB, Kadi W, Walts AE, et al. Next-generation sequencing: A novel approach to distinguish multifocal primary lung adenocarcinomas from intrapulmonary metastases. *J Mol Diagn.* In press.

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