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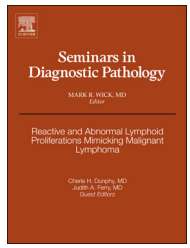

Role of the clinical pathology laboratory in the evaluation of endometrial carcinomas for Lynch syndrome

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ABSTRACT

Molecular diagnostic testing of endometrial carcinomas in the pathology laboratory has recently emerged as a key component of the clinical evaluation of Lynch syndrome in many centers. Testing modalities involve immunohistochemical and PCR-based analyses. This article outlines the routine application of these analyses, provides a practical guide for troubleshooting some of the common technical issues related to their performance, and reviews common pitfalls in their interpretation. Discrepancies between tissue testing and genetic testing results are discussed in the context of the current understanding of endometrial cancer biology. The merits of universal versus targeted tissue testing based on clinical patient history and histological tumor appearance are also addressed.

Introduction

Hereditary cancer syndromes include Li–Fraumeni syndrome, Familial Adenomatous Polyposis Syndrome, BRCA1/BRCA2 Hereditary Breast/Ovarian Cancer Syndrome, Cowden's Syndrome, Juvenile Polyposis, and Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer Syndrome). For nearly all of these hereditary cancer syndromes, identification of affected individuals is dependent on recognition of tell-tale clinical features, such as young age of cancer onset and a striking family history of characteristic cancers, followed by germline sequencing of the suspected affected gene. Such sequencing is typically complex and carried out in only a few large reference genetic laboratories. Most clinical pathology laboratories, thus, do not play substantive roles in the diagnostic work-up of such familial cancer syndromes. For Lynch syndrome, however, the ancillary tests of immunohistochemistry for mismatch repair proteins (MMR) and PCR-based microsatellite instability (MSI) analysis are more widely available and have emerged as key components of the clinical evaluation of this syndrome. Importantly, these tests can be performed using formalin-fixed, paraffin-embedded tissues and do not require special fixatives or frozen tissue preservation. Such ancillary tests have been shown to be a cost-effective first step in patient screening for Lynch syndrome.[1,2] Many pathology practices are reflexively subjecting all colon carcinomas to such immunohistochemical and/or MSI testing, and testing of endometrial carcinomas is starting to increasingly occur. Because these cancer types are common, it is important for pathologists to have a good working knowledge of test interpretation and pitfalls.

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Lynch syndrome

Lynch syndrome occurs due to a germline mutation in one of a family of DNA *MMR* genes, with subsequent loss of associated protein expression. Mutation of *MLH1* or *MSH2* genes is most common, but other important *MMR* genes include *MSH6* and *PMS2*. The hallmark cancers for Lynch syndrome are colorectal adenocarcinoma and endometrial carcinoma. Less common cancer types also associated with this syndrome include ovarian carcinoma, urothelial carcinomas of the ureter/renal pelvis, duodenal adenocarcinoma, and gastric adenocarcinoma. Loss of DNA MMR function typically results in high levels of DNA MSI, which can readily be measured by a PCR-based analysis. Similarly, immunohistochemical expression of *MLH1*, *MSH2*, *MSH6*, and *PMS2* can be performed by routine immunohistochemical analyses. In 15–20% of all sporadic endometrial carcinomas, MLH1 immunohistochemical loss and MSI results from *MLH1* gene promoter methylation with subsequent transcriptional silencing.[3–7] Therefore, PCR-based *MLH1* promoter methylation analysis represents the third component of tissue testing for Lynch syndrome.

Immunohistochemistry for mismatch repair proteins

Immunohistochemistry for MMR proteins MLH1, MSH2, MSH6, and PMS2 is carried out using commercially available antibodies that work quite reliably. Gene mutation of *MMR* genes or methylation of the *MLH1* promoter typically results in loss of immunohistochemical expression of the corresponding protein. For a tumor to be considered as having a loss of an MMR marker, complete absence of nuclear expression should be observed. Strong nuclear staining in the surrounding endometrial stroma, myometrium, lymphocytes, or normal endometrium should serve as an internal positive control (Fig. 1). The MLH1 and PMS2 proteins and the MSH2 and MSH6 proteins act as functional pairs.[8] Loss of the MLH1 protein (due to mutation of the *MLH1* gene or methylation of *MLH1* gene promoter) typically results in loss of immunhistochemical expression of MLH1 and PMS2. Mutation of *MSH2* typically results in immunohistochemical loss of MSH2 and MSH6. On the other hand, mutation of *MSH6* is associated with loss of MSH6 protein but retention of MSH2 by immunohistochemistry. Similarly, mutation of *PMS2* is typically associated with loss of PMS2 protein but retained MLH1 immunohistochemical expression.

While MMR loss and MSI in complex atypical hyperplasia of endometrium have been reported, not all endometrial cancer cases with concurrent complex atypical hyperplasia exhibit these molecular abnormalities in the areas of hyperplasia.[9–11] Although *MMR* gene mutation carriers may show higher levels of concordance between complex atypical hyperplasia and endometrial carcinoma than patients with sporadic endometrial cancers, it is recommended that tissue testing always be carried out in foci of endometrial carcinoma rather than adjacent complex atypical hyperplasia (Fig. 2).

Microsatellite instability analysis

MSI analysis is a PCR-based test that measures errors in DNA replication resulting from absence of MMR protein function. Microsatellites are sequences of DNA composed of repeating units of 1–6 base pairs in length. MSI analysis requires DNA from both the endometrial tumor and normal non-tumor tissues

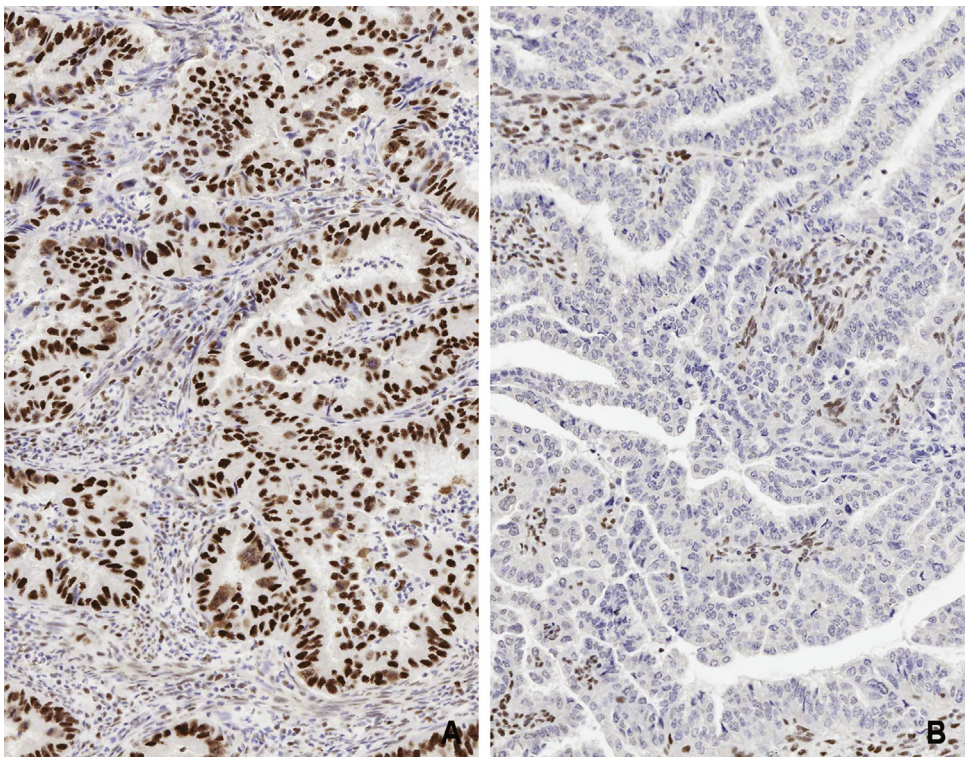

Fig. 1 – (A) Nuclear PMS2 expression with good internal positive control in the stroma, 20×. (B) Loss of nuclear MSH2 expression, with a good internal positive control in the stroma, 20×.