The pathology of Lynch Syndrome

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Abstract

Lynch Syndrome (LS) is a relatively common cause of inherited colorectal carcinoma which have many typical gross and microscopic features. Histopathologists play a critical role in identifying such tumours but also in screening cases for LS using modern molecular techniques. Immunohistochemistry for mismatch repair proteins, microsatellite instability testing and mutation analysis of key genes such as BRAF are important tools in this regard. Pathologists must be aware of the advantages and limitations of all these tests.

Keywords Lynch Syndrome; microsatellite instability; mismatch repair deficiency; MLH1; MSH2; MSH6; PMS2

Introduction

Lynch Syndrome (hereditary non-polyposis colorectal carcinoma) is an autosomal dominant inherited cancer predisposition syndrome associated with a high risk of gastrointestinal (GI) tract cancers and also cancer in other organ systems.[1,2] The most common Lynch associated GI tract malignancy is adenocarcinoma of the colon but Lynch Syndrome is also associated with carcinoma of the stomach, small intestine and biliary tract.[3] It is estimated that between 2 and 7% of all colorectal carcinomas are Lynch Syndrome related. Accordingly, Lynch Syndrome may be suspected in any patient with early onset carcinoma which is not obviously associated with familial adenomatous polyposis. Lynch Syndrome is usually caused by an inherited germline mutation affecting one of the mismatch repair genes. Briefly, there are at least seven known genes which function together as a complex to eliminate DNA base pair errors and abnormal insertion deletion loops which may develop during replication. The commonest genes affected are MLH1, MSH2, MSH6 and PMS2. Of these the vast majority of mutations in Lynch Syndrome are due to mutations in MLH1 and MSH2. Dysfunction of any of these genes, either due to a missense or truncation mutation, will result in repair complex failure and the accrual of numerous DNA replication errors (mutator phenotype). The development of malignancy results from inactivation of other genes including oncogenes and tumour suppressor genes. At the protein level, specific relationships exist between elements of the mismatch repair complex. These include MSH2 and MSH6 which function together as a mutation recognition complex, while MLH1 and PMS2 form a repair complex. Loss of MSH2 results in degradation of MSH6 and similarly loss of MLH1 leads to loss of PMS2.

The average age of patients at diagnosis is approximately 45 years. Usually patients or kindreds are selected for genetic testing utilising clinical diagnostic tools such as the Amsterdam or Bethesda criteria while others have advocated universal screening of colorectal carcinoma patients.[4] Because gene sequencing is relatively expensive and time consuming, further screening suspected Lynch Syndrome patient's tumours by molecular diagnostic techniques such as microsatellite instability (MSI) analysis and mismatch repair protein (MMR) immunohistochemistry have been developed. These may be used either in tandem or in isolation to further select patients for specific gene sequencing. Such 'retrospective' screening for Lynch Syndrome has been carried out in the UK for many years but often only in specialised centres. Recently, new guidance on screening all patients who present with colorectal carcinoma in the 50 years or under age group have been put forward by the Royal College of Pathologists in the United Kingdom.[5] This 'prospective' testing therefore presents a new diagnostic challenge to histopathologists working outwith specialised centres. In this review I will briefly discuss the typical histopathological appearances of both adenomas and adenocarcinomas arising in Lynch patients. We will then focus on the molecular diagnostic pathology with special emphasis on the role of immunohistochemistry.

Colorectal cancer in Lynch Syndrome

The accumulative life time risk of developing colon cancer in Lynch Syndrome is approximately 75%.[6] Adenocarcinomas of the colon arising in Lynch Syndrome are more likely to be right sided than in the general population. The main age at diagnosis is most commonly estimated to be within the fourth decade of life. Multiple colonic adenocarcinomas are also more common. Grossly no specific features are ascribed to these tumours; however a high proportion of them show mucinous change and may demonstrate a glistening gelatinous cut surface. Histologically, these tumours are more commonly poorly differentiated, mucinous and associated with a prominent chronic inflammatory response, variously termed a Crohn's-like reaction or showing high numbers of tumour infiltrating lymphocytes (Figure 1).

It is thought that these carcinomas arise from adenomas. The number of adenomas present varies greatly within families and may relate to the precise genetic abnormality responsible. Histopathologically, despite their molecular genetic similarity to serrated lesions/adenomas, most adenomas detected from Lynch Syndrome patient's are of the conventional type showing either tubular or tubulovillous configuration. Several studies have suggested that colorectal adenomas associated with Lynch Syndrome have a more rapid progression to carcinoma compared to those arising sporadically within the population.[7,8] It is important in practice for histopathologists to remember that individuals from Lynch Syndrome kindreds may also develop sporadic or even serrated pathway derived colorectal adenomas and carcinomas. This latter possibility may lead to misinterpretation of molecular pathological analysis in certain cases.

Other types of gastrointestinal tract cancer

Adenocarcinoma of the stomach occurring in Lynch Syndrome is mostly of the intestinal type with no specific features, occurring most commonly in the 5th decade. Small intestinal adenocarcinoma also shows no specific features and is commonest in the same age group. No specific features are known to associate with

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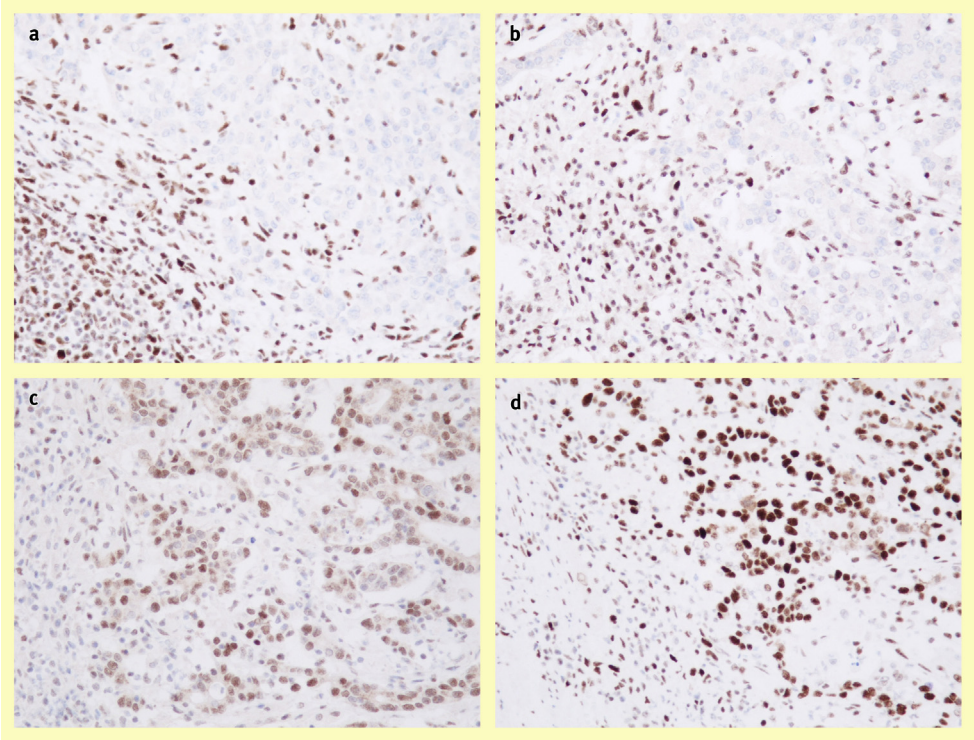

Figure 1 Poorly differentiated colorectal carcinoma with intense lymphocytic response. Tumour cells do not express MLH1 (**a**) or PMS2 (**b**) but strongly express nuclear MSH2 (**c**) and MSH6 (**d**). Note background lymphocytes strongly express all four proteins providing a good internal positive control. This tumour is likely to be mismatch repair deficient. Further analysis of BRAF should be performed to assess whether this tumour is derived from a sporadic 'serrated' pathway or is truly Lynch Syndrome related.

bile duct or pancreatic adenocarcinomas seen in Lynch Syndrome.

Unusual genetics in Lynch Syndrome

While the majority of Lynch Syndrome cases are explained by mutations in mismatch repair genes, several rare additional genetic abnormalities are also described. These include mutation in the EPCAM gene which is responsible for between 3% and 6% of Lynch Syndrome cases.[9] The EPCAM gene, which encodes an adhesion molecule, if mutated may lead to hypermethylation of the MSH2 promoter and epigenetic silencing of the MSH2 gene. In addition very rare examples of Lynch Syndrome due to constitutional epimutation of the MLH1 gene promoter due to hypermethylation have also been described. Finally, recessively inherited mutations in mismatch repair genes are known to be responsible for a rare paediatric syndrome characterised by tumours of the gastrointestinal tract and brain with haematological malignancies and features of neurofibromatosis type 1 including café au lait spots.[10]

Screening strategy for Lynch Syndrome

In recent years universal screening for Lynch Syndrome in all case of colonic and endometrial carcinoma has been advocated. It has been noted that as many as 70% of people with Lynch Syndrome may not meet the diagnostic criteria of commonly used clinical diagnostic algorithms. Which molecular screening methods should be used when considering testing a patient's tumour for Lynch Syndrome has also been the subject of debate and dispute. This has been further complicated as our knowledge base concerning the genetics of Lynch Syndrome broadens and the technology to assess for abnormal genetic signatures/changes.

In the past some have advocated either microsatellite instability analysis alone or immunohistochemistry for mismatch repair proteins alone as the most suitable method for Lynch Syndrome testing. Others have advocated using both approaches in combination with or without the addition of BRAF gene mutation analysis.[11] The relative strengths and limitations of these approaches will be discussed in more detail below.

Prior to embarking on any molecular studies the need for good routine histopathological practices and input from an experienced pathologist remain paramount. In this regard several points must be emphasised. First of all, correct patient identification, confirmation that the material submitted for examination comes from the correct patient, and in particular, that tumour is indeed present in the blocks for testing, are all required. This will require review of an H&E section. A further advantage of reviewing a single H&E section of tumour prior to further analysis is that this allows assessment of how much tumour is present within the tissue block. This is essential for laboratories undertaking MSI analysis as a low amount of tumour submitted may lead to a false negative result due to the sensitivity requirements of most MSI assays. If several blocks have been submitted assessment of the accompanying H&Es will also allow for the elimination of blocks which contain too much autolysis or