Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Hereditary Colorectal Cancer

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Slide 1: Introduction

Hello, my name is Ping Wang. I am Director of Clinical Chemistry at The Methodist Hospital in Houston TX and Assistant Professor at Weill Cornell Medical College. Welcome to this Pearl of Laboratory Medicine on "Hereditary Colorectal Cancer."

Slide 2:

In this presentation, I will first talk about epidemiology and subtypes of hereditary colorectal cancer, which is classified into 3 major syndromes: Lynch Syndrome, or hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, or FAP, and hamartomatous polyposis syndromes. I will then cover each of the 3 subtypes individually.

First, for Lynch syndrome, I will go through criteria for genetic testing, genetic basis, and molecular testing. Similarly, for FAP, genetic basis will be introduced, followed by the description of a clinically similar entity called MUTYH-associated polyposis, and then molecular testing. Lastly, I will briefly touch on hamartomatous polyposis syndromes, the prevalence of which is much lower than the first two syndromes.

The last slide will be a brief summary of molecular testing methods used in clinical labs for these disorders.

Slide 3:

Colorectal cancer is the 3rd most common cancer in both men and women. Hereditary colorectal cancer accounts for 5% of all colorectal cancers. From the definition, one criterion is that several generations, multiple members of the same family have colon cancer. For family members who have not yet been diagnosed, their lifetime risks are significantly increased for both colon cancer and extra-colonic cancers, such as endometrial, renal pelvis, ureter, ovary, stomach, small intestine, etc. Lynch syndrome constitutes about 3%, and familial adenomatous polyposis about 1% of all colon cancers. Both of these are autosomal dominant conditions, and the genetic etiology is relatively well described.

Slide 4:
Lynch syndrome was first characterized as an autosomal dominant disorder in 1966 by Dr. Henry T. Lynch, professor of medicine at Creighton University Medical Center. The mean age of disease onset is 45 yrs, compared to the mean age of 65 yrs for sporadic colorectal cancers. Patients usually present with fewer polyps than those with FAP, but these polyps may behave more aggressively than FAP, meaning they progress to cancer more rapidly. Without intervention, patients with Lynch syndrome have 80% lifetime risk for colorectal cancer. 40-60% patients with Lynch syndrome present with endometrial cancer first. The risks for other cancers in gastrointestinal and reproductive systems are also increased.

Slide 5:
Early detection and intervention is crucial for improved clinical outcomes in Lynch syndrome. Strategies are needed to identify patients who are likely to have Lynch syndrome and should be offered genetic testing. The Amsterdam Criteria were first introduced in 1990 for this purpose. These criteria focus on the number and type of family members and generations affected, and age of disease onset. However, it was later found out that this set of criteria had limited sensitivity in correctly identifying patients. Expansion and revision of the criteria were therefore made to come up with several other criteria, including the Amsterdam II or Modified Amsterdam in 1999, Bethesda in 1997, and revised Bethesda in 2004. As the sensitivity of these criteria increases, however, the specificity decreases, meaning the criteria would increasingly identify patients whose genetic testing results do not fit Lynch syndrome.

Slide 6:
Even with increased sensitivity, not all patients with Lynch syndrome can be identified based on clinical criteria. A universal testing strategy has instead been proposed where all colorectal cancers are screened for Lynch syndrome. This was recommended by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group and has been adopted by many institutions. While this has been suggested as possibly more cost-effective, further studies are needed to determine the cost-effectiveness of the universal screening approach.

Slide 7:
Lynch syndrome is well defined genetically by the common feature of defects in DNA mismatch repair genes. These defects lead to DNA sequence errors, especially in the sequence of microsatellites. Microsatellites are multiple tandem repeats of short nucleotide sequences. Sequence changes in microsatellites in tumor vs. normal tissue are referred to as microsatellite instability.

Microsatellites are frequently located in promoter and other untranscribed regions of genes. Microsatellite instability in these regions affects transcription of genes, including proto-oncogenes and tumor suppressors, such as *APC, ras*, and *p53*.

The mismatch repair genes and related genes implicated in Lynch syndrome are listed below in order of descending frequency of involvement. They are *MSH2, MLH1, MSH6, PMS2,* and *EPCAM*. Of note, *EPCAM* is a gene just upstream of *MSH2*. The deletions in polyA sequences of *EPCAM* silence *MSH2* transcription.

Slide 8:
According to the Colorectal Cancer Screening testing strategy guidelines developed by the National Comprehensive Cancer Network (NCCN), testing is ideally performed in a stepwise manner as outlined in this slide. Step 1 is to test the tumor tissue using immunohistochemistry or MSI testing. Positive cases will proceed to step 2, which differentiates between lynch syndrome and sporadic colon cancers. Finally, if needed, molecular genetic testing of the mismatch repair genes is performed to confirm the genes with mutations.

Slide 9:
Immunohistochemistry testing for Lynch syndrome is performed on tumor tissues. This test looks for lack of mismatch repair gene expression due to mutations. The advantages of this test are: its high clinical sensitivity; it is readily available in most clinical labs; it can identify which MMR gene has mutations, and therefore reducing molecular genetic testing costs. The limitations are: technical issues may result in equivocal staining patterns; it may not identify tumors caused by some missense mutations; and it is less reliable on small tissue samples.

Slide 10:
Microsatellite instability testing is usually performed concurrently with immunohistochemistry testing described in the previous slide. This involves comparison of microsatellite sequences between tumor and non-tumor tissues of the same patient.

The result is positive in virtually all Lynch syndrome cases. However, the results may also be positive in 10-15% sporadic colon cancers. The differentiation between these two requires testing for tumor *MLH1* promoter methylation and somatic *BRAF* mutation. MSI testing usually involves sequence analysis of 5 or more microsatellite markers. Any gain or loss of repeats is considered unstable. If > 2 out of 5 markers or more than 30% markers are unstable, the tumor is considered to have high microsatellite instability, or MSI-high. With 1 unstable marker, or fewer than 30% markers unstable, the tumor is MSI-low. With none of the markers unstable, the tumor is MSI-stable.

Slide 11:
The advantages of MSI testing are: its high clinical sensitivity, including the ability to detect tumors in immunohistochemistry false negative cases; it is highly reproducible; and it requires very little tissue. The limitations are: technical difficulties may be encountered in extremely mucinous tumors and tumors caused by *MSH6* mutations; it does not identify which MMR gene has mutations, and therefore does not reduce molecular genetic testing costs.

Slide 12:
As mentioned in previous slides, 10-15% of sporadic colon cancers are also microsatellite unstable. These tumors may have *BRAF* mutations or *MLH1* promoter hypermethylation, an epigenetic change. Increased methylation of CpG sites in the *MLH1* promoter silences the gene transcription, causing loss of expression and microsatellite instability. However, this is an acquired epigenetic change, is not heritable, and not classified as Lynch syndrome. Of note is that hypermethylation may co-exist with germline *MLH1* mutations, and therefore positive results of hypermethylation do not completely rule out Lynch syndrome.

Slide 13:
Another test that also helps to differentiate between Lynch syndrome and sporadic colon cancer is the *BRAF* mutation testing. This test looks for a change in nucleotide 1799 from *T* to *A*, which results in an amino acid 600 missense mutation from valine to glutamic acid. *BRAF* is part of the *EGFR* signaling cascade. The mutation is associated with decreased response to anti-*EGFR* therapies, such as cetuximab and panitumumab. The mutation is very rare in Lynch syndrome. On the other hand, 70% of sporadic colon cancers with *MLH1* promoter hypermethylation carry the *BRAF* mutation. *BRAF* testing results, therefore, can help differentiate Lynch syndrome from sporadic colon cancers when the tumor is MSI-high and *MLH1* immunohistochemistry negative. The results may also determine the eligibility for anti-*EGFR* therapy.

Slide 14:
Starting from this slide, we will change gears to talk about familial adenomatous polyposis, or FAP. This syndrome is characterized by the presence of more than hundreds or even thousands of polyps, or growths in the large intestine. The onset age is even younger than Lynch syndrome. 50% of people with FAP develop polyps by age 15, and 95% develop polyps by age 35. These polyps start as precancerous adenomas. However, if left untreated, and colon is not surgically removed, there is a 100% chance that some of the polyps will develop into cancer, usually by age 40. Therefore, prophylactic colectomy is the main treatment, with some non-steroid anti-inflammatory drugs being reported to have some chemoprevention effect. There are numerous extracolonic manifestations of FAP, including thyroid and pancreatic cancers, hepatoblastomas, CNS tumors, etc.

A variant form of FAP is called attenuated FAP, in which fewer polyps are found in the large intestine. The average number of polyps is about 30, and it is usually diagnosed at a later age than classic FAP.

Slide 15:
Genetically, FAP is characterized by mutations in the adenomatous polyposis coli, or *APC* gene. This is an autosomal dominant disorder. *APC* is a gene important in the Wnt signaling pathway. Up to 90% of FAP and fewer than 30% of attenuated FAP cases have detectable *APC* mutations. 50-80% of FAP cases have point mutations leading to protein truncation, while 8-12% have large genomic deletions or duplications. About 20% of patients with FAP have apparently de novo *APC* mutations, with no family history. Some of these mutations may be caused by somatic mosaicism, which may not be detected by sequencing.

Slide 16:
A clinical entity similar to attenuated FAP is *MUTYH*-associated polyposis, or MAP. The *MUTYH* gene is the mutY homologue gene, also written as MYH in some reports. It is a base excision repair gene responsible for DNA damage repair. MAP is an autosomal recessive disorder, requiring germline activation of both alleles. This leads to transversion errors, for example, G/C to T/A, in the *APC* gene. Two mutations, tyrosine165 to cysteine and glycine382 to aspartic acid, are most common in northern European populations with MAP. However, 20% of patients with MAP do not have these two mutations. Clinically, MAP is similar to attenuated FAP, although the number of polyps is usually <100.

Slide 17:
Molecular testing for FAP should always start with a diagnosed family member, or a proband. Sequencing and deletion/duplication detection of the *APC* gene should be performed to identify mutations. If mutation is found, targeted *APC* gene analysis should be performed in other family members. If no mutation is found, other conditions such as MAP may be considered. Mutation carriers or at-risk family members with uninformative genetic test results should be offered annual sigmoidoscopy or colonoscopy starting from age 10-12. As the risks for other tumors are also increased in these individuals, extracolonic surveillance is also recommended.

Slide 18:
Finally, I will briefly talk about hamartomatous polyposis syndromes. These are a group of diseases characterized by the development of multiple hamartomatous polyps at a young age. Affected individuals have increased predisposition to intestinal malignancies. There are four major subtypes listed in this slide. The genes responsible for the genetic basis of these subtypes are listed in the brackets. Molecular testing of these subtypes is available at some clinical laboratories.

Slide 19:
Various molecular methods are used in hereditary colorectal cancer testing. This slide shows example of methods that may be used, and is not meant to be a complete list. For point mutations, allele-specific PCR, real-time PCR, PCR followed by sequencing, and pyrosequencing may be used. To detect large deletions and duplications, southern blot, multiplex ligation-dependent probe amplification, and array CGH may be used. Methylation-specific PCR is used for promoter hypermethylation detection. Some clinical labs also offer next-generation sequencing of multiple genes, usually 13-14, covering all hereditary colorectal cancers.

Slide 20: References

Slide 21: Disclosures

Slide 22: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on “Hereditary Colorectal Cancer.” I am Ping Wang.