COMMENTARY

A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: Meeting Highlights and Bethesda Guidelines

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Hereditary nonpolyposis colorectal cancer (HNPCC) is a distinct autosomal dominant syndrome accounting for approximately 5%–6% of the total colorectal cancer burden with clinical and pathologic features caused by defective mismatch repair genes *(1)*. Germline mutations in hMSH2, hMLH1, hPMS1, hPMS2, and MSH6/GTBP have been identified in affected individuals *(2,3)*. HNPCC is characterized by early-onset colorectal cancer (median age at diagnosis 45 years); right-sided predominance; excess synchronous and metachronous colorectal neoplasms; and an increased incidence of extracolonic neoplasms, including endometrial, small-bowel, gastric, renal pelvis and ureter, and ovarian tumors and skin lesions, such as sebaceous adenomas, carcinomas, and keratoacanthomas *(4–10)*.

In 1991, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer *(11)* established minimal clinical criteria for recruiting HNPCC patients for collaborative studies. These criteria, also known as the Amsterdam Criteria, include the following: 1) at least three relatives with histologically verified colorectal cancer, one of them a first-degree relative of the other two (familial adenomatous polyposis excluded); 2) at least two successive generations affected; and 3) in one of the individuals, diagnosis of colorectal cancer before the age of 50. These criteria were pivotal in identifying kindreds that eventually led to the association of the HNPCC syndrome with germline mismatch repair gene mutations (MMR). However, the criteria do not account for extracolonic cancers or for small kindreds.

On November 11 and 12, 1996, the Early Detection Branch of the National Cancer Institute convened an international workshop in Bethesda, MD, entitled ''The Intersection of Pathology and Genetics in the Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Syndrome.'' The purpose of the workshop was to clarify the role of genetics in the pathology of HNPCC. Discussions centered on genomic instability, multistep carcinogenesis and the role of mismatch repair genes in HNPCC, histopathology of HNPCCs and possible relationships to molecular genetic changes, markers of cell proliferation and their relationship to HNPCC as well as their potential use in early diagnosis and prognosis, and, lastly, clinicopathologic criteria that could lead to the identification of additional HNPCC patients. The keynote speaker was Dr. Alfred Knudson (Fox Chase Cancer Center, Philadelphia), who discussed the tumor spectrum of the HNPCC syndrome. Issues that arose during the workshop are discussed below.

Workshop Summary

Genomic Instability and HNPCC

Genomic instability is a fundamental property of tumor cells. One form of genomic instability results from the malfunction of the DNA mismatch repair system. This instability results in the accumulation of mutations, particularly at simple repetitive sequences called microsatellites, and leads to a phenotype that has been termed the replication error (RER) phenotype or microsatellite instability (MIN).

What is the relationship between MIN and colorectal cancer as it applies to HNPCC? Dr. Manuel Perucho (The Burnhan Institute, La Jolla, CA) discussed the following two pathways for colorectal carcinogenesis: 1) the suppressor pathway, where mutational inactivation of two alleles of tumor suppressor genes is required; and 2) the mutator phenotype pathway, or the microsatellite mutator pathway, which involves the mutational inactivation of two alleles of the same gene that is not a suppressor gene but is a mutator gene, (e.g., a member of the mismatch repair gene family). These two pathways are different,

i.e., they result from mutations in different cancer genes. For example, in colon cancer, p53 and K-ras are mutated in the suppressor pathway, whereas TGF-β receptor and BAX genes are mutated in the mutator pathway. The former pathway leads to a tumor that is generally aneuploid and does not have MIN, whereas the latter pathway results in a tumor that is diploid and has MIN. The cell containing the inactivated tumor suppressor gene has a territorial and growth advantage over the neighboring cells, whereas the cell that contains inactivated mutator alleles does not have any territorial advantage. These mutator genes increase the mutation rate and, therefore, increase the probability of mutations occurring from mutations in the suppressor genes or other cancer genes (e.g., TGF-β-RII or BAX) that have a negative role in cell growth and survival, as well as from mutator genes themselves (e.g., MSH3 and MSH6). Tumors of the microsatellite mutator pathway accumulate hundreds of thousands of somatic mutations (insertions and deletions of one nucleotide or a few nucleotides) in simple repeated sequences or microsatellites. The workshop participants also discussed how to differentiate "true" MIN from clonality. The former underlies the mutator pathway, whereas the latter underlies the suppressor pathway. The distinction between MIN and clonality is even more interesting, because it may also diagnose two apparently mutually exclusive types of genomic instability underlying two distinct molecular genetic pathways for cancer.

When does microsatellite instability occur in HNPCC? Dr. Darryl Shibata (The University of Southern California, Los Angeles) presented a study in which HNPCCs had microsatellite diversity (or variance) increased over time between adenoma, cancers, and interval cancers. MIN or loss of DNA mismatch repair is not a gatekeeper mutation that allows clonal expansion, but it allows the gates to be opened and influences the subsequent gatekeepers that occur, which in turn, influence the final pathway for tumor development. In this pathway, MIN and loss of normal repair allele are early events in HNPCC progression.

Should all colorectal tumors be tested for microsatellite instability? Professor P. Meera Khan (The Leiden University, The Netherlands) reported that only two of 75 sporadic tumors tested with 70 different markers showed the RER phenotype, and none had a germline mutation. He concluded that not all tumors should be tested for MIN. An important finding was that different areas of the tumor show differences in the RER phenotypic patterns.

Can RER-positive tumors be characterized in high-risk families? What are the morphologic events leading to colorectal cancer in HNPCC? Dr. Jeremy Jass (The University of Queensland School of Medicine, Brisbane, Australia) presented data from families who met the Amsterdam Criteria and had at least one tumor with instability in one out of six markers (RER positive) versus those Amsterdam Criteria families with two tumors with no instability in any of six markers (RER negative). The RER-negative families were characterized by tumors in the sigmoid colon or rectum (80%), an expected incidence of multiple colorectal cancers, poor differentiation, and mucinous cancers, as well as a paucity of extracolonic cancers. At first colonoscopy, the ratio of adenoma to carcinoma was seven to one in RER-positive families versus 14 to one in RER-negative families. Jass concluded that these Amsterdam Criteria, RER-negative families may represent families with late onset colo-

rectal cancer, which may be an example of familial colorectal cancer caused by unknown autosomal dominant genes (12).

The morphologic events leading to colorectal carcinoma in HNPCC could be explained by the rapid progression of adenomas, *de novo* carcinomas, or a novel pathway. An alternate molecular pathway involving hyperplastic polyps as precursor lesions was discussed. Pathology slides illustrating the origin of colorectal cancer within a hyperplastic polyp were presented. Other discussions included evidence of clonality consistent with rearrangements of chromosome 1 in hyperplastic polyps, MIN in hyperplastic polyps in both HNPCC and non-HNPCC patients, and TGF-β-RII mutations in hyperplastic polyps. Jass concluded that hyperplastic polyps may be particularly sensitive to the mutator effect and, within the context of HNPCC, may serve as precancerous lesions (13).

Clinicopathologic Aspects of HNPCC

Dr. Shozo Baba (Hamamatsu University, Japan) presented data on germline mutations in families not meeting the Amsterdam Criteria but meeting the Japanese criteria for HNPCC. The Japanese criteria include class A, in which there are three or more colorectal cancers within first-degree relatives, and class B, in which there are two or more colorectal cancers within first-degree relatives, and any of the following: (a) early-onset colorectal cancer (age <50 years), (b) right colon involvement, or (c) synchronous or metachronous colorectal and/or extracolonic cancers (14).

Dr. Thomas Smyrk (Creighton University, Omaha, NE) discussed a distinctive tumor that, in his experience, almost exclusively occurs in a subset of HNPCC patients. He described 23 patients from 17 families. In seven families, mismatch repair (MMR) gene germline mutations were identified. These tumors, which have been described as medullary carcinomas or cribriform carcinomas, are characterized by predominantly undifferentiated cells with a fairly solid growth pattern. Focal mucin production can be demonstrated in some cases. The tumors are cytokeratin positive but chromogranin A negative. Their incidence is less than 0.5% of sporadic colorectal carcinomas (15). However, Smyrk stated that this histologic pattern has been noted in at least 10% of the HNPCCs that he has reviewed.

Dr. Miguel Rodriguez-Bigas (Roswell Park Cancer Institute, Buffalo, NY) presented a retrospective review of the pathologic data obtained from a hospital-based registry on Amsterdam Criteria HNPCC families. The salient points were that there was not a marked right-sided predominance of cancers (51% versus 49%). Forty-five percent of the patients had adenomas, of which 60% were left-sided. Unfortunately, some patient details, such as tumor differentiation, mucin production, number of lymph nodes involved, and other characteristics, were not available in the pathology reports. Overall, the review paralleled the published pathology of HNPCC patients; however, because pathologic data were missing from some records, it was suggested that a standardized pathology form be used systematically to report resected specimen characteristics.

Dr. Patrice Watson (Creighton University) spoke on extracolonic tumors in HNPCC. Endometrial and gastric carcinomas, upper urinary tract transitional cell carcinomas, small-bowel carcinomas, as well as sebaceous adenomas, keratoacanthomas, and sebaceous carcinomas are often associated with HNPCC. How-

ever, these extracolonic tumors are not taken into account by the Amsterdam Criteria. A question that needs to be answered is why these patients seem to develop tumors in specific sites and not in others—genotype–phenotype correlation could contribute to such a selectivity *(16)*.

Dr. Lawrence Burgart (Mayo Clinic, Rochester, MN) presented data comparing histopathology to MIN status. There were 20 of 31 tumors with a cribriform or solid growth pattern that were MIN positive (defined as alterations in >30% of the markers). The positive predictive value for these two histologic specific patterns, the cribriform/solid growth pattern and the signet ring cell pattern, was calculated. Assuming a 15% prevalence of MIN at all sites, these tumors had a 58% positive predictive value. Assuming a 30% incidence of MIN positive tumors proximal to the splenic flexure, the positive predictive value of these patterns was 84%. The negative predictive value at all sites was 91%, whereas it was 82% proximal to the splenic flexure. Burgart also discussed the immuno-histochemistry of mismatch repair gene products in HNPCC done in the laboratory of Dr. Steven Thibodeau *(17)*. In all cases in which expression was altered in either MSH2 or MLH1, there was associated MIN. Eight of 14 tumors with abnormal protein expression had a detectable mutation in the corresponding gene. A mutation in either gene resulted in abnormal expression in all but one case.

Dr. David Sidransky (The Johns Hopkins University, Baltimore, MD) discussed microsatellite alterations found in body fluids and in blood. A panel of microsatellites could be devised to look for markers that can detect either initial expansion or even subsequent clonal expansions, which are thought to be synonymous with cancer.

Dr. Stanley Hamilton (The Johns Hopkins University) led a discussion of the pathology of HNPCC. The following conclusions were reached: 1) Even though HNPCCs are more often poorly differentiated, mucin producing, or of the signet ring cell type, there is no specific histologic type diagnostic of HNPCCs; 2) very often, there is a dense lymphocytic infiltrate and a Crohn's-like reaction; 3) undifferentiated cribriform pattern and signet ring cell carcinoma are histologies that may suggest HNPCCs, especially in young individuals; 4) mucin markers (MUC 2 and MUC 1) could potentially be used to delineate the cell lineage in these tumors; 5) there is little information in the literature with regard to the immunopathologic response in RER-positive tumors; 6) there is a remarkable absence of aberrant crypt foci in HNPCC that supports the concept of rapid progression of the malignant transformation once it develops; and 7) there are no molecular findings that distinguish RER-positive sporadic tumors from RER-positive HNPCCs.

Dr. Bernard Levin (The University of Texas M. D. Anderson Cancer Center, Houston) discussed whether the biologic differences between the right and left colon can account for the predominance of right-sided neoplasms with MIN. Cell surface antigens, such as Lewis A, X, and Y, are expressed differentially in fetal and adult colons as well as in colorectal cancer. There are differences in the metabolic gradients of methylhydrazine, ornithine decarboxylase, and gluthatione *S*-transferase, depending on the site of the colon examined. Chromosomal abnormalities and allelic deletions have been reported to be more common in proximal tumors. Levin concluded by speculating that environmental influences, such as bile acid concentrations and fecal flora, or developmental influences within the colon may be significant in modifying the phenotypic expression of abnormal genotypes.

Dr. Henry Lynch (Creighton University) could not explain the longer survival time in patients with HNPCC when compared with the survival time among patients with sporadic colorectal cancers. However, speculation centered on an enhanced immunologic response in patients with HNPCCs, as evidenced by the Crohn's-like reaction and marked lymphocytic infiltrate. Another possible explanation for the enhanced immunologic response was an increase in abnormal products secondary to the increased number of mutations in RER-positive tumors *(18)*. Also put forth was the theory that, because there is a propensity for RER-positive cells to accumulate mutations, there is a paradoxical effect whereby malignant cells may be eventually burned out secondary to the mutational load *(19)*.

Dr. C. Richard Boland (University of California at San Diego) discussed the biology of HNPCC and implications for treatment. He presented data from several experiments in which chromosome transfer was used to correct DNA MMR deficiency in colon cancer cell lines. The following conclusions were reached: (*a*) agents such as O^6-methylguanine, 6-thioguanine, cisplatin, fluorouracil, and melphalan are tolerated by mismatch repair-deficient cell lines, and (*b*) once the mismatch repair system is restored, critical degrees of DNA damage result in G_2/M arrest. The work in cell lines suggests that a careful examination of HNPCC treated with fluorouracil should be performed, since the laboratory data suggest that these colorectal tumors are resistant to the drug.

Panel Discussion

Criteria for the HNPCC Syndrome

After the workshop, an extensive discussion took place. One of the topics considered was whether there is a specific histopathology that could distinguish HNPCCs from sporadic colorectal tumors. Except for the solid cribriform growth pattern and signet ring cell carcinoma in young individuals, the answer was that there is no specific histopathology for HNPCC.

The next topic of discussion was how to identify potential HNPCC patients who are not identified by the Amsterdam Criteria. More than 90% of the colorectal cancers in HNPCC kindreds show MIN, or the RER phenomenon *(2)*. RER has potential utility as a marker of patients and families who need a more detailed study of germline DNA to identify HNPCC individuals. RER testing alone does not identify all familial cases *(20,21)*. Therefore, it may not be cost-effective to study all colorectal cancers for RER, because of the low incidence of HNPCC in the overall colorectal cancer burden and the low prevalence of RER in cases of sporadic colorectal cancer. After much discussion, criteria were developed for the identification of tumors that should be tested for RER phenomena or MIN and, therefore, aid in the identification of HNPCC patients. These guidelines, called the Bethesda Guidelines (outlined in Table 1) will potentially apply to 15%–20% of the total colorectal cancer burden, which in 1997 in the United States has been estimated to be 19 680 to 26 640 new cases *(22)*. Elements of the Bethesda Guidelines include both criteria for assessing colorectal cancer patterns in families meeting the Amsterdam Criteria *(11)* and several other

Table 1. Bethesda Guidelines for testing of colorectal tumors for microsatellite instability

1. Individuals with cancer in families that meet the Amsterdam Criteria
2. Individuals with two HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers*
3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age <45 y, and the adenoma diagnosed at age <40 y
4. Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 y
5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed at age <45 y†
6. Individuals with signet-ring-cell-type colorectal cancer diagnosed at age <45 y‡
7. Individuals with adenomas diagnosed at age <40 y

*Endometrial, ovarian, gastric, hepatobiliary, or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter.

†Solid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.

‡Composed of >50% signet ring cells.

characteristics reported more frequently in the HNPCC syndromes. These characteristics include right-sided predominance of colorectal cancer, undifferentiated pattern of histopathology (solid or cribriform), signet-ring-cell-type colorectal cancer, and early onset of the disease at age less than 45 years [reviewed in (23)]. In addition, affected individuals have a higher incidence of endometrial, hepatobiliary, ovarian, gastric, small-bowel, renal, or pelvic ureteric carcinomas (8).

Tumors from individuals meeting any of the above criteria, as well as tumors from family members, should be tested. If they demonstrate MIN, these patients will be candidates for germline MMR gene testing. It was emphasized that, in these high-risk individuals, genetic counseling is warranted. It is important to note that RER positivity is not specific for the HNPCC syndrome. In addition, 20% of HNPCC families with germline MMR gene mutations do not meet the Amsterdam Criteria, and some cancer-prone families meeting the criteria may not be due to HNPCC.

RER Testing

The panel discussed at length strategies for RER phenomena or MIN testing. A minimum of four markers should be used, with instability defined as alterations in at least two of four markers. However, extensive discussion on which markers to use followed, and no consensus was reached. The panel recommended that a future workshop be considered, so that the definition of RER or MIN, as well as the markers utilized, can be standardized.

Patient Management

Identifying individuals with germline mutations has implications for surgical management, prognosis, follow-up, and surveillance of colorectal and related extracolonic cancers, as well as for surveillance of HNPCC patients and family members at risk. This group of individuals could serve as a model for chemoprevention strategies that could eventually be extrapolated to the general population.

Future Research

The workshop participants agreed that future areas of research in the pathology of the HNPCC syndrome should include the following: 1) evaluation of the immunohistochemistry of hMSH2 and hMLH1 mutations; 2) further evaluation of markers such as the MUC genes and CK20 gene in colorectal carcinoma to determine if they could serve as screening tools for the diagnosis of HNPCCs; 3) initiatives in terms of immunologic characterization of HNPCCs and host inflammatory response and association with survival; and 4) evaluation of the response of RER-positive tumors to chemotherapeutic agents.

In summary, the following conclusions were made: (a) At the present time, there are no specific histopathologic characteristics that differentiate HNPCCs from sporadic colorectal cancers, with the possible exception of those in young individuals with right-sided undifferentiated solid/cribriform pattern or signet ring cell cancer; (b) in order to identify HNPCC, the Bethesda Guidelines, based on histopathology and family history, should be applied for RER testing of about 15%–20% of the total colorectal cancer burden in the population; (c) standardized methodology for tissue handling, collection, and reporting should be adopted; (d) future areas of research should include the characterization of the immune response in HNPCCs, and the evaluation of markers for the histologic diagnosis of HNPCC neoplasms; and (e) the definition of RER should be standardized.

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Notes