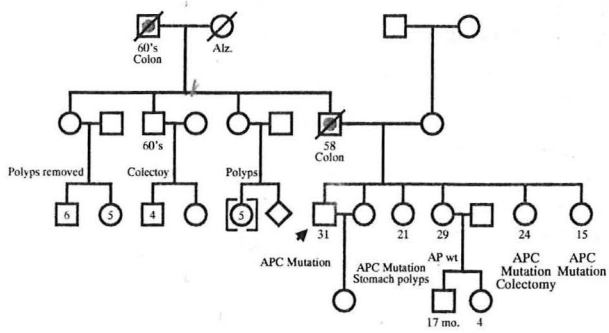
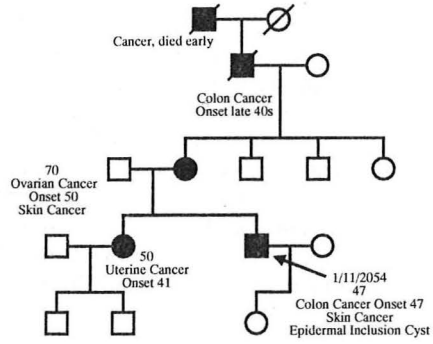
COLORECTAL CANCER SYNDROMES: CLINICAL IMPLICATIONS, DIAGNOSTIC OBLIGATIONS AND CHEMOPREVENTION OBSERVATIONS



FAP Pedigree



HNPCC Pedigree

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Introduction

Colorectal cancer is an important cause of morbidity and mortality in the United States. According to the SEERS data we will have 148,750 new colorectal cancers in the United States for the year 2003 representing the 4th leading cause of cancer and the second leading cause of cancer related death[1]. With the incorporation of chemotherapy, radiation therapy and improvement in surgical techniques, the 5 year survival for patients with diagnosed colorectal cancer has improved in the past 3 decades (Figure 1)[1]. Much progress has been made in understanding the genetic events involved in the development of colorectal neoplasms and well characterized mutations involved in colorectal carcinogenesis has been elucidated[2]. Although the hereditary gene mutations involved in colorectal neoplasms only explain around 6% (Figure 2) of the total patient population with colorectal cancer, it is very important for clinicians to recognize the phenotypic characteristics of these patients since it has implications for patient surveillance and family screening. Moreover many of the same mutations are also present in patients with sporadic colorectal neoplasms.[2] In today's presentation I will describe the known mutations involved in familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC), the clinical consequences of these mutations, the screening recommendations, treatment for patients affected with the mutations and finally the potential chemoprevention strategies for this patient population.

Figure 1

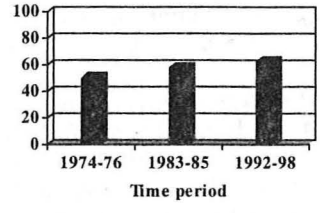
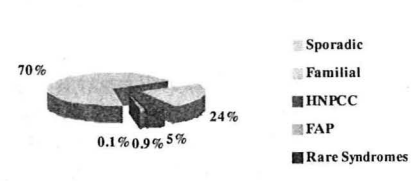


Figure 2



Familial Adenomatous Polyposis Syndromes

Molecular features

The gene responsible for the familial adenomatous polyposis syndrome was localized to chromosome 5q21 by Kinzler and collegues in 1991[3]. One year later, a mouse lineage that exhibited autosomal dominant predisposition to multiple intestinal neoplasia was found to have a missense mutation in the human equivalent of the APC gene[4]. In clinical studies, approximately 80% of patients with FAP have a truncated mutation of the FAP gene. An additional 15% have an inactivating mutation of one allele detected by monoallelic mutation analysis[5]. Gene dosage experiments have estimated that

constitutional 50% decrease in expression of one APC tumor suppressor gene is sufficient for the phenotypic expression of the syndrome[6].

The APC is considered a tumor suppressor with functions that include regulation of cell growth, cell migration, signal transduction, and chromosomal stability[5]. The gene is constituted by 8535 base pairs arranged in 21 exons. Exon 10A is subject to alternative splicing which adds 18 more amino acids to the APC 2843 amino acid protein. In sporadic colorectal neoplasms, the APC mutation tends to occur early in the process of carcinogenesis[2]. Studies performed in the azoxymethane rat model, and preneoplastic tissues obtained from patients, have revealed mutations in the APC gene at the stage from normal to hyperproliferative epithelium (aberrant crypt foci)[7]. Subsequent mutations in other genes such as K-ras, p53, deletions of 18q and others follow conferring the cell its malignant phenotype[2,8,9].

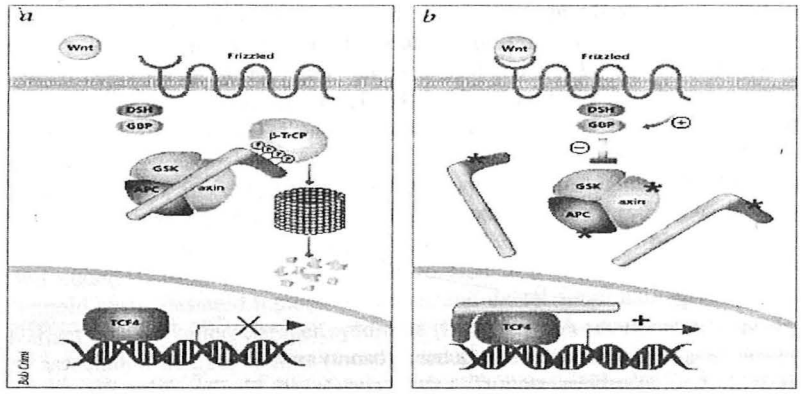
The APC Pathway

Although still incomplete, knowledge of the APC pathways has helped elucidate the mechanisms involved in cellular malignant transformation. The APC protein forms a complex with Axin II and interacts with free b catenin in the cell cytoplasm promoting glycogen-synthetase kinase 3b (GSK3b) phosphorilation of b-catenin[10,11]. When there is a mutation of APC, b-catenin is not degraded[12]. Moreover, b catenin translocates to the nucleus and can interact with tissue coding factor (TCF) and lymphoid enhancer-binding factor 1 (LEF-1), two transcription factors[13,14]. This interaction results in upregulation of genes involved in tumor development such as c-MYC and cyclin D 1. Increases in the level of b catenin can be also enhanced by increasing signaling via the WNT pathway or by loss of cell to cell adhesion and e cadherin dysregulation (figure 3)[14]. The importance of b catenin as a transforming protein was realized with transfection experiments in fibroblast cell lines and subsequent transformation of the cells[15].

A second important event that may explain the chromosomal instability is the realization that the APC protein is involved in chromosomal segregation[16,17]. The protein links the chromosomal kinetochore to microtubules during mitosis via two gene products Bub 1 and Bub 2. In the absence of a functional APC gene product secondary to mutations, there are chromosomal segregation defects that lead to chromosomal instability and an increase in mutation rate, a hallmark of malignancy.

The second event leading to neoplastic transformation depends on the site of mutation in the APC gene. Thus if the mutation is located between exons 1194 and 1392 then the second event that leads to neoplastic transformation is most frequently a loss of heterozygosity. On the other hand, mutations outside of this region will lead to a truncating mutation in the mutation cluster region.

Figure 3



Relationship between specific mutations and phenotypic expression in FAP

The site of mutation in the FAP gene determines the length of truncated protein product and it is related to the resultant phenotypic expression of the syndrome. Most somatic mutations occur in exon 15 with greater than 50% occurring between codons 1286 and 1513 the so-called mutation cluster region[18]. This specific region contributes more than 70% to the open reading frame and is the region involved in binding and down regulating of b-catenin[19].

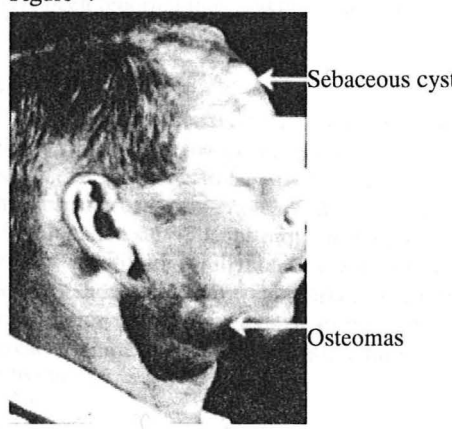
An attenuated variant of FAP is also known as hereditary flat adenoma syndrome[20]. This syndrome is characterized by a small number of colonic adenomas that arise later in life[21]. Although CHRPE is not present, there is increased risk of upper GI lesions. This subtype of FAP arises from germline mutations of the APC gene at the 3' and 5" end region[21]. It is unclear why the truncated proteins from the protein product give rise to FAP. A possible explanation is that the protein product from the mutated allele interferes with the function of the proteins from the normal allele. In the past, the protein truncation assay to test for the mutation could yield false negative results. Currently this test is seldom used when performing molecular analysis to confirm the diagnosis of FAP.

A polymorphysm in the APC gene at I1307k has been detected in the Ashkenazi Jewish population[22]. This polymorphysm appears to create DNA sequences that are hypermutable. This leads to somatic, truncating mutations in adjacent nucleotides. This polymorphism, present in 1 in 16 individuals, is the most common cancer-related founder mutation thus far in the Ashkenazi population. Population based studies have shown that 6% of Ashkenazi's have the mutation, 10% in patients with colorectal cancer and no family history and 28% with colorectal cancer and a family history[22]. The incidence of

the mutations and its presence in patients with colorectal cancer suggest that the penetrance of this mutation is low.

In FAP at least 75% of patients have extracolonic manifestations of the disease[23]. The mutations region seems to correlate with the extracolonic manifestation of the syndrome. Mutations between codons 543 to 1309 are associated with high risk of developing congenital hypertrophy of the retinal pigment epithelium (CHRPE)[24]. Mutations located downstream of codon 1309 are associated with a 6 fold risk for desmoid tumors and mutations between codons 976 and 1067 are associated with a 4 fold risk of duodenal adenomas. Other manifestations of the syndrome include osteoid osteomas, dental abnormalities, intra and extraabdominal tumors, gastric adenomas and other neoplasms such as stomach cancer, adenocarcinomas of the papilla of vater and biliary tree including pancreas, thyroid cancer, hepatoblastoma and medulloblastoma[23]. Mutation of the gene beyond codon 1256 is associated with a constellation of physical findings including epidermoid cysts, desmoid tumors, osteomas, and dental abnormalities which constitute the variant of FAP called Gardner syndrome (figure 4)[25]. A second less frequent variant is the association of CNS tumors (mostly medulloblastomas but also glioblastomas and astrocytomas) with colonic neoplasms called Turcots syndrome[26]. The relationship between genotype and phenotype correlations is not exact and the final phenotypic expression of the APC mutation is modulated by environmental factors (diet) and, presently unknown, modifier genes[27].

Figure 4



Clinical characteristics in Gardner syndrome

Clinical Characteristics

The estimated occurrence of FAP is 1/8,300 to 1/14,025 live births[28]. About one third of the mutations occur spontaneously. Males and females are affected equally and the occurrence of FAP is worldwide. Mutations on the APC gene will invariable lead to the development of hundreds to thousands of colorectal adenomas in the large intestine and virtually 100% of patients will end up developing colorectal cancer at a median age of 40

unless prophylactic colectomy is performed (much earlier than the general population with a median age of colorectal cancer of 63 years)[29,30](figure 5 and 6).

Figure 5

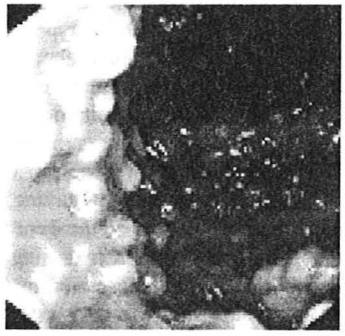
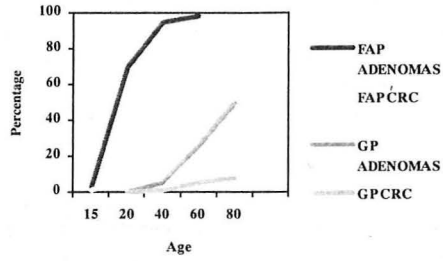


Figure 6



The histological appearance of adenomas in the FAP patients is indistinct from that of sporadic adenomas in the general population[28]. The adenomas can be either tubular tubulovillous or villous on light microscopy. The adenomas are usually found distally in the colon and progress proximally as the disease evolves[28]. Adenomas can start appearing at an early age and cases of adenomas and carcinomas occurring in preadolescent years have been reported thus making it important to start screening patients with a diagnosis of FAP at an early age.

Mutational analysis

The test for mutational analysis should be ordered in individuals who already have the phenotypic expression of the disease (ie presence of greater than 100 adenomas)[31]. The purpose of the test is to identify the mutations so that family members can be screened. In the absence of a mutation in the APC gene, the family member can be spared the recommended surveillance program for patients with FAP. Unfortunately APC mutations are not identified in all families with the classic phenotypic findings of FAP. These families should still have appropriate surveillance procedures recommended for patients with a known mutation. Because of the high incidence of patients with de novo mutations (greater than 1/3 of the patients), a family history is not required to test for affected individuals.

Once a family member is diagnosed with FAP the rest of the family should be tested for the presence of the gene mutation. The results of the test should be discussed with the patient by a multidisciplinary team that includes a geneticist and genetic counselor. It is important for the genetic counselor to have the results of the gene mutation at hand as well as the possible penetrance of the mutation since the penetrance varies from 10-20% for I1307K APC mutation in Ashkenazi Jews to 100 percent penetrance in classic FAP caused by truncating mutations in the APC gene. The test for the mutation analysis is commercially available and involves obtaining a sample of 3cc of peripheral blood. The

mononuclear cells are separated from the rest of the blood and sent to a company for sequence analysis of the gene.

Surveillance

Based on the age of onset of polyps in patients with FAP the current recommendation is to start surveillance on patients at age 12 with a yearly flexible sigmoidoscopy since the polyps tend to occur in the distal colon[32]. Sigmoidoscopy can be decreased to every 2 years by age 25 and 3 years after age 35. After 50 years the patients should follow recommendations for screening average at -risk populations. Surveillance procedures to detect extracolonic neoplasms are directed towards the detection of upper gastrointestinal neoplasms specifically adenomas and carcinomas localized to the ampulla of vater[33].

Treatment

Surgery:

Surgery is the only known intervention that reduces the risk of colorectal cancer[34]. Since the whole colon and rectum are at risk in patients with FAP the recommendation is for patients to undergo a total proctocolectomy. Depending upon the evolution of the syndrome and the number and pathological characteristics of the polyps detected, surgeons might elect to delay colectomy in patients especially in the early teenage years. Some surgeons opt to treat patients with a subtotal colectomy with the understanding that the remaining rectal mucosa is at risk of developing cancer[35]. Close surveillance and frequent biopsies of the rectum in cases of subtotal colectomy are mandatory in order to detect recurrent polyps or early rectal cancer.

Chemoprevention:

In an attempt to delay or prevent polyp formation, investigators have used nonsteroidal anti-inflammatory agents (NSAIDS)[36]. The mechanism of action of NSAIDS in FAP is unclear at present but believed to involve blocking of peroxisome proliferator delta (PPAR delta)[37]. PPAR delta is a ubiquitously expressed member of ligand activated transcription factors that also include PPAR alfa and gama[38]. PPARs bind to sequence specific DNA response elements as heterodimers with the retinoic acid receptor. Prostaglandin I2 (PGI2) and its metabolite carbaprostacyclin can up regulate PPAR delta. PPAR delta is believed to have antiapoptotic functions in the cells[37]. Thus blocking the COX 2 enzyme with COX 2 inhibitors will decrease production of PGI2, carbaprostacyclin and down regulation of PPAR delta thus facilitating apoptosis.

The most widely studied agent has been sulindac, a nonspecific nonsteroidal anti-inflammatory agent that targets both COX 1 and 2 enzymes. Initial reports on a small number of patients with FAP showed regression in the number and volume of polyps[36]. Based on this small report, clinicians have reported use of sulindac in patients with FAP. Unfortunately after long term follow up and in spite of initial regression of polyps, there has been clear recurrence of polyps and the development of rectal adenocarcinomas in spite of close follow-up in this patient population[39]. More recently cyclooxygenase 2 inhibitors has been employed in patients with documented FAP syndrome in a small phase III study[40]. In this study, patients were randomized either to placebo, low dose celecoxib (100 mg PO BID) or high dose celecoxib (400 mg PO BID). The high dose

celecoxib arm had a statistically significant reduction in the number and volume of polyp formation compared to the control and to the low dose celecoxib arm. Based on these results, the FDA approved celecoxib for use in patients with FAP.

Attempts at improving the results of the COX 2 trial have stemmed on the observation that alternative pathways are also aberrantly signaled in colorectal neoplasms. One of the pathways involves signaling via the epidermal growth factor receptor. This receptor is overexpressed and the signal is abnormal in at least 80% of colorectal neoplasms[41]. This aberrant signal leads to change in the behavior of the cell thus increasing proliferation, invasion, angiogenesis and metastasis along with inhibition of apoptosis[42,43]. Therefore combinatorial chemoprevention might enhance the therapeutic effect of the COX 2 inhibitors. This concept has been tested in min mice and proven to be effective[44]. By combining a COX and EGFR inhibitor, the investigators were able to decrease polyp formation in mice by 97% even when using 25 to 50% of what would be considered a therapeutic dose for both agents. This model will be explored in a phase I/II study at our institution in patients with a diagnosis of FAP. Hopefully we will be able to induce polyp regression in this patient population, delay time to colectomy at a reduced risk of side effects by using lower doses of a COX 2 agent and EGFR inhibitor. Nonetheless the encouraging results with NSAIDS and its more specific COX 2 derivatives must be tempered by the fact that use of sulindac in patients with a molecular diagnosis of FAP in a randomized study failed to delay polyp formation or time to colectomy[45].

Colonic neoplasms with normal germline APC gene

A recent report studied a Welsh family with multiple colorectal adenomas and a recessive pattern of inheritance[46]. The patients' neoplasms had an increase in the somatic mutation rate which consisted of a substitution in the thymine-adenine pair for the guanine cytosine pair in the APC gene, changes typical of oxidative DNA damage. This finding led investigators to perform mutation analysis of the enzymes involved in the repair of DNA induced by oxidative damage.

There are 3 genes responsible for the repair of the oxidative damage: MTH1, OGG1 and MYH[47]. These 3 genes interact and prevent mutagenesis induced by the by product of oxidative damage, 8-oxo-7,8 dyhydroxy-2 deoxyadenosine. MYH removes adenine mispaired with 8-oxo-7,8 dyhydroxy-2 deoxyadenosine or guanine, MTH1 converts 8-oxo-7,8 dyhydroxy-2 deoxyadenosine triphosphate to monophosphate and OGG1 detects and removes 8-oxo-7,8 dyhydroxy-2 deoxyadenosine incorporated into DNA. When the genes are defective, there is an increase in hyper mutability of other genes including APC and b-catenin.

A recent study in individuals with personal or family history of large number of colonic polyps but no germline mutation in the APC gene revealed a mutation frequency in the MYH gene of 7.5%for patients with the classic polyposis syndrome[48]. When only the patients with 15 to 100 adenomas were considered, then 29% of the patients had biallelic mutations of the MYH gene.

Clinically these patients manifest their disease with a slower onset of disease progression as compared to FAP or H NPCC[48]. Similar to FAP, they have extracolonic manifestations of the disease such as CHRPE and duodenal adenomas. The polyps resulting from a mutation of the MYH gene have similar pathologic characteristics to those of patients with FAP.

Testing for MYH gene mutation should be considered for individuals who have personal or family history of polyposis and a recessive pattern of inheritance. Patients should have regular close surveillance for their colon and upper gastrointestinal tract once a mutation in the MYH gene is confirmed. Some patients will need to have prophylactic colectomy if the polyposis cannot be controlled with polypectomy.

Hereditary Non Colorectal Cancer Syndrome

Initial studies of HNPCC date back to 1913 when Dr Alfred Warthin reported on the high incidence of uterine and gastrointestinal cancers in the family of his seamstress[49]. Dr Henry Lynch subsequently studied this association of colorectal and extraintestinal adenocarcinomas in two extended kindreds[50]. This autosomal dominant disease later became known as the Lynch syndrome or HNPCC. Currently there are two sub classifications of this syndrome; families with site-specific colorectal cancer or Lynch type I syndrome, or families in with colonic and extracolonic adenocarcinomas called Lynch II syndrome[51]. Epidemiological studies have revealed that this syndrome accounts for about 5% of the colorectal cancers in the general population.

Molecular features

Errors of DNA replication during S phase are predicted to occur at a frequency of 10^3 to 10^4 base pairs[52]. The DNA polymerase keeps the fidelity of the DNA replication process in check by excising the mispaired nucleotide from the new DNA strand and replacing it with the correct nucleotides. If the DNA polymerase fails to correct the mismatch defect, then the mismatch repair complex detects and corrects the defect[53]. The system corrects both single base pair mismatches (i.e. AG to AT) and mispaired loops of DNA resulting from replication errors in microsatellite tracts.

The mismatch repair complexes involves the formation and interaction of a complex structure constituted by genes from mutS (hMSH2, hMSH3, hMSH6) and mutL (hMLH1, hMLH3, hPMS1 and hPMS2)[54,55]. The presence of a mismatched defect is first detected by hMSH2 were it binds to DNA at the site of the mismatch defect. Depending on the type of mismatch repair defect present, hMSH2 binds to either hMSH6 (for single base pair mismatch defects) or to hMSH3 (for 2 to 8 nucleotide insertions or deletions). A second complex constituted by hMLH1 and hPMS2 is subsequently recruited to excise the mismatch areas in the DNA. Both hMLH1/hPMS1 and hMLH1/hMLH3 complexes have also been detected but presently their role in the mismatch repair process remains undefined. Table 1 delineates the characteristics of the genes involved in the mismatch repair process.

Table 1

Gene	Chromosome location	Function	Mutation frequency	Comments
hMSH2	2p16	DNA MMR	++++	Represents 35% of cases
hMLH1	3p21	DNA MMR	++++	Represents 60% of cases
hMSH6	2p16	DNA MMR	++	Late onset CRC + endometrial cancer
hPMS1	2q32	DNA MMR	+	One kindred w germline mutation
hPMS2	7p22	DNA MMR	+	Two kindreds w germline mutation
hMLH3	14q24.3	DNA MMR	?	Missense mutation
EXO1	1q42-32	Exonuclease Interacts with MSH2	?	Missense mutation
hMSH3	5q11-13	DNA MMR	0	No germline mutations identified so far

Initial studies in yeast revealed similarities between microsatellite instabilities observed in tumors to that of bacteria with mutations in mismatch repair genes such as mutS and mutL[56]. Further evidence that mutations in mutS and mutL resulted in mismatch repair defects and carcinogenesis was provided by experiments in which extracts of tumors with microsatellite instability were found to be deficient in mismatch repair activity in-vitro[57,58]. Moreover, transfer of a human chromosome with an intact copy of hMLH1 into a human cancer cell line with a mutant MLH1 restored the mismatch repair activity and reversed microsatellite instability[59]. The search for homologues of mutS and mutL in humans resulted in the discovery of at least 6 genes (hMSH2, hMSH, hMLH1, hPMS1, hPMS2 and possibly hMLH3) involved directly or indirectly in the DNA mismatch repair process. Although mutations of any of the 6 genes can lead to the development of HNPCC, mutations in hMLH1 and hMSH2 are the most frequent and are found in approximately 80% of the patients with HNPCC[60].

Mutations in the genes involved in the DNA mismatch repair process determine the degree of mismatch repair dysfunction present in the complex. For example mutations in either hMSH2 or hMLH1 result in high degree of microsatellite instability (MSI-H phenotype)[61]. On the other hand, mutations in genes like hMSH6 result in only partial dysfunction of the complex[62]. When only one allele carries the mutation, the phenotypic expression of the disease might not be evident. The mutation or inactivation of the second allele increases the risk of the phenotypic expression of the disease. These findings are in keeping with Knudson's 2 hit hypothesis whereby patients inherit a mutated allele and the second allele is mutated after exposure to environmental carcinogens. It is important

to note that missense mutations with single amino acid substitutions and negligible functional consequence on the protein make up to 35% of hMLH1 gene alterations[63].

The consequences of a mutation in the mismatch repair process are what have been known as the mutator phenotype where cells are unable to repair the mismatches in DNA[56]. This leads to an accumulation of growth regulatory gene mutations in the cell and malignant transformation. In other words the MMR defect is not tumorigenic per se but sets the stage for further mutations in specific genes that deregulates gene function. Specific genes that are known to undergo mutation with the MSI-H phenotype include receptors for growth factors, regulators of cell cycle and regulators of apoptosis[64-66]. In the specific case of mutations in hMLH6 the mutator phenotype is MSH-L[62]. In this case the cells have an increased frequency of point mutations in genes known to be involved in colorectal carcinogenesis such as the APC gene[67,68]. It is possible that there are MSI-independent mechanisms that lead to malignant transformation of cells. Recently yeast cells with a defect in mismatch repair were still able to proliferate in spite of lacking telomerase[69]. This finding suggests that an increase in chromosomal recombination compensates for a lack of telomerase in these cells.

Clinical Characteristics of HNPCC

The age of onset of colorectal cancer in patients with HNPCC is around 45 years, a much younger age than that of the general population (age 63 in the US)[51]. These patients have an 80% lifetime risk of developing colorectal cancer[51]. The initial lesion is a flat adenoma that is located in the right side of the colon in 70% of the patients[51]. This adenoma progresses to adenocarcinoma at an accelerated pace with an estimated time to progression of 2 to 3 years[70].

The suspicion of a patient harboring HNPCC is helped by assessing the patient's age of onset of the disease, a positive family history of certain malignancies and location of the neoplasm. The clinical characteristics of the tumors in HNPCC has been summarized into the Amsterdam criteria: 1) Three or more relatives with histological verified colorectal cancer one of whom is first degree relative of the other 2) Colorectal cancer involving at least 2 generations 3) One or more colorectal cancers diagnosed before the age of 50[71]. Some experts have considered these criteria to be too strict so they developed a modified version of the Amsterdam criteria (called the Bethesda criteria) that in addition to the Amsterdam criteria would also include extracolonic manifestations of the syndrome such as: 1) Persons with two types of HNPCC-related cancers (includes synchronous or metachronous cancers) 2) Persons with colon cancer and a first degree relative with colon cancer and/or HNPCC associated extracolonic cancer and /or adenoma (cancer < 45 years of age and adenoma <40 years of age) 3)Persons with colon or endometrial cancer before the age of 45 4) Persons with right sided colon cancer with undifferentiated pattern on histology before the age of 45 5) Persons with colonic cancers that have signet ring features and occur before the age of 45 6) Persons with colonic adenomas that occur before the age of 40[72].

The pathology of the colorectal carcinoma is quite distinct[73,74]. The lesions have an intense inflammatory response with prominent presence of tumor infiltrating

lymphocytes. Additional characteristics include high production of mucin, a poorly differentiated histology, pushing borders of the tumor instead of infiltration, and high frequency of diploid cells (as compared to high frequency of aneuploidy detected in sporadic tumors). In addition there appears to be less frequency of tumors metastasizing to the lymph nodes as compared to sporadic neoplasms. This distinct pathologic characteristic of colorectal neoplasms in HNPCC is not seen in extra-colonic manifestations of the disease.

The spectrum of phenotypic expressions in patients with HNPCC almost invariably involves neoplastic proliferation of colonic lesions. Additionally these patients are at risk of developing adenocarcinomas in other organ sites such as the stomach, ovaries, small bowel, bladder, brain, kidneys, renal pelvis, biliary tract and endometrium[52]. The higher incidence of endometrial cancer in women with HNPCC is associated with mutations in hMSH6[58,75]. These patients also have a tendency to have a late onset of colorectal carcinoma as compared to other HNPCC mutations[76]. It is important to realize for surveillance purposes that a mutation in hMSH6 patients might first lead to endometrial adenocarcinomas before colorectal neoplasms develop; thus a colonoscopy should be part of the screening procedures for these patients. The reports on increased incidence of breast cancer in patients with HNPCC are equivocal at present. Table 2 illustrates the lifetime risk of developing colonic and extra colonic cancer in patients with HNPCC as compared to the general population[52].

Table 2

Type of Cancer	HNPCC	General Population
Colorectal	80%	5%
Endometrial	60%	3%
Gastric	13%	1%
Ovarian	12%	2%
Small bowel	4%	0.01%
Bladder	4%	3%
Brain	4%	0.6%
Kidney/renal pelvis	3%	1%
Biliary tract	2%	0.6%

Genetic analysis

Commercially available genetic testing is available for hMSH2, hMLH1 and hMSH6. These 3 genes represent around 80% of the mutations found in patients with HNPCC[60]. Since there are no genetic hot spots for mutations in HNPCC full sequencing of the gene is required[63]. The mutation product usually includes truncating, frameshift and missense mutations.

The approach to testing is to first evaluate the proband (patient) for the mutation[52]. If a specific mutation in the gene is found then one proceeds to test the rest of the family

looking for mutations in the same spot as the proband. If there is no mutation found in the family members, then it should be assumed that the family member has the same risk as the general population. This way the family member can be spared the intense surveillance measures in place for individuals with the mutation.

In patients with family clustering of colorectal cancer, the frequency of mutations in hMLH1 or hMSH2 is 25 to 34%[77,78]. The incidence is higher in patients who fulfill the Amsterdam criteria, 39 to 45%[78]. Since investigators are unable to detect a mutation in greater than 50% of this patient population, the implications are that there are unidentified genes involved in the neoplastic transformation or that the techniques used are not sensitive enough to detect mutations in the mismatch repair genes. A recent technique was developed to analyze alleles separately and avoid the masking effect of the normal allele on the mutated allele by converting diploid to haploid cells[79]. This technique was employed in 10 patients with a clinical diagnosis of HNPCC but no evidence of mutations present by sequence analysis. The investigators were able to show presence of mutations in all 10 patients by analyzing the alleles separately. Although promising, this technique is not commercially available and can only be performed in highly specialized laboratories.

A different approach to define at risk population is to perform a screening test to determine the status of micro satellite instability using 5 different markers as defined by the US National Cancer Institute[71]. This approach will increase the yield in the mutation detection rate. In patients who are MSI H (at least 2 of 5 markers detected) the frequency of mutations detected will be 38-73%, in MSI L (1 of 5 markers detected) the mutation detection rate in MSH6 will be 22% and in microsatellite stable the mutation detection rate is only 3-8%.

An alternative approach is to determine the level of mutated protein present in the tumor by performing immunohistochemistry (IHS) directed against the 2 most common mutations, hMLH1 and hMSH2[80]. As a consequence of hMLH1 or hMSH2 gene mutation the protein product will be absent by IHS with close to 100% concordance to MSI –H results. A few mutations can lead to persistence of the protein product but it is believed to occur rarely (<8% of samples). This method is simple to implement, less costly than MSI testing and readily available for use in pathology labs. In addition, the method can be used as a screening tool and genetic analysis performed on patients with absence of the protein.

At least on third of patients with the mutator phenotype do not have any family history of malignancy[81]. The majority of these patients have de novo mutations. This finding has implications in the way we approach patients with no family history but suspected HNPCC. These patients should all be offered testing for the common mutations and professional counseling for the patient and his family.

Surveillance

Based on the natural history of the disease, the currents recommendations for surveillance in families with HNPCC include a colonoscopy performed every 1 to 2 years beginning

at age 20[51]. Women should have annual gynecologic exam including transvaginal ultrasound and endometrial aspirate. Guidelines for other extracolonic manifestations of the disease depend on the phenotypic expressions for the specific kindred. For example, families with a high incidence of gastric adenocarcinomas would undergo yearly upper GI endoscopy in addition to colonoscopy as part of their surveillance program.

Current recommendations have proven to be effective in improving the overall outcome in patients with HNPCC. Surveillance programs will detect neoplasms at an earlier stage of disease (Dukes stage A 50% vs. 15%, stage B 35% vs. 50%, stage C 15% vs. 16% and stage D 0% vs. 19%)[82]. In addition there is a gain in life expectancy with surveillance programs of 13.5 years and with proctocolectomy of 15.6 years at age 25[83]. This benefit gradually diminishes with later age of surveillance initiation with little benefit noted after age 40.

Prognosis

In spite of the poorly differentiated histology, patients with HNPCC who eventually develop colorectal cancer have lower mortality rate independent of tumor stage when compared to sporadic colorectal cancer. Patients who develop colorectal cancer and have involvement of lymph nodes (stage III disease or Dukes stage C) have improved recurrence-free survival compared to non-HNPCC patients (90% vs. 32%)[84]. These results are supported by a second study showing similar results in patients with stage III patients with MSI-H tumors who received 5FU based chemotherapy (Elsaleh H Clinical Cancer Research 2001;7:1343-9)[85]. A possible explanation for this improved survival is that mutations necessary for cell survival accumulate in the cell initiating a self-destructive program.

Chemoprevention strategies for patients with HNPCC

The role of chemoprevention for patients with HNPCC is not well established. There are some pre-clinical indications that NSAIDS might help delay the phenotypic effects of HNPCC. Cell lines with hMLH1, hMSH2, and hMSH6 exposed to ASA and sulindac showed a marked reduction in microsatellite instability[86]. This effect was time and concentration dependent, appeared independent of proliferation rate and cyclooxygenase function. In MSH2 knockout mice, ASA did not modify the mutator phenotype but weakly extended survival in mice but in APC and MSH2 knockout mice, ASA significantly delayed onset of intestinal and mammary neoplasms[87].

In clinical studies expression of COX 2 by immunohistochemistry has been assessed in patients with HNPCC. The expression COX 2 was less prevalent and of lesser intensity in patients with HNPCC (67%) compared to sporadic colorectal cancers or colorectal cancers in the setting of FAP (92-100%)[88]. Based on this preliminary data, investigators have proposed use of COX 2 inhibitors as part of chemoprevention strategies for patients with HNPCC but currently there are no completed clinical trials evaluating the benefit of NSAIDS as a chemopreventive agent for patients with HNPCC.

Sporadic Colorectal Cancer and its relationship to FAP and HNPCC

The molecular events that lead to sporadic colorectal cancer are believed to be similar to those events involved in the FAP population. These events in sporadic colorectal cancer involve early mutations in the APC gene in approximately 80% of tumors with subsequent mutations involving 6 or more genes (such as p53 and k-RAS) as originally proposed by Vogelstein and colleagues[9,89]. Mutations in the APC gene lead to chromosomal instability in addition to accumulation of b-catenin in the cytoplasm. The resultant of mutations in APC is loss of tumor suppressor genes and mutation of proto-oncogenes that eventually lead to tumorigenesis. Of the approximately 15% of tumors that do not have a mutation in the APC gene, half will have a mutation in b catenin at exon 3, the site involved with b catenin coupling to the APC protein[90]. As a result, b catenin will not be degraded leading eventually to carcinogenesis.

In approximately 10-15% of sporadic tumors there will be a defect in the genes involved in the DNA mismatch repair process[91]. The majority of the alterations will be at the level of hMLH1 gene silencing via methylation[92]. In addition MSI-L is more frequent in sporadic colorectal neoplasms compared to HNPCC tumors. Pathologically the tumors from sporadic colorectal cancer with defects in MMR genes have similar characteristics to those of patients with HNPCC; tendency of tumors to occur proximately, large mucinous component, diploid tumors, presence of infiltrating lymphocytes and improved survival. Thus the end result of the MMR gene dysfunction will be the same whether there is presence of mutations (more frequent in HNPCC) or of gene silencing (more frequent in sporadic colorectal neoplasms) and cells will continue to accumulate additional mutations that will lead to a colorectal neoplasm.

Conclusions

Careful laboratory studies in patients with the two most frequent colorectal cancer syndromes have lead to significant progress in understanding the molecular events involved in carcinogenesis. This understanding of the molecular events has translated into powerful laboratory techniques that help detect mutations at an earlier stage of the disease. At present we have only one established method to reduce the frequency of colorectal cancer in patients with FAP and HNPCC, prophylactic colectomy. Unfortunately this intervention is mutilating and leads to a permanent colostomy. Moreover surgery does not address the significant risk of neoplasm at other organ sites.

Chemoprevention strategies are still at an early stage of development with both disease entities but with knowledge acquired in molecular biology and more specific chemopreventive strategies we might be able to delay or even stop the process of tumorigenesis in FAP and HNPCC. These same chemopreventive strategies might also be employed in the future for patients with sporadic polyposis and in secondary chemoprevention after the diagnosis and treatment of colorectal cancer to reduce the risk of tumor recurrences.

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