Molecular genetic alterations in gastrointestinal polyposis syndromes: with emphasis on the Peutz-Jeghers syndrome
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Chapter 3

Molecular and Phenotypic Markers of Hamartomatous Polyposis Syndromes in the Gastrointestinal Tract

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KEY WORDS: Polyposis; Hamartoma; FAP; Cowden; Peutz-Jeghers; Genotype; Phenotype; Juvenile polyposis

ABBREVIATIONS: Gastrointestinal (GI); Familial Adenomatous Polyposis (FAP); Adenomatous Polyposis Coli (APC); Mismatch Repair (MMR); Colorectal Cancer (CRC); Deleted in Colorectal Cancer (DCC); Deleted in Pancreatic Cancer (DPC4); Hereditary Non-polyposis Colorectal Cancer (HNPCC); Juvenile Polyposis Syndrome (JPS); Hereditary Mixed Polyposis Syndrome (HMPS); Peutz-Jeghers Syndrome (PJS); Cowden’s Disease (CD)

SUMMARY

Hamartomatous gastrointestinal polyposis syndromes have always been considered as non-neoplastic. Nevertheless, an increased cancer risk both within and outside the gastrointestinal tract may exist in these syndromes. The hamartomatous polyps may sometimes harbor dysplasia, but their neoplastic potential is unknown.

The genetic defects causing the hamartomatous syndromes are less well established than, for example, familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). The genetic studies on the Mendelian inherited syndromes FAP and HNPCC have made a major contribution to the identification of genes involved in colorectal tumorigenesis. The genes involved in colorectal cancer development may also contribute to cancer development in the hamartomatous polyposis syndromes, and are currently under investigation. Furthermore, new insights into the development of various cancers may be obtained by the isolation and characterization of genes involved in Mendelian inherited hamartomatous polyposis syndromes.

This report summarizes the available literature on this subject, and describes the pheno- and genotypic features of the hamartomatous syndromes of juvenile polyposis, Peutz-Jeghers syndrome, and Cowden’s disease.

INTRODUCTION

Several genetic disorders are associated with an increased susceptibility for colorectal or extra-colonic gastrointestinal (GI) malignancies. These inherited GI disorders can be subdivided into polyposis syndromes and non-polyposis syndromes (Table 1). Most are inherited in an autosomal dominant fashion. The phenotypic hallmark of the polyposis syndromes is the occurrence of multiple polyps, which can be subdivided histopathologically into adenomatous and hamartomatous types. An adenoma is defined as a macroscopically visible lesion that carries a dysplastic mucosa and is therefore, by definition, neoplastic. In contrast, a hamartoma is non-neoplastic and is considered as a congenital malformation composed of tissue elements normally present at that particular site. Polyps found in familial adenomatous polyposis (FAP) patients are adenomas and predispose to colorectal cancer with virtually 100% certainty.
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### TABLE 1 Inherited Polyposis and Non-Polyposis Syndromes Associated with (Extra-) Gastrointestinal Carcinomas

<table>
<thead>
<tr>
<th>Polyposis syndromes</th>
<th>hamartomatous syndromes</th>
<th>chromosomal location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile polyposis</td>
<td>10q23.3, 18q21</td>
<td></td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>19p13.3</td>
<td></td>
</tr>
<tr>
<td>Cowden’s disease</td>
<td>10q23.3</td>
<td></td>
</tr>
<tr>
<td>Bannayan-Zonana syndrome</td>
<td>10q23.3</td>
<td></td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>5q21</td>
<td></td>
</tr>
<tr>
<td>Gardner’s syndrome</td>
<td>5q21</td>
<td></td>
</tr>
<tr>
<td>HMPS</td>
<td>6q</td>
<td></td>
</tr>
</tbody>
</table>

**Non-polyposis syndrome**

<table>
<thead>
<tr>
<th>Inherited non-polyposis colorectal carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMSH2</td>
</tr>
<tr>
<td>hMLH1</td>
</tr>
<tr>
<td>hPMS1</td>
</tr>
<tr>
<td>hPMS2</td>
</tr>
<tr>
<td>hMSH6</td>
</tr>
</tbody>
</table>

(1). FAP is caused by a germline mutation in the adenomatous polyposis coli (APC) gene. APC encodes a large protein of 2843 amino acid residues, organized in several domains with different functions. Mutation analysis showed somatic mutational ‘hot-spot’ regions (2). In the Ashkenazi Jewish population, a unique APC germline sequence at codon 1307 is observed which leads to a hypermutable spot (3). There is increasing evidence that different classes of APC mutations correlate with a specific phenotype. When FAP is accompanied by extracolonic manifestations such as sebaceous cysts, osteomas or desmoid tumors, it is referred to as Gardner syndrome. The Turcot syndrome is applied to the combination of brain tumors and adenomatous polyps, and can be caused by either germline mutation of the APC gene or by altered mismatch repair (MMR) genes, discussed below.

Tumor progression in FAP follows a similar pathway as the adenoma-carcinoma sequence in sporadic colorectal cancer (CRC). In addition to the APC gene, the ras oncogenes, the deleted in colorectal cancer (DCC) gene, deleted in pancreatic cancer (DPC4) gene, and the p53 gene are the major genetic factors in this progression model that follows a preferential order (Table 2). The APC gene, located on chromosome 5q21, is a tumor suppressor gene. Somatic APC mutations have been found in early adenomas. APC protein modulates levels of β-catenin, a downstream effector of the Wnt pathway (4). APC is thought to play an important role in colonic and extra-colonic tissue proliferation. The cytoplasmatic proto-oncogene ras family has signal transduction functions, leading to growth advantage.

### TABLE 2 Oncogenes and Tumor Suppressor Genes in Colorectal Carcinogenesis Pathway and in FAP and HNPCC

<table>
<thead>
<tr>
<th>Normal epithelium</th>
<th>APC (FAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative epithelium</td>
<td>K-ras</td>
</tr>
<tr>
<td>Early adenoma</td>
<td>DCC/DPC4</td>
</tr>
<tr>
<td>Intermediate adenoma</td>
<td>p53</td>
</tr>
<tr>
<td>Late adenoma</td>
<td>(HNPCC)</td>
</tr>
</tbody>
</table>

Activating mutations of ras oncogenes are frequently found (approximately 50%) in CRC. The DCC and DPC4 genes are both located on chromosome 18q21, a region commonly lost in CRC. DCC encodes a cellular adhesion molecule. Expression of DCC is thought to maintain cell adhesion. Mutation and allelic loss of DCC is frequently found in late stages of colorectal carcinoma development. DPC4 (also known as SMAD4) encodes an important cytoplasmatic mediator in the TGF-β (transforming growth factor) signaling pathway (5). The most common event in human and colorectal tumorigenesis is mutation and allelic loss of the p53 tumor suppressor gene on chromosome 17p. p53 Is considered the guardian of the genome, and the...
p53 protein prevents the cell cycle from progressing from G1 to S-phase to allow DNA repair (6). When DNA damage leads to inaccurate DNA repair, p53 can induce cellular apoptosis (6). Mutations of the p53 gene lead to loss-of-function and tumor progression. Hereditary non-polyposis colorectal cancer (HNPCC or Lynch syndrome) is caused by mutations in one of several MMR genes (7). Affected family members develop colon cancer at a young age, mostly in the proximal colon, and the tumors have a typical biology and characteristic microscopic features. The tumors are not accompanied by numerous colorectal polyps and, in some families, malignancies in other organ systems also occur at an increased frequency in patients carrying the genetic alteration (7). Four MMR genes, hMSH2, hMLH1, hPMS1 and hPMS2, responsible for HNPCC, have been identified. The gene products of these MMR genes and the hMSH6 gene (8) form protein complexes which recognize, excise and repair mismatched sequences on newly synthesized strands of DNA in a coordinated fashion. The precise role of the new MMR gene, hMSH3, in the repair mechanism is as yet not fully unraveled. Mutations in MMR genes are also found in sporadic GI tumors. Because of their benign histological nature, the polyps of patients with hamartomatous polyposis syndromes have always been considered as non-neoplastic. However, several hamartomatous polyposis syndromes are thought to have an increased risk for carcinoma development both inside and outside the GI tract and, on rare occasions, neoplastic (dysplastic) foci are found in hamartomatous polyps. However, the risk of malignancy in these hamartomatous polyposis syndromes is less well defined than in FAP and HNPCC. Recently, a number of underlying genetic defects causing GI hamartomatous polyposis syndromes have been identified (9-11). Meanwhile, several genes involved in CRC development and identified through the genetic studies of HNPCC and FAP (Table 2) are also under investigation in hamartomatous polyps, because a similar progression as in the adenoma-carcinoma sequence may also contribute to the dysplasia-carcinoma sequence in hamartomatous polyps. Further, the isolation and characterization of genes involved in hamartomatous polyposis syndromes may provide new insights in the development of various cancers.

This report provides a summary of our current knowledge of the geno- and phenotypic features of the most well known hamartomatous polyposis syndromes in the GI tract.

**JUVENILE POLYPOSIS**

Juvenile polyposis syndrome (JPS) is characterized by the occurrence of multiple juvenile polyps in the GI tract. The number of juvenile polyps found in a patient with JPS distinguishes the inherited syndrome from sporadic juvenile polyps; this number ranges from three to hundreds in the familial syndrome. The JPS polyps are hamartomatous lesions predominantly found in the colon but also at other sites of the GI tract. The polyps typically have edematous mucosa, cystically dilated mucus-filled crypts, an abundant mesenchymal stroma, and superficial erosion. Most affected individuals present in early childhood with rectal bleeding, anemia and anal prolapse of a polyp. Patients with JPS are at an increased risk for colorectal cancer. The risk is ill defined but may be increased three-fold compared with an unaffected patient of similar age. The polyps can show dysplastic foci and are potentially premalignant. There is evidence for genetic heterogeneity in JPS. Subsets of families have mutations in PTEN located at 10q23.3 or in DPC4 located at 18q21 (9,12). Interestingly, the molecular change causing JPS may be harbored in the subepithelial mesenchymal stroma, and not in the epithelium (13). This corresponds with the microscopic observation that the proliferation may be limited to the stromal component and that the epithelial changes are primarily due to mechanical insults and inflammation. In a study of 46 juvenile polyps of 8 patients, collected from the Johns Hopkins Polyposis Registry, 14 polyps harbored dysplastic foci (14). Immunohistochemical analysis in JPS polyps of p53 and p21 protein, which mediate the p53 tumor suppressor pathway, showed that the p53 tumor suppressor pathway can also be disrupted in JPS polyps, especially when these lesions are dysplastic (14). Moreover, in the majority of the dysplastic polyps, there were alterations in the topographical distribution of the progenitor zone and terminally differentiated cells, similar to the changes found during the adenoma-carcinoma sequence. These results
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indicate that the molecular genetic cascade leading to CRC in patients with JPS may correspond to the conventional one. On the other hand, cytogenetic study in 4 JPS polyps showed a normal karyotype, which contrasts with the abnormal karyotypes in most sporadic and FAP colorectal neoplasms (15). Other malignancies in the digestive tract of JPS patients at a young age have also been described. For example, in the JPS pedigree from Iowa, with germline SMAD4 (DPC4) mutations, a pancreatic cancer in addition to several CRCs was also observed (12). Hereditary mixed polyposis syndrome (HMPS) may be a variant of JPS, but its phenotype appears distinct from JPS. HMPS is ill defined and is characterized by atypical juvenile polyps, colonic adenomas and carcinomas. HMPS has been mapped to chromosome 6q through linkage analysis (16). Further studies are needed to identify the genetic basis of JPS and to explore the route leading to neoplastic growth in these patients.

PEUTZ-JEGHERS SYNDROME

Peutz-Jeghers syndrome (PJS) is an autosomal dominant condition characterized by multiple GI hamartomatous polyps. The polyps have a distinct histopathology and are found primarily in the small intestine but may also occur in the colon and stomach. PJS patients usually have melanin pigmentation of the lips, buccal mucosa and, occasionally, of the hands and eyelids. Pigmentation, seen in the first years of life, varies per individual and can fade or disappear in late adolescence. The clinical presentation of PJS includes colicky abdominal pain due to intestinal obstruction and anemia caused by GI bleeding. Histopathologically, the epithelium of the polyps consists of the components which are normally found at that particular site of the GI tract; there is abundant stroma with smooth-muscle proliferation in an arborisation-like pattern. Furthermore, pseudo-invasion is often present; very rarely is dysplasia seen in PJS polyps. The smooth-muscle proliferation and pseudo-invasion may be due to mechanical forces, since these features can sometimes be encountered in other GI polyps. Although neoplastic transformation apparently occurs infrequently in PJS polyps, patients with PJS have an increased risk for developing intestinal and extra-intestinal carcinomas at a relatively young age (17). The extra-intestinal carcinomas can include some unusual forms, including ovarian sex-cord tumor with annular tubules, adenoma malignum of the cervix, Sertoli cell tumor of the ovary and feminizing Sertoli cell tumor of the testes. Life-expectancy of patients with PJS is significantly less than the general population and they often die from cancer-related death (17). A recent study showed a relatively higher cancer risk for female PJS patients (18).

The genetic defect causing PJS has recently been identified. The mutated gene LBK1/STK11 encodes a serine/threonine kinase and is located at 19p13.3 (10). The physiologic function of this protein is as yet unknown. Interestingly, mutational inactivation of a protein kinase in a cancer susceptibility syndrome has not been previously described. Not all PJS families seem to link to 19p13.3. Linkage to 19q has been reported in a PJS family (19), and a case has been described of a karyotypic analysis in a patient with PJS which showed a pericentric inversion on chromosome 6 (20). The 19q locus and the breakpoints of this inversion (inv(6)(p11.2;q25.1)) are potential loci for alternative mutated genes predisposing to PJS.

We have recently observed that a PJS polyp harboring dysplasia also carried an activating K-ras codon 12 mutation. Interestingly, however, this mutation was not found in the dysplastic epithelium of the polyp but in the non-dysplastic epithelium after microdissection of the polyp and analysis of these 2 areas separately. The implication of this finding is unclear but it questions the role of K-ras in dysplasia formation in hamartomatous PJS polyps. Similarly, in the colorectum aberrant crypt foci may carry ras mutations in the absence of dysplasia (21). We have not observed other K-ras codon 12 mutations in a large series of additional PJS polyps. Thus, these mutations appear to be rare events in PJS polyp formation, in contrast to adenomatous polyps. In two PJS polyps a normal karyotype was noted, again contrasting with adenomatous polyps (15). In some PJS polyps with dysplasia, we have seen overexpression of the p53 protein, and studies of mutations of the p53 tumor suppressor gene are in progress. Further analysis of chromosome 5q, 17p and 18q will also help to elucidate the neoplastic potential of PJS polyps and clarify whether tumor
progression in that event follows the conventional pathway to CRC.

COWDEN'S DISEASE ("Multiple Hamartoma Syndrome")

Cowden's disease (CD) or multiple hamartoma syndrome is associated with GI polyposis and characterized by multiple hamartomas of the ectodermal, endodermal, and mesodermal origin. The syndrome is associated with GI polyposis and the conventional pathway to CRC.

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