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Biomarkers in familial adenomatous polyposis: role and significance

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1. ABSTRACT

A biomarker, according to a generally accepted definition, is a substance or a manifestation used as indicator of a biologic state. It has the characteristic to be objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacological responses to therapeutic interventions. Biomarkers are important tools available to the clinicians with escalating perspectives in oncologic field. Clinical and genetic biomarkers are essential to properly individuate the disease, to address patients to specific surveillance programs and therapeutic strategies. An ideal biomarker should be absent in normal tissue/condition but present in precancerous lesions like dysplasia and so able to recognize early cancer. Coming from these considerations, several of the known genetic pathways in cancer pathogenesis could be considerate potential candidate biomarkers. In this review, we have reported clinical and molecular biomarkers helpful to manage the Familial Adenomatous Polyposis (FAP), a dominantly inherited colorectal cancer predisposition syndrome. Biomarkers, both clinical and molecular, are essential to reduce the high potential morbidity of FAP giving the opportunity to develop innovative diagnostic and therapeutic protocols.

2. INTRODUCTION

Familial Adenomatous Polyposis (FAP, OMIM N175100) is a dominantly inherited colorectal cancer predisposition syndrome in which hundred to thousands of precancerous colonic polyps (adenomas) and extracolonic manifestations and/or neoplasms (tumours) are variably present. FAP is generally caused by germline inactivating mutations in the Adenomatous Polyposis Coli gene (APC) at 5q21, which encodes a protein of 2843 aminoacids (1). APC is a tumour suppressor gene, member of the WNT pathway. Normally, the WNT pathway leads to changes in gene expression profile; in fact, APC is able to form a multiprotein complex with glycogen synthesis kinase-3ß and axin, and to bind ß-catenin, which in turn is phosphorylated by glycogen synthase kinase-3ß and subsequently degraded by the proteasome. If APC is mutated, the multiprotein complex could not be formed and, therefore, ß-catenin accumulates into the cytoplasm and then translocates to the nucleus, where it activates the T-cell factor, which in turn causes transcription of target genes, influencing different cellular processes such as cell migration, cell cycle control, differentiation and apoptosis (2).

Table 1. classification of FAP severity[1]

	Phenotype	No. of colorectal adenomas	Age of onset
Classical (n. >100)	Profuse Intermediate	Thousands Hundred to thousands	1st and 2nd decade 2nd and 3rd decade
Attenuated	Attenuated	< 100	4th and 5th decade

[1] (Adapted with permission from 5)

Table 2. Spigelman Classification for duodenal polyposis in FAP

Criterion	1 point	2 point	3 point
Polyps number	1-4	5-20	> 20
Polyps size (mm)	1-4	5-10	> 10
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Stage 0, 0 points; stage I,1-4 points; stage II, 5-6 points; stage III, 7-8 points; stage IV, 9-12 points

APC gene is considered at high penetrance activity so, patients carrying a germline mutation, if not adequately treated, have theoretically the 100% of risk to develop at early age a colorectal cancer.

The standard prophylactic approach is still surgical. Generally a total colectomy (extended to the rectum in specific pathological conditions) is required to interrupt the sequence from adenoma to cancer and frequent endoscopic screening of the individuals at risk is mandatory from the age of 10-14 years. However, it is imperative to have the best risk estimation and to submit to endoscopy only individuals that with high probability could develop colorectal lesions (1).

Biomarkers, by definition, are important tools for the clinicians and their role is even more fundamental. A biomarker is a substance or a manifestation used as an indicator of a biologic state. It is a characteristic that can be objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacological responses to therapeutic interventions. Clinical and genetic biomarkers are essential to individuate correctly the disease, to address patients to specific surveillance programs and to opportune therapeutic strategies. An ideal biomarker should be absent in normal tissue/condition but present in precancerous lesions like dysplasia and so able to recognize early cancer (early stages) This is the rationale for the utilization of several of the known genetic pathways involved in the pathogenesis of cancer as candidate biomarkers (3).
For FAP syndrome, we can divide the known biomarkers in two categories: clinical and molecular.

3. CLINICAL BIOMARKERS

The most important clinical biomarker in defying and classifying FAP syndrome is represented by the presence and the number of large bowel polyps. The polyps, at least hundreds, are located in the colon and rectum, typically adenomatous and less than 1 cm size, peduncolate or sessile, with tubular, villous or tubulo-villous histology (4).

Profuse polyposis is defined as severe polyposis with over than thousand polyps and young age of onset (first and second decades of life). The average age of onset of colorectal cancer is approximately 34 years (4).

I the classical and sparse phenotype patients develop hundred to thousands of colorectal adenomas in their second decades of life. Mean age of colon cancer in untreated individuals is about 40 years. At last, in the attenuated phenotype (AFAP) patients, generally older than twenty years, present less than 100 polyps and cancer onset is delayed (Figure 1) (4).

Although classifying FAP phenotype, according to the number of adenomas, may seem arbitrary, it might be useful for directing genetic testing, estimating colorectal cancer risk and to define the right therapeutic approach. It is important to notice that grouping the different phenotypes can be difficult, in fact it is often impossible to count the exact number of polyps especially if they are very small. Therefore, generally the classification is simplified considering attenuated polyposis if polyps are less than 100 and without any other affected family member with 100 or more adenomas, and classical if they are more than 100, as reported in Table 1 (5). Currently, there is a lack of consensus regarding the exact diagnostic criteria that should be used for AFAP. Nielsen *et al.* (6) propose the following diagnostic criteria for AFAP: no family member with more than 100 polyps before the age of 30 years and at least two individuals with 10 to 99 adenomas diagnosed after the age of 30 years or one individual with 10 to 99 adenomas diagnosed after the age of 30 years and a first-degree relative with colorectal cancer with few adenomas. This proposed definition takes into account the phenotype variability seen in AFAP (i.e. some individuals may have ≥100 polyps at a later age, although the most have <100 polyps) (7). One limitation in the proposed criteria is that APC mutation status is not taken into account.

The gastrointestinal tract can be also affected in FAP: duodenal adenomas, particularly water papilla and gastric polyps seem to be important and typical manifestations of FAP subjects and so it is not wrong to consider their presence as a peculiar clinical biomarker (8).

At least 60% of FAP patients develop duodenal adenomas on the ampulla or in the periampullary region and age appears to be the most important risk factor, because they become to be more evident after the third decade of life (9). The severity of duodenal polyposis is assessed using Spigelman classification (Table 2). This system describes five (0-IV) stages. Points are accumulated for number, size, histology and severity of dysplasia of polyps. Stage I (1-4 points) indicates mild disease; stage III

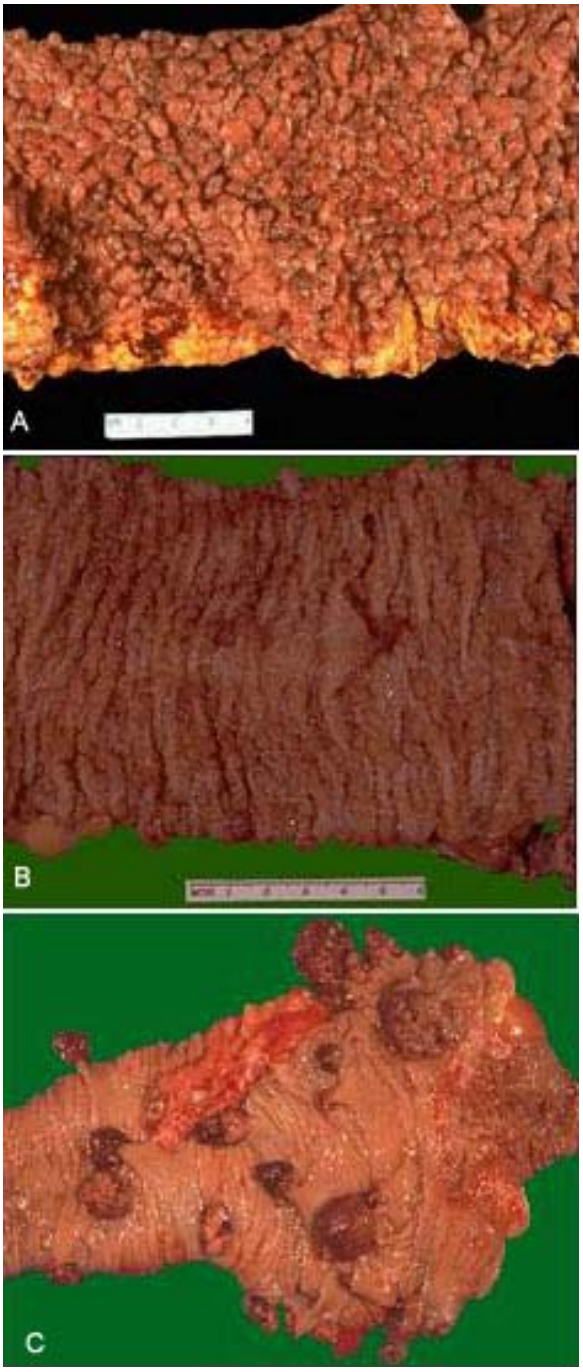

Figure 1. The variability of FAP phenotype: a) severe polyposis b) classical polyposis c) attenuated polyposis.

and IV (> 6 points) implies severe duodenal polyposis. Almost 80% of the patients have stage II or III, and 10-20% have stage IV (9). Recently, Saurin and colleagues reported a 43% cumulative risk for developing stage IV duodenal polyposis after 60 years and 50% by age 70 (10).

The adenoma-to-carcinoma sequence of colorectal neoplasia is evident also in upper gastrointestinal polyps. Different studies have demonstrated slow progression of duodenal polyps in size, number and histology: the risk for duodenal cancer as 36% for Spigelman stage IV, 2% for stages III and II and 0% for stage I polyposis, however the early identification of such patients is important to address them to specific intensive surveillance and early treatment (9).

Also gastric polyps are diagnosed in FAP patients with rates as high as 81% to 84%. Most are fundic gland polyps with histological features including cystic dilatation, irregular budding of the fundic gland and foveolar epithelial dysplasia (in 25% of cases), but cancer is rare. Gastric adenomas can be found, but less frequent than fundic gland polyps. Furthermore, there is no evidence linking gastric polyposis and gastric cancer in FAP patients (4).

Other important clinical FAP-related manifestations are represented by benign lesions. The most common is the congenital hypertrophy of the retinal pigment epithelium (CHRPE). It refers to discrete, flat, pigmented lesions of the retina that are not age dependent and do not cause clinical problems. Visualization of CHRPE may require examination of the ocular fundus with an indirect ophthalmoscope through a dilated pupil. Observation of multiple or bilateral CHRPE may be an indication that an at-risk family member has inherited FAP, whereas isolated lesions may be seen in the general population (11). Other benign lesions include osteomas of the skull and mandible, dental abnormalities such as supernumerary and impacted teeth, multiple epidermoid cysts and lipomas (4, 12). Although not thoroughly studied, a statistically significant association between adrenal masses and FAP has been reported. Adrenal masses are found in 1-3% of the general population; a retrospective analysis identified adrenal masses in 7,4% of individuals with FAP (13) and a proscpective study of 107 individuals with FAP found in 13% an adrenal mass greater than or equal to 1 cm on abdominal CT scan. Most of these masses appeared to be adenocortical adenomas without endocrinopathy or hypertension (14). It is important to recognize in patients these benign alterations, in fact their presence can be used as markers to identify high risk subjects, particularly in FAP families without a known APC mutation.

FAP patients are also associated with desmoid tumours, one cause of morbidity and the first cause of death for extracolonic manifestations in FAP (4, 15). Desmoid tumour is a rare clonal fibroblastic proliferation that may arise in abdominal or extra-abdominal sites (deep soft tissues), characterized by infiltrative growth and a high risk of local recurrence even after complete surgical excision. Although desmoids can arise from fibroblasts throughout the body, intra-abdominal desmoids are most frequent in FAP with several and important complications like obstruction of the small bowel or ureters, occlusion of mesenteric blood vessels, thrombosis of larger veins and compression of peripheral nerves (15). The causes of desmoids are attributed to surgical trauma, hormonal exposure or to genetic alterations that bring to WNT pathway deregulation. The risk of desmoid development

Table 3. frequency of different FAP manifestations in affected patients[1]

Extracolonic manifestations	Frequency
Duodenal Adenomas	90%
Osteomas	80%
Papillae Adenomas	75%
CHRPE	70%
Epidermoid Cysts	53%
Benign Fundic Polyposis	51%
Dental Anomalies	38%
Desmoid Tumors	12%
Thyroid Carcinoma	1%
Hepatoblastoma	0,5-1%
Medullablastoma	0,5-1%

[1](Adapted with permission from 5)

seems to be particularly related to mutations in specific region of the APC gene, beyond codon 1444 (5). Several studies reported a significantly higher prevalence of desmoid tumours in females than males (15).

Thyroid cancer, hepatoblastoma and brain cancer have also been associated with FAP. Also pancreatic adenocarcinoma, intraductal papillary neoplasm, mucinous pancreatic tumors and high-grade pancreatic intraepithelial neoplasia have been reported in the FAP disease spectrum (4).

It is important to notice that these malignancies as well as desmoids could not be considered as specific clinical FAP biomarkers, but important FAP-related aspects that could help in identifying FAP subjects, in fact, often their diagnosis can occur before that of the large bowel adenomas, giving good reason to suspect a diagnosis of FAP.

4. MOLECULAR BIOMARKERS

As previously described, FAP is generally caused by germ-line inactivating mutations in the APC tumour suppressor gene (1). FAP patients inherit one germline mutation and develop tumors from those cells in which a second hit or loss of other allele of APC is somatically acquired (4, 5). The identification of APC germ-line could be considered the most important molecular biomarker in identifying a FAP subject. APC is a large gene with 15 exons. Truncating mutations in the APC gene on chromosome 5q was demonstrated to be the cause of the vast majority of FAP cases (4). It's important to notice that somatic mutation of the APC gene were demonstrated to be early events in 60% to 80% of sporadic adenomas or colorectal cancer and for this reason APC is evident to play a key role in colorectal carcinogenesis not only in genetic hereditary related patients (16).

Actually, more than 825 germline mutations have been identified (database: http:// perso.curie.fr/Thierry.Spossi/APC.html). The vast majority of germline mutations in the APC gene result in a truncated non functional protein (17). For this kind of deregulation the protein-truncation assay was originally used to detect truncated protein products using DNA and RNA from peripheral blood lymphocytes. Additional diagnostic techniques include conformation-sensitive gel electrophoresis, denaturing HPLC, denaturing gradient gel electrophoresis, single-stranded conformational polymorphism and sequencing of the coding region (4).

Besides common truncating mutations in FAP, a non-truncating missense mutation, caused by a nucleotide substitution in the gene I1307K was described in approximately 6% of the Ashkenazi Jewish population, and seems to be related to an increased risk of colorectal adenomas or cancer in heterozygous carriers. This variant seems to cause an impairment of the APC protein and to increase rate of somatic APC mutation in carriers. However, the lifetime risk of developing cancer is only 10% to 15 % in carriers and the age of onset is not significantly different in carriers compared to the general population (18). Generally, APC mutational hotspots are located at codons 1309 and 1061. Because of the accumulation of APC germline mutations from codon 1250 and 1464, this region is termed mutation cluster region (MCR) (4, 5). The most frequent APC germline mutation at codon 1309 represents 'only' the 16% of overall mutations. Substantially, FAP is a disease characterized by the involvement of only one gene (APC) but with a large variety of mutations. Different authors indicate that the type of APC germline mutation can also determine the nature of the second hit (Kundson theory) (19), an important and fundamental step to the carcinogenesis and to the phenotypical appearance. If a germline mutation occurs between codons 1194 and 1392, there is a strong selection for loss of heterozygosis as the second hit in the development of colorectal adenoma. If the germline mutation is outside this region, the second hit is likely to be a truncating mutation in the MCR (19, 20).

In several studies an association between the location of APC mutation and the phenotype in FAP patients was described (Figure 2) (5). For this reason, it is possible to hazard that APC germline mutation could be considered not only a diagnostic tool discriminating FAP patients from patients not affected by FAP, but also a prognostic tool about the development and the progression of the disease. In fact, number of adenomas, age of onset and occurrence of extracolonic manifestations seem to be strongly correlated with specific APC mutation sites (5, 21). Each particular extracolonic manifestation usually affects a fraction of FAP patients and patients can also show a sort of heterogeneity further reflected in a familial heterogeneity (Table 3). Studies have shown that genotype-phenotype correlation can be strongly related to the position of the inherited mutation of the APC gene, thus, at the present could be an important approach to better cope FAP disease (5).

A relation between a truncating mutation between codons 1250 and 1464 and a classical profuse type of polyposis was observed; codon 1309 mutations are particularly associated with severe polyposis and with early onset (5, 21). Diagnosis and mortality for colorectal cancer in patients with 1309 mutation is on the average 10 years earlier compared to FAP patients with other mutations. APC mutations are located between codon 157 (exon 4) and codon 1595 (exon 15) (excluding the Mutation Cluster

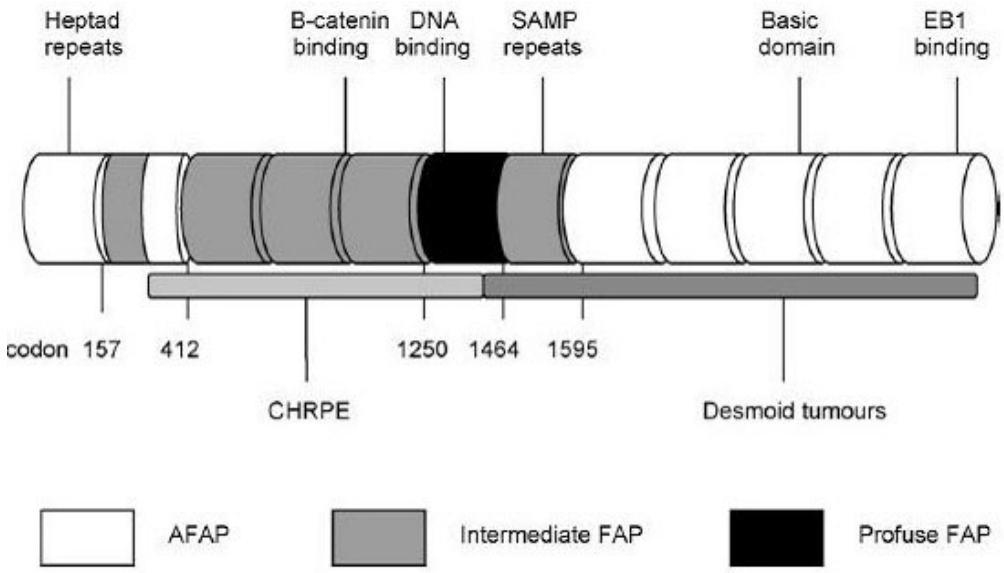

Figure 2. Structure of APC and related disease (Adapted with permission from 5).

Region) seem to be correlated to an intermediate or attenuated phenotype (5, 21, 22).

For the upper gastrointestinal tumours that, as we have just affirmed, is common in classical FAP as well as in attenuated FAP, different locations of APC mutations have been indicated related and some studies suggested mutations at the 3' end, beyond codon 1395 (5). Exon 4 and codons 564-1465 seem to be associated with this type of extracolonic manifestation too, in particular to gastric and duodenal polyps (23, 24, 25). However, actually, the relationship between germline APC genotype and the severity of upper gastrointestinal polyposis is controversial or not well defined (5).

The occurrence of CHRPE, present in 3 of 4 FAP patients, is generally related to a specific distinct region of the APC gene between codon 311 and codon 1465 (23, 26). Giardiello *et al.* and Gebert *et al.* demonstrated that CHRPE status could be used to direct APC mutation analysis to a specific region of the gene, concluding that this combined molecular and clinical screening was an efficient strategy for identifying APC germline mutations (27, 28).

Other benign lesions can occur in FAP patients: osteomas, dental abnormalities, epidermoid cysts and lipomas are often present many years before colorectal adenomas development, but their specificity can be quite low because they are not strictly correlated with FAP but also present in healthy individual of general populations. Clinical relevance and genotype-phenotype correlation of these extracolonic manifestations have not been well established because not always deeply evaluated and reported (5).

The occurrence of desmoids in FAP patients is linked to APC mutations, generally at the 3' end , downstream codon 1400 (5). Bertario *et al.* indicates that mutations between codons 1310 and 2011 were generally associated with a six-fold risk of desmoid tumors relative to the low-risk reference region (159 to 495) (21). This correlation for other authors does not appear always to be consistent. Mutations beyond codon 1400 are often associated to other extracolonic manifestations (5, 24, 25).

Moreover, as previously described there is an increased risk for other malignancies too, including thyroid cancer, hepatoblastoma and brain tumors, but genotype-phenotype correlations have not been estabilished (5).

The mutations located at 5' end of the APC gene, within exon 9 and at the 3' distal end appear to be associated with an AFAP phenotype (< 100 adenomas) (25), but the specific limits are different in referring literature. Several studies have shown a great variability of mutations located in the AFAP regions of APC. It might be noticed that some authors consider uncertain the significance of genotype-phenotype correlation and have questioned whether a better understanding of APC mutations would influence therapeutic decisions, that should be instead strictly based on clinical features (5). Some advise prophylactic colectomy with ileorectal anastomosis instead of restorative procto-colectomy with ileal-pouch anal anatomises (IPAA) for patients with a mutation before codon 1250 or for patients with a mutation in codon 0-200 or beyond 1500, while others caution that decisions should be based only on the clinical findings (1).

If genotype is taken in consideration to support therapeutic approaches, it is important to become aware that in 30-50% of patients with FAP or AFAP phenotype no APC germ-line mutations are found (21). In 10 – 15 % of not-mutated patients with classical FAP large genomic deletions were described; to detect them can be very difficult if a standard molecular testing is applied. These

defects are not reported in AFAP cases, explained by the fact that large gene deletions may cause a more complex and severe phenotype (29).

Another polyposis-causing gene was detected on chromosome 1p33-34, the mutY homolog (E. coli) (MUTYH) gene (OMIM n. 608456). Mutations in this gene have been found to be associated with milder form of polyposis. MUTYH germ-line mutations are related to an attenuated phenotype and have been reported in 10-30% of patients without an APC mutation (4). For these reasons it could be considered another important biomarker in identifying polyposis and in particular AFAP patients. Recent studies have also demonstrated that germ-line MUTYH mutations predispose to colorectal cancer with an autosomal recessive pattern, accounting for up to 1% of these neoplasms. In this setting, biallelic MUTYH mutations have been found to be associated with a 93-fold excess risk of colorectal cancer, with almost complete penetrance by 60 years of age. Interestingly, in up to one third of these patients, no associated adenoma was found (1, 4). In contrast, the influence of monoallelic MUTYH mutations on colorectal risk remains controversial, although recent studies suggest a modest effect (30) The MUTYH gene encodes a member of the base excision repair system. This system is composed of 3 enzymes (MUTYH, OGG1, and MTH1) that contribute to protect cells against the mutagenic effects of aerobic metabolism. MUTYH is a DNA glycosylase, which acts at a third level of defense, and is responsible for the removal of adenines mispaired with 8-oxoguanine, one of the most mutagenic DNA products of oxidative DNA damage. Failure to correct these mispairs leads to somatic G:C→T:A transversions in target genes, namely, APC and KRAS. Somatic G:C→T:A transversions in the APC gene were described and in addition, G:C→T:A transversions in the KRAS gene were also observed in adenomas from AFAP patients (31).

Although AFAP patients have milder disease, starting later in life, it should be considered that colorectal cancer was frequently found in these subjects and so they need strict surveillance programs like classical FAP patients, to reduce risk of developing cancer. Some reports have identified cases of upper gastrointestinal adenomas/polyps also in AFAP patients, so also upper gastro-intestinal tract needs controls (32).

4.1. COX-2: a strategic molecular biomarker for therapy?

Actually, prophylactic total colectomy and endoscopic surveillance represents the most effective policy for FAP and AFAP patients (1).

However, the research of biomarkers as target for pharmacological therapies as suggested by pioneer studies on sulindac, represents an important alternative way tool for local control of the disease and the management of serious extracolic manifestations as desmoids tumours (33). The inflammatory cascade is the theoretical interpretation of a possible role of the nonsteroidal anti-inflammatory drugs (NSAIDs). The cyclooxygenase-2 (COX-2) is the inducible form of the COX enzyme family, whose members regulate the conversion of arachidonic acid to prostaglandins, which play critical roles in a large number of biological processes including immune function regulation, kidney development, reproductive biology, gastrointestinal integrity and inflammatory processes. COX-2 is overexpressed in various cancer tissues, and it has been found that its activity contributes to tumorigenesis by inhibiting apoptosis, stimulating angiogenesis and invasiveness, and modulating cell proliferation by increasing the expression of growth factors (33, 34). COX-2 overexpression was detected in colorectal adenomas of FAP patients as well as in AFAP patients APC or MUTYH associated (35). It is also well known as a major pharmacologic target in chemoprevention and its inhibition by NSAIDs seems to reduce the risk of developing cancer (33, 34). Celebrex (Pfizer), a selective COX-2 inhibitor, is demonstrated in a prospective randomized trial to reduce the number and the size of adenomas in FAP and AFAP patients (36) confirming that anti-COX drugs can have an effective implication on treatment. However, management guidelines of these patients indicated NSAIDs drugs as a therapeutic option that can be offered only in peculiar situations and never as alternative to the prophylactic surgery (1).

COX-2 was recently suggested as target in Desmoids' treatment too (37). Control of this important extracolonic manifestation in FAP patients, one of the most important causes of death after prophylactic surgery is a challenge for oncologists because it is a sort of iatrogenic disease, result of an effort to prevent the carcinogenetic, otherwise inexorable, transformation of large bowel adenomas. Surgery and radiotherapy are currently the principal modes of treatment, but some resections may be mutilating (and not resolutive) and radiotherapy has several drawbacks (38). Recently, there have been reports of responses to the oral kinase inhibitor Imatinib (Novartis), which are mediated by the inhibition of PDGF receptor ß (PDGFRB) kinase activity rather than KIT (39). In our previous studies we suggested that aggressive fibromatosis is characterized by WNT/oncogene pathway alterations triggering the COX-2–mediated constitutive coactivation of PDGFRA and PDGFRB, and may therefore be suitably treated with a combination of NSAIDs and tyrosine kinase inhibitors (40).

5. CONCLUSIONS

In conclusion, FAP disease is not difficult to recognize when the classical phenotype, characterized by hundred to thousands of large bowel adenomas, is present. FAP clinical management instead can be difficult in terms of therapy and surveillance due to the high penetrance of APC mutations, the large spectrum of possible extracolonic manifestations and the modality of hereditary transmission. FAP biomarkers are essentially represented by the presence of adenomas and the detection of APC or MUTYH germline mutations. While clinical features of the adenomas as number (> or < 100) and histology have a direct impact on the diagnosis and therapy, APC or MUTYH mutations principally select high risk subjects to

address to specific and intensive surveillance programs. However, genetic tests, in peculiar situations, can strongly influence also therapy; for example, APC mutation at codon 1309, due to its correlation with an aggressive phenotype, demands an early proctocolectomy to prevent colorectal cancer development at young age.

6. PERSPECTIVES

Futures researches on biomarkers will be addressed:

- to better define a correlation between genotype and phenotype, to determine a tailored risk profile management in particular for extracolonic neoplasms as UGI carcinoma.

- to select a subset of FAP patients to submit to chemoprevention treatment (i.e. NSAIDs).

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