Role of p53 in different colorectal carcinogenesis pathways

Is it relevant for clinical practice?

Papel da p53 nas diferentes vias de carcinogénese colo-rectal

Qual a sua relevância para a prática clínica?

Maria Luísa Noites Sacramento

Dissertação para obtenção do Grau de Mestre em

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Orientador: Prof. Doutora Maria Paula Guerreiro Chaves Pascoal

Co-orientador: Prof. Doutora Isadora Alexandra Luz Rosa

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Dedicatória

Dedication

Aos meus pais e irmã, por me ensinarem perseverança.

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Resumo

A p53 é uma proteína que regula o ciclo celular, a apoptose e a estabilidade genómica. Pensa-se que seja responsável pela progressão desde neoplasia intraepitelial (displasia) para neoplasia invasiva, nos cancros esporádicos. Na Doença Inflamatória Intestinal (DII), o cancro colo-rectal (CCR) pode surgir como resultado do estado de inflamação crónica ou pode ser um evento esporádico, evoluindo de adenomas (lesões precursoras). Nestas duas vias distintas, as mutações da p53 ocorrem em estádios diferentes. Em doentes com cancro associado a colite, a mutação da p53 é um evento precoce. Em contraste, na carcinogénese esporádica, a mutação da p53 é um evento tardio no processo. Será proposto um projeto de um estudo com o objetivo de analisar lesões de uma coorte de doentes com DII e displasia/CCR seguidos no Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG). Será usada Imunohistoquímica para avaliar a expressão da p53; – lesões endoscopicamente ressecáveis classificadas como adenoma-like (controlos) serão comparadas com lesões não-adenoma-like (casos). Usando a análise imunocitoquímica da p53 poderá ser possível distinguir entre CCR esporádico e cancro associado a colite, no contexto de DII. Esta distinção tem implicações quer para a terapêutica, quer no prognóstico destes doentes.

Palavras-chave

Proteína p53; vias de carcinogénese; doença inflamatória intestinal; cancro colo-rectal; displasia; adenomas.

Resumo alargado

A proteína p53, codificada pelo gene *TP53*, é uma proteína que regula o ciclo celular, a apoptose e a estabilidade genómica. Quando há mutação desta proteína, a carcinogénese é facilitada e ocorre acumulação de proteínas p53 mutadas. Pensa-se que a p53 seja responsável pela progressão de displasia até cancro colo-rectal (CCR) nos cancros esporádicos. A Doença Inflamatória Intestinal (DII), caracteriza-se por um estado de inflamação crónica da mucosa intestinal que facilita a acumulação de proteínas mutadas como a proteína p53, contribuindo para um defeito de campo. Esta acumulação progressiva facilita o aparecimento de CCR, que tende a ser mais frequente no contexto de DII, nomeadamente nos casos com atingimento do cólon e de longa duração.

Na DII, o CCR pode assim surgir como resultado do estado de inflamação crónica ou pode ser um evento esporádico, evoluindo a partir de adenomas (lesões precursoras). Como os adenomas esporádicos são comuns na população em geral, eles são também lesões expectáveis em doentes com DII. Nestas duas vias distintas, as mutações da proteína p53 ocorrem em estádios diferentes. Em doentes com cancro associado a colite, a mutação da p53 é um evento precoce na sequência displasia-CCR. Em contraste, na carcinogénese esporádica, a mutação da p53 é um evento tardio no processo adenoma-carcinoma. Além disso, as mutações da proteína p53 na neoplasia associada a DII podem ser encontradas mesmo em mucosa sem displasia, pois toda a mucosa intestinal afetada pela inflamação apresenta um defeito de campo, com risco de transformação neoplásica. No CCR esporádico, as lesões precursoras apresentam-se como adenomas e normalmente são únicas, ou separadas por mucosa normal (com p53 *wild-type*).

Será proposto um projeto de estudo com o objetivo de analisar lesões de uma coorte de doentes com DII e displasia/CCR seguidos no Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG). Será usada Imunohistoquímica para avaliar a expressão da p53; – lesões endoscopicamente ressecáveis classificadas como adenoma-like (controlos) serão comparadas com lesões não-adenoma-like (casos). Usando a análise por imunocitoquímica da proteína p53 poderá ser possível distinguir entre lesões desenvolvidas no contexto de CCR esporádico e lesões desenvolvidas no contexto de carcinogénese associada à DII. Perante uma lesão displásica secundária à DII, o risco de outras lesões síncronas ou metacrónicas é muito mais elevado do que no contexto esporádico, pelo que a resseção endoscópica da lesão pode não ser uma terapêutica suficiente.

Abstract

p53 is a protein that regulates cell cycle, apoptosis and genomic stability. It is thought to be responsible for the progression from dysplasia to colorectal cancer (CRC), in sporadic cancers. In Inflammatory Bowel Disease (IBD) patients, CRC can occur as a result of the chronic inflammatory state or it may simply be sporadic, evolving from adenomas (precursor lesions). In these two distinct pathways, p53 mutations occur in different stages. In patients with colitis-associated cancer (CAC), p53 mutation is usually an early event. In contrast, in sporadic carcinogenesis, p53 mutation will only occur later in the process. A project will be proposed regarding the analysis of lesions from a cohort of patients with IBD and any form of dysplasia/CRC diagnosis followed at Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG). Immunohistochemistry will be used to assess p53 protein expression – lesions endoscopically classified as adenoma-like (controls) will be compared to non-adenoma-like lesions (cases). By using p53 immunoexpression analysis, it may be possible to distinguish between sporadic CRC and CAC in the context of IBD. These have different implications regarding therapeutic decisions and patient prognosis.

Keywords

p53 protein; carcinogenesis pathways; inflammatory bowel disease; colorectal cancer; dysplasia; adenoma.

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Abbreviations

Lista de acrónimos

AMPK- AMP-activated Protein Kinase
APC gene- Adenomatous Polyposis Coli gene
CAC- Colitis-Associated Cancer
CIMP pathway- CpG Island Methylator Phenotype Pathway
CIN pathway- Chromossomal Instability Pathway
CRC- Colorectal Cancer
IBD- Inflammatory Bowel Disease
IHC- Immunohistochemistry
GI- Gastrointestinal
HGD- High Grade dysplasia
H-MSI- High Microsatellite Instability
LGD- Low Grade dysplasia
L-MSI- Low Microsatellite Instability
LOH- Loss of Heterozigoty
MAPK- Mitogen-activated Protein Kinase
MLH- MutL Homologue
MMR- DNA Mismatch Repair
MSH- MutS Homologue
MSI pathway- Microsatellite Instability Pathway
TCF- T-cell Factors
TP53- Tumor Protein p53
TSG- Tumor Suppressor Gene
UC- Ulcerative Colitis
wt-p53- Wild-type p53

Chapter 1

Introduction

p53 protein, which is encoded by TP53, intervenes in cell cycle regulation, DNA repair and apoptosis. When the gene is mutated, carcinogenesis is facilitated and mutated p53 proteins accumulate. This protein is the most commonly mutated protein in human carcinomas.

Inflammatory Bowel Disease (IBD) is characterized by a state of chronic inflammation of the intestinal mucosa, in which the constant renewal of the intestinal epithelium cells [1] can facilitate the accumulation of mutated proteins (such as p53 mutated proteins) throughout the colon, contributing to a field defect [2]. Prolonged exposure to inflammation leads to extensive cytokine stimulation, activation of multiple signaling pathways and injury to the mucosal barrier, promoting the initiation of dysplasia [3]. This progressive accumulation of injuries can lead to CRC, which is more frequent in the context of long duration colonic IBD. Of note, p53 mutations are an early key event in the IBD related colorectal carcinogenesis [4]. Furthermore, p53 mutations in IBD associated neoplasia can be found in nondysplastic intestinal mucosa, since the entire colonic mucosa carries risk for neoplastic transformation, which can be multifocal, opposing to sporadic cancers that usually arise as single lesions that evolved from adenomas [5]. However, sporadic adenomas are common in the general population, so they are to be expected in patients with IBD as well [6].

Sporadic adenomas can usually be managed by either endoscopic resection or segmental surgical resections, opposing to IBD-related CRCs which are commonly an indication for total colectomy. Given the fact that both synchronous and metachronous lesions are significantly more frequent in IBD related colorectal carcinogenesis than in the sporadic context, understanding both molecular pathways involved in sporadic CRC as well as CAC carcinogensis is important. Although these molecular alterations are similar, they seem to differ in sequence and frequency.

Regarding sporadic CRC, there are 3 major molecular pathways involved in sporadic carcinogenesis: the Chromosomal Instability (CIN) pathway, the Microsatellite Instability (MSI) pathway and the CpG Island Methylator Phenotype (CIMP) pathway [5].

1.1 Sporadic CRC Carcinogenesis

1.1.1 The Chromossomal Instability Pathway

The CIN pathway represents the most common pathway for CRC, contributing up to 85% [5]. It is the main pathway on the conventional sequence adenoma-carcinoma. Progression to CRC usually involves mutations in the *APC*, *KRas* and *TP53* genes. The key event is the mutation of the Adenomatous Polyposis Coli (*APC*) gene. The inactivation of the *APC* gene leads to an increase in WNT signaling, through the failure of degrading β-catenin, which accumulates in the cell cytoplasm, leading to its translocation into the nucleus. In the nucleus, it activates specific transcription factors (namely T-cell factors – TCFs), that lead to the expression of several genes implicated in increased proliferation, differentiation, migration and cell adhesion.
K-Ras mutations allow the cell to escape apoptosis, lending them an advantage. The *Ras* mutation is also involved in other signaling pathways that are critical for the initiation of carcinogenesis such as the interaction between AMP-activated Protein Kinase (AMPK) and Mitogen-activated Protein Kinase (MAPKs). AMPKs regulate cell proliferation and growth through the activation of p21 and p53.
Mutation of p53 contributes to high proliferative activity of the cell through the loss of cell cycle control and apoptosis. The p53 gene also interacts with COX-2, which plays a role in promoting inflammation and cell proliferation in CRC.

1.1.2 The Microsatellite Instability Pathway

The MSI pathway is responsible for 15% of sporadic CRCs. It results from inactivation of the DNA Mismatch Repair (MMR) system, which identifies errors in DNA replication and repairs the mismatched nucleotides. The genetic basis for instability in MSI tumors is an alteration in any one of the implicated MMR genes: MSH2, MLH1, MSH6 and PMS2. Microsatellite unstable tumors can be divided into two distinct MSI phenotypes: MSI-high (MSI-H) and MSI-low (MSI-L). Sporadic MSI-H CRC most often arise from the epigenetic silencing of the mismatch repair gene MLH1, leading to inactivation of target genes, in particular tumor suppressors having a microsatellite sequence in their coding region. Most of the CRCs arising from this pathway show typical characteristics: loss of expression of the protein corresponding to the mutated gene and MSI-H. MSI corresponds to altered number of repeats in several scattered repetitive sequences in the DNA.

1.1.3 The CpG Island Methylator Phenotype Pathway

The CIMP pathway is responsible for 20% to 30% of CRC and is related to the serrated pathway. It consists on the hypermethylation of CPG nucleotide sequences localized in the promoter regions of genes that regulate cell cycle, apoptosis and DNA repair, causing loss of gene expression. The CIMP phenotype can be classified into CIMP-high and CIMP-low, according to the number of methylated markers. Of note, *BRAF* oncogene mutation is an early event in the serrated pathway and is often identified in CIMP-high CRC [7].

1.2 CAC Carcinogenesis

In CAC carcinogenesis, DNA damage and genomic instability contribute to the dysplasia-carcinoma sequence. Epigenetic factors can also contribute by damaging the cell-cycle control [8]. Chronic inflammation contributes to predisposing the tissue to dysplasia by inducing gene mutations, by the inhibition of apoptosis or through the stimulation of angiogenesis and cell proliferation [9].

1.2.1 Genomic Instability

Genomic instability happens both in the MSI pathway associated with mutant phenotypes and in the CIN pathway characterized by chromosomal abnormalities. In patients with pancolitis, genomic instability precedes neoplastic transformation and may be related to the extension and severity of chronic inflammation.

1.2.2 DNA Damage

Free radicals, produced by oxidative stress during chronic inflammation, can act as initiators of DNA damage and can activate oncogenes and inactivate oncosuppressor genes.
In tissues with chronic inflammation, the resulting increase in oxidative stress results in enhanced mutation of DNA, thereby promoting the early stages of tumorigenesis.

1.2.3 Epigenetic Changes

Aberrant methylation events occur in the early stages of colorectal carcinogenesis, resulting in profound modifications in gene expression. Hypermethylation of oncosuppressor and DNA mismatch repair gene promoter regions leads to the accumulation of epithelial cells with genomic damage in inflamed mucosa and may contribute to the initiation of the neoplastic process [8].

There has been much investigation regarding CRC in the context of IBD. However, it is important to consider the possibility of sporadic CRC arising in IBD patients, especially when considering multiple risk factors such as age, sex, tobacco smoke, alcohol, obesity and lack of physical activity [10][11]. It is known that CRC progression through the different pathways is promoted by the accumulation of mutated proteins, namely the p53 protein. However, this mutation occurs in a late phase in previously healthy patients. In IBD patients, this mutation occurs early in the progression of CRC. Taking that into consideration, we can search for the moment in which the mutation occurred and learn more about the nature of the CRC in an IBD patient. If it was an early mutation, we are probably facing an IBD-related lesion in an inflammatory background in the intestinal mucosa. If it was a late mutation we should conclude that a CRC deriving from a sporadic conventional adenoma is more likely.

Chapter 2

Objective

The aim is to elaborate a scientific project to be conducted in the future regarding the role of p53 protein as a possible biomarker to differentiate CRCs/dysplastic lesions related to IBD from those arising from the sporadic pathway.

Chapter 3

Materials and Methods

A literature search using the website PubMed was used for articles published until December 20th of 2019. The searched terms included CRC, IBD, p53, carcinogenesis pathways and sporadic adenoma. Articles were considered when written either in english or portuguese. No limits were applied concerning the year of publication. Abstracts were read to select the most relevant articles, which appear in the final reference list.

Chapter 4

Results

To evaluate the possible role of the p53 protein as a biomarker to distinguish between different carcinogenesis pathways that can lead to CRC in IBD patients, a scientific study was planned. The scientific project proposed will be conducted at Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG).

4.1 Study Presentation

4.1.1 Introduction

p53 is a protein that regulates the cell cycle, apoptosis and genomic stability. It is one of the major suppressor genes, frequently mutated in CRC. It is thought to be responsible for the progression from dysplasia to CRC in sporadic cases. Nevertheless, there are different carcinogenesis pathways that can lead to CRC. In IBD patients, CRC may be secondary to the chronic inflammatory disease or may simply be sporadic, with no direct relation to the disease. In these two distinct pathways, p53 mutation occurs in different stages. Sporadic adenomas are usually well circumscribed polyps, whereas Ulcerative Colitis-associated (UC) dysplastic lesions have a variable macroscopic appearance and are difficult to recognize endoscopically. Histologically, these two lesions are very similar making difficult the distinction between UC-associated dysplastic lesions from adenomas in inflamed mucosa. Analyzing p53 expression in precursor lesions may point to their origin, influencing therapeutic decisions and patient prognosis. Sporadic adenomas can usually be managed by polypectomy, in contrast to IBD-related dysplastic lesions that may be an indication for colectomy, namely if high-grade. So it is important to select the most appropriate treatment when dysplasia of any grade is found in an IBD patient.

IHC detects the accumulation of stabilized p53 protein. Wt-p53 protein in normal cells has a short half-life being present in low concentrations that are, generally, not detected by IHC. However, a small proportion of regenerating non-dysplastic cells, with accumulation of wt-p53 in its interior, may be positive. Mutation of *TP53* stabilizes the protein, increasing its half-life. The abnormal p53 protein accumulates in the cell becoming detected by IHC, with a p53 positive staining. Another scenario is the inactivation of both alleles of a tumor suppressor gene (TSG), such as *TP53,* resulting in the absence of protein. In fact, the sequential inactivating mutation of one allele followed by allelic loss of

heterozigoty (LOH) results in a null or absent pattern of staining.

4.1.2 Objective

To evaluate the ability of p53 protein immunoexpression to distinguish between sporadic CRCs/dysplastic lesions occurring in IBD patients and IBD-related dysplastic lesions.

4.1.3 Materials and Methods

For this study, a cohort of patients with IBD and any form of dysplasia/CRC diagnosis followed at IPOLFG will be used. Patient's data, including demographic and clinical data, will be collected from an established database from previous studies. Patients with an IBD diagnosis with colonic involvement and with colonic dysplasia and/or CRC, diagnosed more than 12 months after the IBD diagnosis, will be selected. Lesion's samples previously collected at routine procedures (endoscopic or surgical) will be studied by IHC to assess p53 protein expression. Staining will be classified as +(weak), ++(moderate) and +++(strong).

Adenomas or CRC in previously healthy mucosa or adenoma-like lesions with dysplasia will be used as controls. Dysplasia and/or CRC in flat mucosa or non-adenoma-like lesions will be used as cases.

The pattern of staining will be compared between samples defined as controls and as cases.

Considering this a pilot study and taking into account the central limit theorem, a minimum sample of 30 patients will be considered adequate.

For statistical analysis, continuous variables will be expressed as means and standard deviations. Qualitative variables will be expressed as absolute and/or relative frequencies and associations tested by Chi-square or Fisher's Exact tests. Multivariate analysis will be done by logistic regression. A significance level of 0,05 for bilateral testing will be accepted. SPSS 19 will be used for the analysis.

4.1.4 Timing and Calendar

Table 4.1 – Study Project's Timing and Calendar

3 months	Project submission for approval, by the Ethics Committee and by the Scientific Council at IPOLFG
3 months	Patient selection and sampling collection
6 months	p53 immunohistochemistry technique
6 months	p53 immunoexpression histological analysis
3 months	Data collection and statistical analysis
3 months	Revision, discussion and results presentation

4.1.5 Budget

Table 4.2 – Study Project's List of Reagents for 30 Cases

Designation	Brand	Reference	Quantity	Needed quantity	Price
p53	CELL MARQUE	453M-85/D07	0,5 ml	1	500 €
Visualization kit	VENTANA	760-700	250	1	1230 €
Superfrost lamin	TOMO	8082286001	100	1 box	120 €
24x50 mm Lamelas	THERMO SCIENTIFIC	631-0901	1000	1 box	30 €
TOTAL					**1880 €**

Table 4.3 - Total Budget for the Project

Reagents and materials	1880 €
Divulgation	1000 €
TOTAL	**2880 €**

4.1.6 Funding

This Project will be totally financed by a grant from Grupo de Estudos na Doença Inflamatória Intestinal (GEDII).

Chapter 5

Discussion

According to previous studies it is expected that *TP53* gene mutations occur earlier in the carcinogenesis sequence related to IBD [2] than in sporadic cases. Indeed, p53 mutations occur even before aneuploidy. On the contrary, in sporadic CRC, these mutations occur later and lead the progression from advanced adenoma to carcinoma [12]. Sada *et al* stated that TP53 mutations occur approximately 8 months before low grade dysplasia (LGD), 26 months before high grade dysplasia (HGD) and 38 months before CRC [13].

Many of the molecular alterations responsible for sporadic CRC may also play a role in colorectal carcinogenesis that complicates IBD. The two major pathways involved in sporadic CRC, which are the CIN pathway and MSI pathway, are also involved in CAC and in similar proportions [14].

According to Yin *et al* it is the inactivation of tumor suppressor genes, such as protein p53, combined with the alteration of DNA repair genes that results in proto-oncogene mutations that produce oncogenes. These oncogenes give rise to abnormal cellular proliferation and invasion. The activity of *TP53* may be downregulated through mutations, alterations of upstream factors, or modifications of the downstream components that mediate *TP53* signals [15].

The typical mechanism by which the inactivation of tumor suppressor genes occurs, specially in colorectal neoplasms, is the sequential inactivating mutation on one allele, followed by allelic LOH of the other allele [16].

The tumor-suppressor *TP53* ensures the quality and genomic stability of stem cells, specially those located in intestinal crypts. When mutated, p53 protein loses its tumor-suppressor functions and gains additional oncogenic functions, a phenomena called p53 gain of function [17].

Several biomarkers have been proposed for prediction of IBD-related CRC such as DNA ploidy, mutations of the *APC* gene, *K-Ras* gene and *TP53*. *APC* loss of function is less frequent in IBD when compared to sporadic CRC, and typically occurs later in the process, but currently, *TP53* gene mutations seem to be the most important biomarker.

Gerrits *et al* showed that prior to the development of CRC, an increase in the proportion of p53 immunopositivity in the colonic biopsies can be detected [18]. Also, altered p53 immunoexpression was detected in biopsies without neoplasia [19], contributing as a significant predictor for development of advanced neoplasia.

Finally, according to Du *et al*, IBD patients that present with *TP53* mutations are more likely to develop CRC in the context of IBD [20].

Hussain and colleagues also showed that there was an increased frequency of *TP53* mutations in non-neoplastic IBD mucosa, increasing the susceptibility to cancer, compared to non-IBD affected regions in UC patients. In fact, a substantial subset of patients with UC carry a high p53 mutation load. The high frequency of these mutations in inflamed mucosa when compared to the non-inflamed mucosa of the same colon supports the hypothesis that p53 can be used to predict the development of CRC [21].

Thus, altered expression of p53 in colonic mucosa can be used by pathologists as a useful biomarker to predict the risk of evolution toward malignancy. Popp *et al* showed that architectural distortion was significantly correlated with p53 overexpression in epithelial cells [22].

According to Choi *et al*, increased frequency and early onset of *TP53* mutations might provide an additional reason for the higher proportion of flat dysplastic and cancerous lesions seen in IBD [23].

The mutant *TP53* gene acts as an oncogene, increasing the aggressiveness, survival and the ability of the tumor to metastasize. The product of the mutated gene is a mutated p53 protein that can be detected by IHC. The assessment of the anti-p53 antibody by IHC is lower in cost but also effective and efficient [24].

IHC analysis of the p53 protein is a useful method for early detection of risk areas since there is concordance between expression of p53 mutated protein and *TP53* [25]. A positive staining does not always reflects the accumulation of mutated p53 but may result from stabilization of wt-p53. Potential mechanisms that stabilize wt-p53 include binding to viral proteins (such as large T antigen SV40) or binding to *mdm2* gene which functionally inactivates it [26].

In addition, data seems to show that IBD-associated early stage neoplasia has a different pattern of p53 immunoexpression than sporadic adenoma. The immunoreactivity in sporadic adenomas has a scattered or sporadic pattern of p53 expression, while early-stage CAC exhibits a “bottom-up” pattern characterized by p53 immunoreactivity in the basal half of the crypts [25]. IHC is a well-established method in Pathology and a useful method in the assessment of p53 inactivation and it was proved by Kaserer *et al* that assessing the pattern of p53 expression is superior to quantitative analysis to determine p53 inactivation. Thus, in CRCs, positive p53 IHC can be regarded as a good indicator of the functional status of p53 (after sampling errors have been excluded). Negative p53 IHC, however, does not indicate retained p53 function [27]. p53 has a short half-life so does not accumulate and is present in low concentrations, indetectable by IHC [28].

The definition of a positive p53 immunoexpression is not straightforward. In one study, to define true p53 positivity, analysis of the localization of p53 staining was required. Restriction of p53 staining to the basal third of the crypts should not lead to the diagnosis of dysplasia because some p53 expression is common in highly proliferative epithelium, with high levels of wt-p53 protein, such as those of IBD patients [29]. The low concentration of wt-p53 in normal cells confers a weak staining. Mutated p53 protein accumulates in the cell so that it becomes detected by IHC, revealing p53 positive staining. The inactivation of TSGs, such as *TP53*, is the sequential inactivating mutation of one allele followed by allelic LOH which results in a null or absent pattern of staining [26].

Therefore, the best discriminative power of p53 staining resides in the absence of "upper third" p53 positivity in non-dysplastic biopsies.

Mutant p53 can be detected uniformly or heterogeneously in malignant tissues but, in some cases, particularly in the early phases of malignancy, accumulation of mutant p53 is observed in the basal region or edges of tumors and is accompanied by high Ki67 expression [30].

Mutant p53 gain of function confers sustained proliferation, cell death resistance, tumor-promoting inflammation as well as the ability of invasion and metastasis. Therefore, mutated p53 promotes the tumorigenic potential, confers drug resistance mechanisms, such as protection from chemotherapy-induced apoptosis, and leads to undifferentiated tumors, contributing to enhanced CRC aggressiveness [15].

Regarding the treatment of dysplastic lesions and CRC in IBD, a personalized approach is clearly needed. Since CAC lesions have a worse prognosis and are more frequently multifocal and/or associated with metachronous lesions, a total colectomy must always be considered and discussed with the patients as a therapeutic option. On the contrary, sporadic lesions may generally be endoscopically resected or safely submitted to segmental colonic resection surgeries.

Therefore, p53 altered expression would be extremely useful as a biomarker if it was proven that it could be used for stratification of IBD patients into risk categories - those with IBD-associated lesions vs those with sporadic lesions.

Chapter 6

Conclusions

Colonic IBD carries an increased risk of inflammation-associated neoplasia in the colon. Nevertheless, sporadic adenomas are common in the general population so they have to be expected in patients with IBD as well.

p53 is the most commonly mutated protein in human carcinomas and it is mutated earlier in the carcinogenesis sequence related to IBD. On the contrary, *TP53* gene mutations are a late event in the sporadic CRC pathway.

The detection of dysplastic areas in IBD patients, macro and microscopically, can be challenging for gastroenterologists as for pathologists, respectively, so multiple biopsies are required. In addition, and despite dysplasia arises as the most reliable marker of CRC risk, the morphological features of LGD and regenerative epithelium can overlap, making the differential diagnosis between dysplasia and reactive changes a challenge. Molecular biomarkers, namely the immunohistochemical procedures, may provide diagnostic methods useful to detect early neoplastic colonic mucosa and p53 immunoexpression emerges as a strong putative candidate.

Also, and according to published studies, it may be possible to use p53 immunoexpression to distinguish the two carcinogenic pathways, CAC and sporadic CRC in IBD. This would have important prognostic and therapeutic implications, since CAC is known to have increased risk of both synchronous and metachronous neoplastic lesions.

Further studies are needed to confirm the use of p53 immunoexpression as a reliable biomarker of CRC in the context of IBD.

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Annexes

Annex A

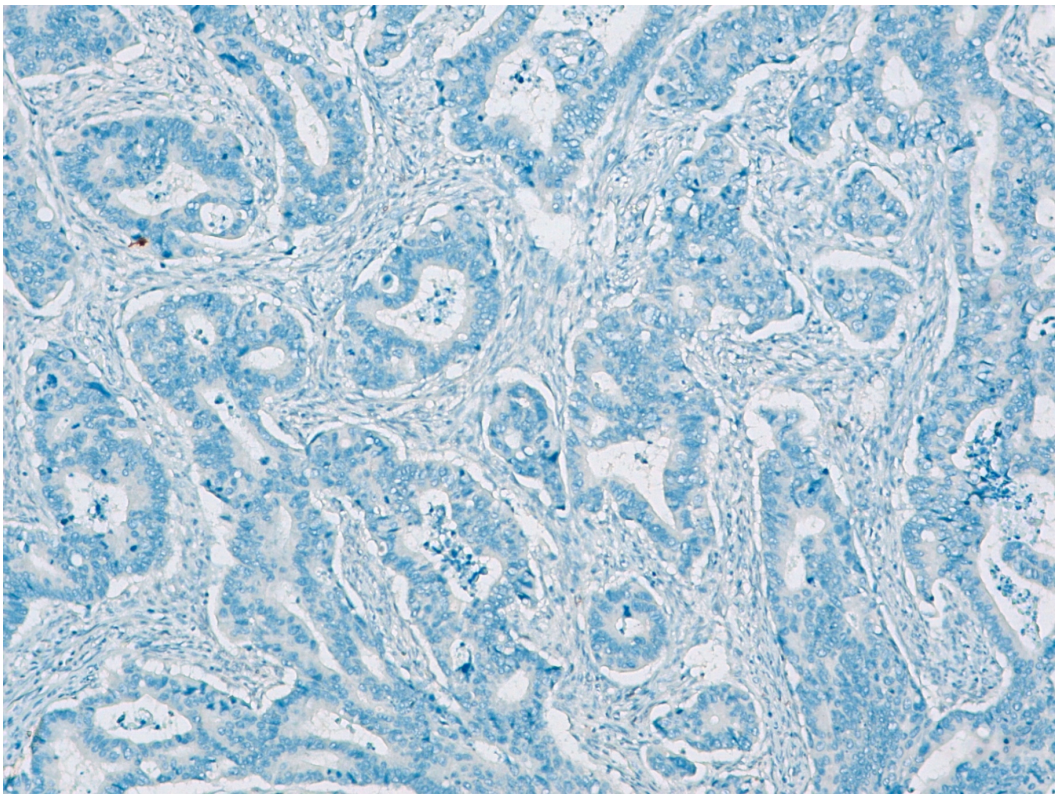

Figure A1 - Null/absent p53 staining. Mutation of one allele followed by allelic LOH which results in a null or absent pattern of staining.

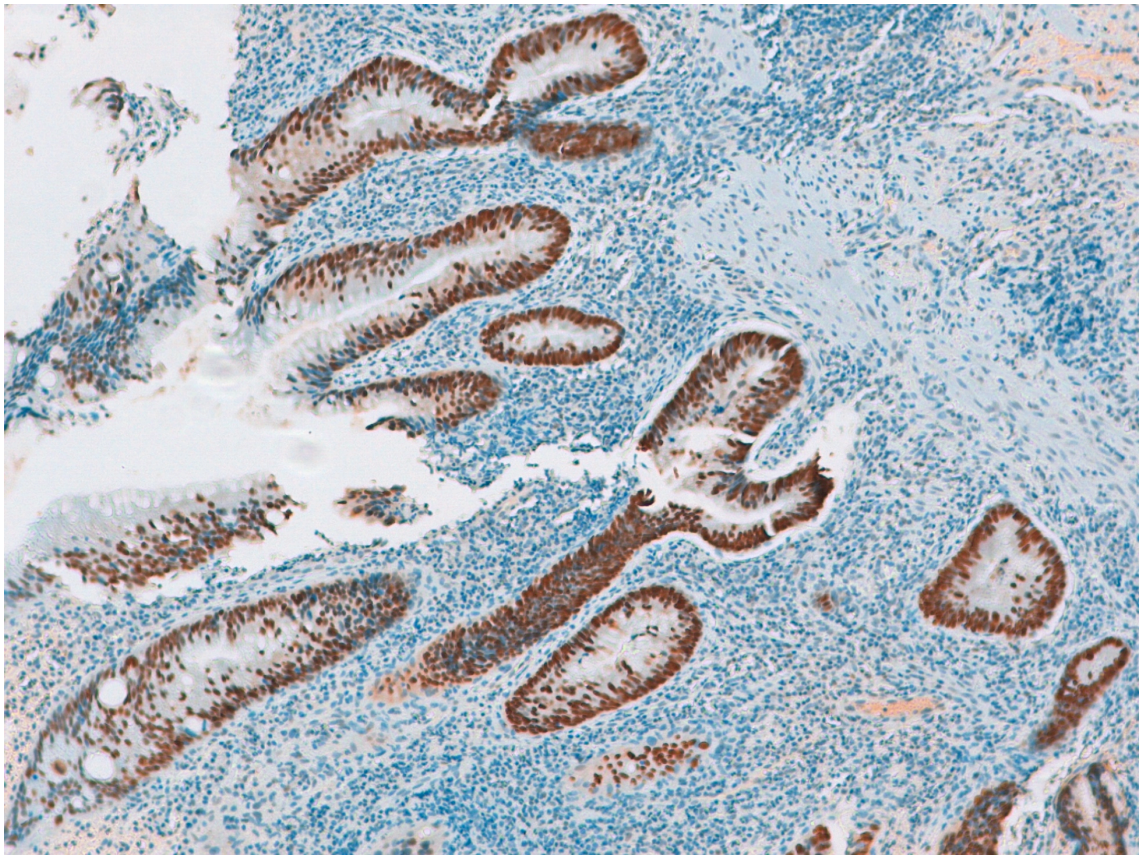

Figure A2 - p53 positive staining. Mutated p53 has an increased half-life and accumulates in the cell.

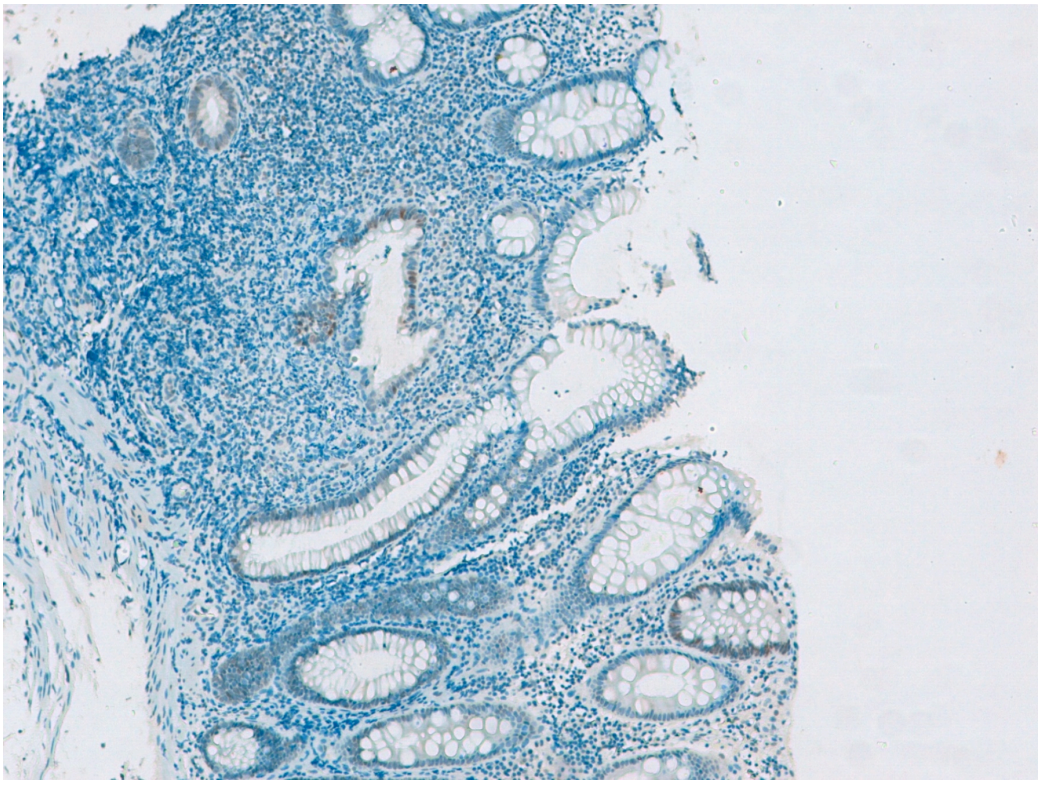

Figure A3 - wild-type p53. Wt-p53 protein in normal cells has a short half-life so it is present in low concentrations and generally is not detected by IHC. However, a small proportion of regenarating, non-dysplastic cells may also be positive.

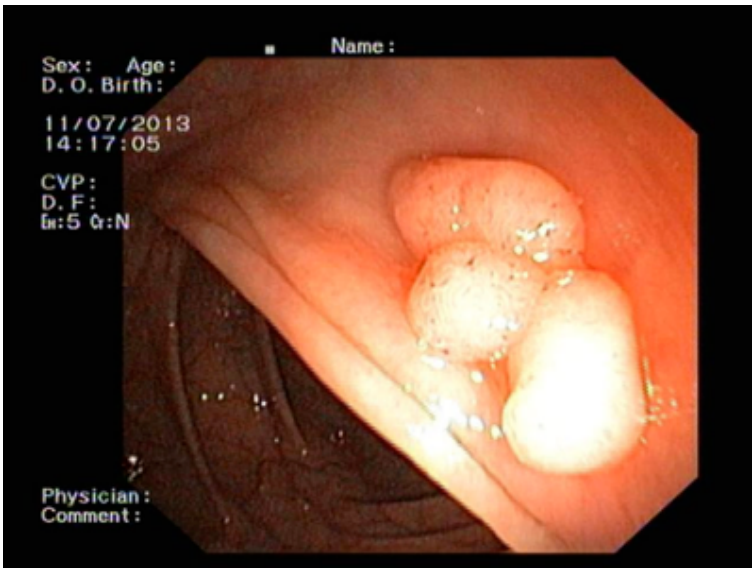

Figure A4 - Adenoma-like lesion.

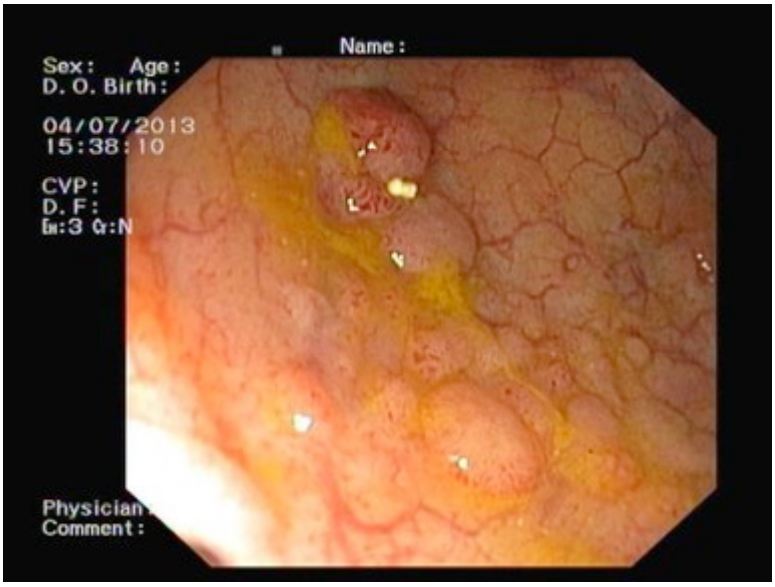

Figure A5 - Non-adenoma-like lesion.