ORIGINAL

Study of APC and β-catenin protein expression in polyps and colorectal adenocarcinoma

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Abstract

Objective: To study part of the Wnt signaling pathway for carcinogenesis in colorectal cancer and polyps, through adenomatous polyposis coli (APC) and β-catenin protein expression. **Methods:** We studied the immunoexpression of APC protein and β-catenin in the adenocarcinoma and polyps (adenoma) from ninety-one patients who underwent resection of sporadic colorectal cancer (CRC) with curative intent from 1993 to 2004 at the Hospital do Câncer de Barretos (Brazil). Each patient was resected within a period 3 months before or after diagnosis. The polyps or adenocarcinoma was considered positive for APC when more than 10% of the cells showed cytoplasmic staining and for β-catenin when more than 5% of the cells showed nuclear stainning. **Results:** Among the 91 patients, we found association among APC protein expression in the polyps and in the respective colorectal adenocarcinoma (p = 0.014). Similarly, there were significant association with nuclear expressions (p = 0.001) of β-catenin in the polyps and colorectal adenocarcinoma. Nuclear β-catenin expression was associated with villous adenoma (86%, p = 0.042). There was no association among APC and β-catenin expression and any other histological finding. **Conclusion:** There is an association of APC and β-catenin protein expression in polyps and in colorectal adenocarcinoma. This finding allows us to speculate that in the same patient the same WNT pathway or another is activated in both lesions; polyp and adenocarcinoma. Villous adenomas occur most frequently via the Wnt signaling pathway.

Keywords: adenomatous polyposis coli, beta catenin, colorectal carcinoma, familial adenomatous polyposes.

INTRODUCTION

Colorectal cancer (CRC) is one of the most common forms of cancer in the Western world. It can occur in a hereditary manner (i.e. in individuals with some predisposing syndrome)[1,2].

The syndromes that predispose towards CRC may present with or without polyposis. Syndromes with polyposis account for less than 1% of CRC cases and are mainly represented by familial adenomatous polyposis (FAP), Gardner's syndrome and Turcot's syndrome. Hereditary non-polyposis colorectal cancer (HNPCC) is responsible for around 10% of the cases of CRC[1-4].

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Submitted: 02/05/2011.
Aproved:30/05/2012.

The great majority of CRC consist of sporadic cases in which no hereditary condition is identified in the patients. Thanks to the development of molecular and genetic biology over recent years, it has become possible to understand some of the mechanisms involved in the carcinogenesis of these neoplasms and, in particular, in CRC[4-6].

FAP is a dominant autosomal hereditary disorder characterized by the presence of thousands of adenomatous polyps throughout the colon. All patients with this syndrome develop CRC if they are not treated. The APC gene is a tumor-suppressing gene and when it is inactivated, the formation of adenomatous polyps can begin. It is considered to be a key "gatekeeper" for colorectal neoplasm, when it undergoes mutation in the germinative lineage (i.e. hereditary lineage). All the epithelial cells of the colon in patients with FAP contain an allele of the APC gene that undergoes mutation because the cells are already affected by mutation in the germinative lineage. Inactivation of the remaining normal copy of the APC gene, by deletion or mutation, completely removes the tumor-suppressing function of this gene, with growth of adenomatous polyps. Normal APC protein promotes apoptosis in the cells of the colon. Its most important function seems to be to impede the cell gro-

wth stimulation action of the protein β-catenin. The latter activates the genes associated with growth together with tissue coding factors. Therefore, loss of APC function would impede apoptosis and allow β-catenin protein to accumulate in the intracellular medium and stimulate rapid cell growth[1,4,7-9].

Another function of β-catenin protein is in cell adhesion, which is important for maintaining cell architecture, polarity, movement limits and proliferation[4,10]. The proteins E-cadherin and β-catenin are the principal elements in cell adhesion. Thus, alteration of the expression or function of the cadherin-catenin complex results in low cell adhesion, with possible cell transformation and tumor progression[4,11].

Because the proteins APC and β-catenin are related to carcinogenesis via the Wnt pathway, it has been sought to study the association between the expressions of these two proteins in patients who have CRC in association with adenomatous polyps. This would have the aim of finding out whether the gene alterations that may be responsible for CRC might also occur in the associated adenomatous polyps. Through this it would be possible to verify whether the pathway for carcinogenesis in CRC cases was already present in the benign lesions represented by the polyps.

The aim of this study was to study part of the Wnt pathway for carcinogenesis in CRC and polyps, through APC and β-catenin protein expression.

MATERIAL AND METHODS

This was a retrospective study developed in the Departments of Oncological Surgery and Pathological Anatomy of *Hospital do Câncer de Barretos - Fundação Pio XII*, (Barretos, Brazil).

The biological samples were collected from the archives of the Pathology Laboratory of *Fundação Pio XII* and the clinical data were collected from SAME (Medical and Statistical File Service). The sample comprised of 91 patients with colorectal adenocarcinoma in association with adenomatous polyps who were treated between 1993 and 2004.

This study included patients admitted for surgical treatment who had colorectal adenocarcinoma with associated polyps that were removed three months before or after the diagnosis.

Patients were excluded from the study if they had FAP, intestinal inflammatory disease, or clinical characteristics suggestive of HNPCC.

In the sample analysis, the following were considered: gender, age group, location of the neoplasm, anatomopathological staging (TNM), and the histological grade and type of the colorectal adenocarcinoma. The number, size and degree of atypia of the polyps were studied. The analytical data on the sample are shown in Table 1.

Table 1. Sociodemographic, clinical and therapeutic characteristics of 91 patients* with colorectal adenocarcinoma.

Characteristic	N	%
Gender		
Female	46	50.5
Male	45	49.5
Tumor location		
Rectum	54	59.3
Sigmoid	15	16.5
Ascending colon	11	12.1
Descending colon	6	6.6
Transverse colon	5	5.5
Stage		
I	33	36.3
II	30	32.9
III	16	17.6
IV	12	13.2
Histological grade		
I	19	20.9
II	67	73.6
III	5	5.5
Histological type		
Tubular	67	73.6
Tubular-villous	20	22.0
Mucinous	4	4.4
Polyp: histological type		
Tubular	47	51.6
Tubular-villous	30	33.0
Villous	14	15.4
Polyp: degree of atypia		
Slight	40	44.0
Moderate	23	25.3
Marked	20	22.0
No atypia	8	8.7

* Mean age of the patients was 64 years.

Immunohistochemical technique

The expression of the proteins APC and β-catenin was evaluated by means of the immunohistochemical method. For this, primary monoclonal antibodies (*Novocasta Labs*, Newcastle, UK) were used: APC at a dilution of 1:20 and β-catenin at a dilution of 1:1000.

The paraffin blocks were sectioned using a rotary microtome, to obtain histological sections of 2 to 3 microns in thickness. These were placed on slides that had previously been treated using silane (3 -aminopropyl-triethoxysilane; cat. no A-3648; SIGMA, USA), with a reaction using the avidin-biotin-peroxidase (ABC)/streptavidin--biotin-peroxidase complex (StreptABC).

Evaluation of the results obtained from the immunohistochemical method

The evaluation of the proteins was carried out by reading the histological slides under an optical microscope at 100x magnification. This was done by three pathologists who had no knowledge of the clinical-pathological staging or histological grade and type of the material analyzed. The criterion adopted for defining the results was the immunohistochemical reaction, with the percentage of cancer cells stained. The nucleus was considered to be positive for the protein β-catenin when 5% or more of the cells were stained by the marker, for adenocarcinoma and for the polyps.

The cytoplasm was considered to be positive for the protein APC when 10% or more of the cells were stained by the marker, for adenocarcinoma and for the polyps.

Figures 1 to 3 show the expression of these proteins after using the immunohistochemical method.

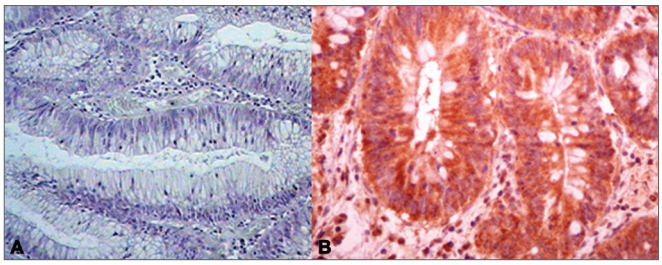

Figure 1. (A) Adenomatous polyps: negative (100x); and (B) positive for APC protein expression (400x).

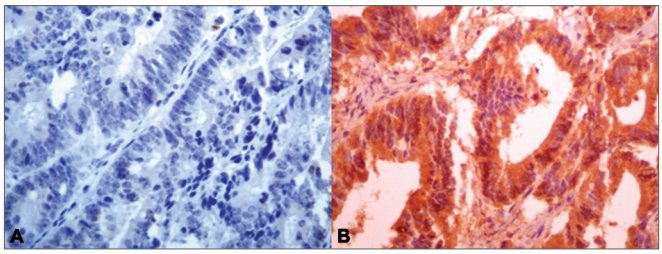

Figure 2. (A) Colorectal adenocarcinoma: negative (400x); and (B) positive for APC protein expression (400x).

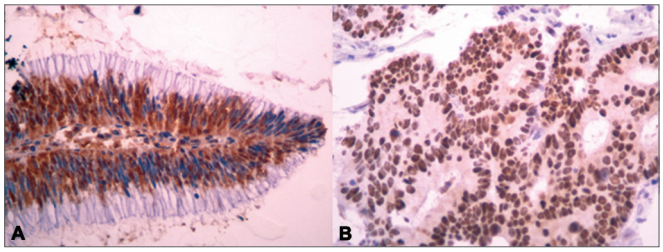

Figure 3. (A) β-catenin protein expression in nuclei, in adenomatous polyps (400x); and (B) colorectal adenocarcinoma (400x).

Statistical analysis

The results were subjected to statistical treatment with the aim of determining whether there was a correlation between APC and β-catenin protein expression in the colorectal adenocarcinoma and the associated adenomatous polyps and the expression of these markers in relation to the stage, histological grade and histological type of the adenocarcinoma, and also the histological type and degree of atypia of the polyps.

The Pearson chi-squared test was used for comparing the data collected with the results obtained from the immunohistochemical method for APC and β-catenin expression. The significance level was set at $p \leq 0.05$.

RESULTS

Study of APC protein expression

Table 2 shows the APC protein expression in polyps. Fifty patients (55%) were negative for APC protein expression and 41 (45%) were positive.

Table 2. Distribution of APC protein expression in polyp patients.

APC protein	Patients	
	N	%
Negative	50	55.0
Positive	41	45.0
Total	91	100.0

This study of the correlation between APC protein expression in polyps and the histological type showed that there was an association, since the APC protein tended to be expressed more in the tubular and tubular-villous types. On the other hand, no correlation was found between the expression of this protein and the degree of atypia of the polyps.

Fifty-nine patients (65%) were negative for APC protein expression in adenocarcinoma and 32 (35%) were positive. These data are shown in Table 3.

Table 3. Distribution of APC protein expression in adenocarcinoma patients.

APC protein	Patients	
	N	%
Negative	59	65.0
Positive	32	35.0
Total	91	100.0

Sixty-five percent of the patients were negative for APC protein expression in adenocarcinoma. There was no correlation between APC protein expression and the stage, histological grade and histological type of the adenocarcinoma.

Table 4 shows the correlation of APC protein expression between the polyps and adenocarcinoma.

The correlation of APC protein expression between the polyps and adenocarcinoma showed that these variables were associated, since there was a tendency for positive and negative findings to coincide ($p = 0.014$).

Table 4. Correlation of APC protein expression between polyps and adenocarcinoma.

APC protein	Polyps					
	Positive		Negative		Total	
Adenocarcinoma	N	%	N	%	N	%
Negative	21	51.0	38	76.0	59	55.0
Positive	20	49.0	12	24.0	32	35.0
Total	41	100.0	50	100.0	91	100.0

(Chi-squared test: $p = 0.014$).

Study of β-catenin protein expression

Table 5 shows the β-catenin protein expression in nuclei, in polyps. Fifty-five percent of the polyps were positive for β-catenin protein expression in nuclei.

Table 5. β-catenin protein expression in nuclei, in polyps.

	Patients	
	N	%
Negative	41	45.0
Positive	50	55.0
Total	91	100.0

The correlation between β-catenin protein expression in nuclei in the polyps and the histological type showed that there was an association between the expression of this protein and villous histological type, which was 86% positive ($p = 0.042$).

There was no correlation between β-catenin protein expression in nuclei in the polyps and the degree of atypia.

Table 6 shows the distribution of β-catenin protein expression in nuclei, in adenocarcinoma. Eighty percent of the cases were positive for β-catenin protein expression in nuclei, in adenocarcinoma. The correlations between β-catenin protein expression in nuclei and the stage, histological type and histological grade of the adenocarcinoma did not present statistical significance.

Table 6. β-catenin protein expression in nuclei, in adenocarcinoma.

	Patients	
	N	%
Negative	18	20.0
Positive	73	80.0
Total	91	100.0

Table 7 shows the correlation of β-catenin protein expression in nuclei between the polyps and adenocarcinoma. This table shows that there was an association for β-catenin protein expression in nuclei between the polyps and adenocarcinoma, i.e. there was a tendency to positivity and negativity ($p = 0.001$).

Table 7. Correlation of β-catenin protein expression in nuclei between polyps and adenocarcinoma.

β-catenin	Polyps					
	Positive		Negative		Total	
Adenocarcinoma	N	%	N	%	N	%
Negative	3	6.0	15	37.0	18	20.0
Positive	47	94.0	26	63.0	73	80.0
Total	50	100.0	75	100.0	91	100.0

(Chi-squared test: $p = 0.001$).

DISCUSSION

Activation of the Wnt signaling pathway plays an important role in the process of colorectal carcinogenesis. The normal APC protein promotes appoptosis in the colon cells. It's most important function seems to be to prevent the action of stimulation over β-catenin protein cellular growth, which activates genes associated with growth, together with tissue encoders. The loss of APC function would allow the β-catenin protein to be accumulated in the intracellular means with translocation to the nucleus and to activate genes such as Cyclin D1, C-myc and others, stimulating cell growth with rapid and unplanned development of colorectal neoplasm[8,10].

Evaluation of the APC protein expression

Initially identified in patients with polyposis, familial adenomatous was called APC (adenomatous polyposis coli). Its amendment is considered a 'trigger' for the appearance of proliferative disorders in the colonic mucosa, whether on stage or adenomatous polyp invasive carcinomas. Mutations of this protein are present in 80% of adenomas in the initial phase; such mutations are considered today as the earlier alteration in the carcinogenese of the colonic mucosa, which is why it is assigned the role of guardian or 'gatekeeper'. Its functions are linked to cell adhesion and proliferation, and also suppressive function by inhibiting cell division[4,9]. Consequently, several studies in the literature have demonstraded the importance of Wnt pathway with gene mutation APC and nuclear expression of β-catenin in polyps and hereditary and sporadic colorectal adenocarcinomas.

The correlation of APC protein in polyps and adenocarcinoma performed in this study has shown statistical association with the tendency to positivity and negativity. This demonstrates that when there is an overexpression of this protein in polyps, the same happens to the colorectal adenocarcinoma, favoring the possibility that the mutation of the APC gene is associated with colorectal carcinogenesis not depending on histopathologic variables.

In relation to adenomatous polyps, positive cytoplasmic APC was 45% in this study and 62% in the literature, according to Iwamoto et al.[7]. However, unlike the

literature, in this study we found statistical correlation in tubular histological types (20/43%) and tubulo-villous (18/60%) (p = 0.05). Conversely, there was no statistical correlation of expression of APC with the atypia degree of polyps, which shows that the mutated APC is present in the polyps adenomatous of patients, regardless the atypia degree.

As for the expression of the APC in adenocarcinomas in the present study, the protein expression on APC colorectal adenocarcinoma has shown to be negative and positive at 65% in 35% of patients, a percentage lower than those found in the studies mentioned, ranging from 78.3%[9] to 88%[12].

In all studies reported in the literature, such as Lugli et al.[11], Aust et al.[12] and Ho et al.[13] as well as this study, no statistically significant correlation was observed in colorectal adenocarcinoma and stage, histological grade and histological type of adenocarcinoma, which demontrates that the mutation of APC acts early in colorectal carcinogenesis.

Evaluation of the β-catenin protein expression

β-catenin seems to have a central role in balancing proliferation of colonic mucosa and, by extension, the very mechanism of colorectal carcinogenesis. It is related to the adhesion between cells as a binding protein with transmembrane, known as E-cadherin[4,11]. It can also be observed as a free form in the cytoplasm and still within the nucleus. The mutation in the APC gene has been demonstrated to be associated with an increasing β-catenin nuclear, which represents a significant stimulation of cell division. This proliferative stimulus is a consequence of positive action of β-catenin nuclear for the expression of other proteins which play an important role in cell division, such as Cyclin D1, Gastrin, C-myc, Cox-2 and MMP-7, which the last two are clearly related to angiogenesis and stromal division, respectively[9,10].

There is also a great diversity in the literature in the results found when studying the expression of β-catenin protein by immunohistochemistry in colorectal cancer and polyps[14]. Hao et al.[15] in their study showed a positive nuclear β-catenin in most adenomatous polyps and the expression was directly proportional to the degree of atypia of polyps. In the present study, the nuclear expression of β-catenin in the polyps was 55% and the correlation of this protein statistically significant with only villous histological, which was shown 86% positive (p = 0.042). Concerinng the degree of atypia of polyps, there was no statistical correlation, as well as the positivity of cytoplasmic β-catenin in these tumors and their variables.

These data demonstrate that nuclear expression of β-catenin is correlated with colorectal carcinogenesis, since villous adenoma is more likely to be malignant than the tubular and tubular-villous types.

Evaluation of the β-catenin protein expression in the colorectal adenocarcinoma

Several studies in the literature have demonstrated the importance of nuclear expression of β-catenin in colorectal carcinomas correlated with lymph node and liver metastases[2,9,16].

In this study, nuclear expression of β-catenin protein in colorectal adenocarcinoma was 80%. In the literature, the rates ranged from 42%[14,15], but none of the studies found correlation of nuclear expression of this protein with stage, grade or histological type of tumor.

Moreover, in this study a correlation was performed between nuclear expression of the β-catenin protein in the polyps and adenocarcinoma, which showed statistical association with the positivity and negativity trends, that is, where there was positivity in the polyps, the same occurred in adenocarcinoma; supporting the hypothesis that the pathway of colorectal carcinogenesis is the same either for polyps or adenocarcinoma.

CONCLUSION

In this study there was a correlation between cytopasmic expression of APC protein expression and nuclear β-catenin in the polyps and colorectal adenocarcinoma, which makes us think that these two neoplasms, originating from the same patient show the same pathway of carcinogenesis.

The lack of correlation of both APC expression of β-catenin nuclear on the degree of atypia and polyps os the stage, degree of cellular differentiation and the histological type of adenocarcinomas shows that these proteins act at the beginning of colorectal carcinogenesis.

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