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ORIGINAL ARTICLE

Expression and Mutation Pattern of β-Catenin and Adenomatous Polyposis Coli in Colorectal Cancer Patients

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Background and Aims. β-Catenin and adenomatous polyposis coli *(APC)* are major components of the Wnt pathway. This study aimed to investigate the expression of β-catenin and APC in tumors and lymph nodes in colorectal cancer (CRC) patients and the mutational spectrum of the genes coding these proteins.

Methods. Expression of APC and β-catenin was examined in 124 tumors and 41 lymph nodes. Exon 3 of *CTNNB1* and the *mutation cluster region (MCR)* in exon 15 of the *APC* gene were screened for mutation by PCR-sequencing.

Results. Nuclear/cytoplasmic immunostaining of β-catenin was detected in 58.1 and 48.8% in tumors and lymph nodes, respectively. In tumors, abnormal expression of β-catenin correlated with tumor size and with those in lymph nodes. Membranous β-catenin expression occurred in 41.9 and 14.6% of tumors and lymph nodes, respectively. In tumors, lack of membranous β-catenin correlated with high invasiveness and metastatic potential. Positive immunostaining for APC was observed in 2 and 14% of tumors and lymph nodes, respectively. Overexpression in nucleus/cytoplasm and lack of membranous β-catenin significantly correlated with a reduced overall survival. Among 25 tumors, four harbour mutation in Ser33 and Ser47 and overexpress the β-catenin in the nucleus/cytoplasm. Mutations were identified in the *APC* gene in 13 tumors and six mutations were novel.

Conclusions. Positive association between aberrant expression of β-catenin in the nucleus/cytoplasm of tumors and lymph nodes was observed. Nucleus/cytoplasmic accumulation of β-catenin and loss of membranous expression are related to reduced survival and could serve as a candidate prognostic predictor.

Key Words: Colorectal carcinoma, β-Catenin, APC, Immunohistochemistry, Prognostic markers.

Introduction

Colorectal cancer (CRC) remains the most prevalent gastrointestinal cancer worldwide (1). In Tunisia, the incidence of CRC was 2.5–4.5/100000 (2). During colorectal carcinogenesis, genetic and epigenetic changes occur gradually that trigger the transformation of normal colonic mucosa (3,4). Chromosomal and microsatellite instability pathways constitute the major genetic instability events in CRC (5,6), and aberrant methylation of cancer-related gene promoter is responsible for transcriptional silencing *of tumor suppressor genes (TSG)* in tumor tissues (7–9). In the pathogenesis of CRC, the Wingless (Wnt) pathway is often deregulated, in particular the tumor suppressor Adenomatous Polyposis Coli *(APC)*, which is inactivated by allelic loss, aberrant methylation or mutations (10–15).

In normal cells, free β-catenin anchors to the Axin/APC/GSK3-β complex and undergoes phosphorylation by GSK3-β, leading to its degradation via the proteasome

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(16,17). On the other hand, loss of APC results in destruction of the Axin/APC/GSK3-β complex leading to the accumulation of β-catenin in the cytoplasm and its translocation to the nucleus, where it activates the expression of target genes such as Cyclin D1, c-Myc, CD44 and matrix metalloproteinase 7 jointly with the TcF/LEF (T cell factor/lymphoid enhancer factor) transcription molecule family (17−19).

There are several reports regarding APC and β-catenin alteration and expression in CRC. Lugly et al. showed in a large series of patients that increased expression of nuclear β-catenin and loss of membranous E-cadherin are independent prognostic factors in the group of MMR-proficient CRC (20). Most studies concern Western populations and only few data on North African patients were available. A recent study conducted on 150 Tunisian patients reported that disruption of β-catenin/E-cadherin complex may be considered as a dependent predictor of disease outcome (21). On the other hand, the majority of studies focused on the expression of APC and β-catenin in tumor samples, whereas in the present work we also analyzed the expression in corresponding lymph nodes.

In this study we investigate the clinical significance of two major components of the Wnt signaling pathway, namely, the β-catenin and APC proteins by analyzing their expression in 124 primary tumors and the corresponding lymph nodes of 41 cases. Correlation between APC expression and β-catenin distribution with major clinicopathological features and overall survival were investigated. Furthermore, the mutation cluster region of the APC gene where >60% of somatic mutations occur and exon 3 of the CTNNB1 were investigated in order to better characterize these 2 members of the Wnt signalling pathway in the pathogenesis of CRC.

Materials and Methods

Patient Characteristics

A total of 124 primary sporadic CRC and the corresponding lymph nodes of 41 cases were collected between January 2003 and December 2007 from patients who underwent radical surgical resection at the Department of Digestive Surgery of Habib Bourguiba University Hospital (Sfax, Tunisia). All patients gave informed consent prior to specimen collection according to institutional guidelines. None of the patients had pre- or postoperative chemotherapy. At the time of surgery, patient ages ranged from 25−85 years (mean: 62.9 years). Histological subtypes were classified using the World Health Organization (WHO) criteria (22).

Immunohistochemical Staining

Before immunostaining, two pathologists (LA and AK) reviewed hematoxylin- and eosin-stained slides in each case, and blocks containing adenocarcinoma were selected. Briefly, 4 μm-thick sections were cut from each paraffin block, mounted on poly-L-lysine-coated slides, fixed in acetone for 10 min, and left to dry overnight at 37°C. Slides were deparaffinized in xylene followed by ethanol and subsequent rehydration in graded ethanol. The sections were then pre-treated with 3% hydrogen peroxide for 10 min to inactivate endogenous peroxides and washed in phosphate-buffered saline (PBS) solution. Heat-induced antigen retrieval was performed using epitope retrieval solution (DAKO, Carpinteria, CA) at 95°C for 40 min. After heating, slides were allowed to cool to room temperature, briefly washed in PBS and then incubated in blocking solution (Protein Block Serum: 0.25% Casein in PBS containing Carrier Protein and NaN3; DAKO) for 5 min. Immunohistochemical staining was performed using the streptavidin-biotin peroxidase system. Slides were incubated for 30 min at room temperature with anti-APC (F3, sc-9998 Santa Cruz Biotechnology, Santa Cruz, CA) and anti-β-catenin (12 F7 sc-59737 Santa Cruz Biotechnology) antibodies diluted at 1:100, washed with PBS before applying the biotinylated secondary antibody (anti-rabbit, DAKO) for 5 min. Sections were incubated with the streptavidin-biotin complex reagent (Universal Quick Kit, DAKO) for 15 min and developed with 3,3′diaminobenzidine tetrahydrochloride (DAB) for 30 min. Finally, tissues sections were counter stained by Mayer's hematoxylin, dehydrated, and mounted (DAKO).

The immunostained slides were scored as described previously (23). Briefly, the initial scoring was graded according to the extent of immunostaining as follows: 0: no staining or <5%; 1: 5−25% staining; 2: 26−75% staining; 3: 76−100% staining. In addition, staining intensity was also evaluated as follows: 0: negative; 1: weak; 2: moderate; and 3: intense. When tumors were heterogeneous in staining intensity, each component of the tumor was scored independently. The sum of the intensity and staining extent scores was used as the final immunoreactive score (0−12). The final scores were regarded as negative (0−1 score) and positive (2−12 score). The same immunostaining assessment was used for tumors and lymph nodes.

Mutation Analysis

Genomic DNA was prepared from 25 frozen specimens using standard proteinase Kdigestion and phenol/chloroform extraction after homogenization. All 25 frozen cases were also represented as paraffin-embedded samples. The quantity of DNA was checked by nanodrop (Thermo Fisher Scientific, Wilmington, USA) and stored at −20°C for further use.

Exon 3 of *CTNNB1* and the mutation cluster region (MCR) of the APC gene, were investigated by direct sequencing of genomic DNA. Briefly, PCR reactions containing 0.2 μM of each primer, covering the exon 3 of