The value of histological changes and immunohistochemical markers Ki67 and p53 in the assessment of ulcerative colitis related dysplasia

Ovidiu C. Fratila¹*, Tiberia I. Ilias², Dana Puscasiu¹

¹ University of Oradea, Faculty of Medicine and Pharmacy, 1st of December Street, 410073, Oradea, Romania
² Oradea Clinical County Hospital, Republicii Street, 410167, Oradea, Romania

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Abstract: The risk of carcinoma increases in patients with a 10-year or longer duration of ulcerative colitis (UC). To search for a more objective parameter to assess epithelial dysplasia. The study comprised 25 cases of longstanding UC: 7 cases with regenerative atypia, 7 with low grade dysplasia, 7 with high grade dysplasia, and 4 cases indefinite for dysplasia. The colonic biopsies obtained during endoscopy were stained with H&E to identify the aforementioned categories. Seventy-five sections from biopsy specimens were stained immuno-histochemically to detect differences in the frequency and pattern of nuclei positive for the proliferation marker Ki67 and p53. In high grade dysplasia, the distribution of Ki67 positive cells was diffuse throughout the full length of the crypt, whereas low grade dysplasia and epithelium indefinite for dysplasia, as well as regenerative epithelium, showed an expanded basal zone. None of the regenerative atypia cases showed strong intensity p53 staining compared to dysplasia cases. None of the high grade dysplasia cases showed restricted p53 staining to the lower two thirds of the crypt. All the cases of HGD showed extension of Ki67 and p53 staining above the basal two thirds of the crypt. Ki67 and p53 immunostained cell assessment combined with routine histological evaluation of colorectal mucosa can improve the diagnostic accuracy, as well as the assessment of malignant transformation risk.

Keywords: Ulcerative colitis • Histopathology • Immunohistochemistry • Chronic inflammation • Dysplasia • Carcinoma

1. Introduction

Ulcerative colitis (UC) is commonly characterized by continuous inflammation or cyclic episodes of relapse and remission. On the histological level, the cycling or ongoing disease activity is accompanied both by increased numbers of apoptotic cells throughout the crypt axis and by corresponding epithelial lining of regenerating areas [1].

Colorectal carcinoma is a dreadful complication of UC. The risk of developing cancer increases in patients with longstanding UC (7 to 10 years) with a rate of approximately 0.5-1% per year and therefore surveillance colonoscopy has been widely recommended [2]. In most instances, cancer evolves through a neoplastic process called dysplasia. Dysplasia (intraepithelial neoplasia) is present in more than 70% of patients with chronic UC and cancer, coinciding with the location of the cancer, which arises from chronically inflamed mucosa [3].

Dysplastic lesions are difficult to recognize by means of endoscopy. It is also a tough task to differentiate them histologically from inflammatory regenerative changes of the epithelium. The efficacy of current surveillance remains unsatisfactory and thus, it becomes clear that we need additional methods to improve the early detection of cancer.

Evaluation of dysplasia in long-standing UC is a difficult and often subjective task. Although the routine pathological examination provides important information...
about the chronic inflammation-carcinoma sequence, the relationship between the morphological changes or remodeling of regenerated mucosa, and carcinoma development, is yet to be clarified.

In order to overcome these difficulties, adjunctive modalities for diagnosing UC-associated neoplasia, chromo- and magnifying endoscopy for endoscopic diagnosis, and analysis of p53 and Ki67 alteration for histological diagnosis, have been introduced.

Furthermore, if it were possible to differentiate UC patients with long-standing and extensive forms of colitis into subgroups with a high and a low risk of neoplasia, it would enable physicians to conduct more intensive surveillance using these modalities for patients at higher risk [2].

Protocols for the immunohistochemical analyses of Ki67 and p53 expression in fixed, paraffin-embedded tissue, are well established. Antibodies for these techniques are readily available and many laboratories are experienced in the use of such antibodies, particularly the MIB-1 antibody to Ki67 [4].

Ki67 is a huge nuclear protein that plays an important role in cellular proliferation. The monoclonal antibody Ki67 detects a nuclear antigen, which reflects cell proliferation and identifies the growth fraction of tissues and tumors [5]. Recently, Ki67 like antibodies (MIB 1) were developed that are reactive in sections of formalin fixed, paraffin wax embedded tissue after antigen retrieval.

p53 is a protein that plays an important role in regulating the cell cycle and in controlling cellular proliferation. A mutation in this protein is the most common finding in human cancers [6].

The aim of our paper was to find a more objective parameter to help distinguish regenerative changes from epithelial dysplasia and to correlate these results with the routine histological evaluation of colorectal mucosa. We were also interested in determining whether such staining techniques could be used to distinguish between low-grade dysplasia and high-grade dysplasia.

2. Material and Methods

The morphological and immunohistochemical study concerning UC related dysplasia comprised 25 cases of longstanding UC (more than 7 to 10 years); 7 cases with regenerative atypia, 7 cases with low grade dysplasia, 7 cases with high grade dysplasia and 4 cases interpreted as indefinite for dysplasia as stated by the IBD Morphology Study Group criteria (1983).

The biopsies obtained during colonoscopy were fixed in formaldehyde (10%) for 24-48 hours, embedded in paraffin, then cut into 3 to 4 µ thick sections. Then, we made serial multiple sections (8-10 per case). In order to establish the pathological diagnosis and to include the case into a group of lesions, the first sections were stained using routine H&E histological methods. The examination of the H&E stained slides was made using a light microscope Carl Zeiss Ergaval type (ob. ×40, ob. ×100). The microscopic examination of these biopsies allowed us to classify and group the lesions.

From this assessment, a collection of 75 sections from biopsy specimens obtained from these patients were selected and then stained immunohistochemically to detect differences in the frequency and pattern of nuclei positive for the proliferation marker Ki67 and p53.

Three-micrometer thick sections were cut from the tissue blocks, dewaxed and rehydrated. For Ki67 immunostaining, antigen retrieval was carried out and then the sections were stained using the MIB-1 antibody to Ki67 at a dilution of 1:100, using Dako ChemMate™ reagents and detection agents as described in the manufacturer’s operating manual. For p53 immunostaining, antigen retrieval was carried out using citric acid buffer (1.05 g in 500 ml distilled water, pH 6) and microwaving at full power for 20 minutes. Sections were pretreated with 1.5% hydrogen peroxide for 15 minutes followed, after antigen retrieval, by 1:5 normal rabbit serum for 10 minutes and then the DO-7 antibody to p53 (Dako, UK) at a dilution of 1:100 for 60 minutes. Visualization was achieved by incubation with rabbit antimouse antibody (1:400) for 30 minutes. A single observer assessed all sections blindly.

Cases were divided into four groups depending on the extent of Ki67 staining: ‘basal zone’ (staining restricted to the basal third of the crypt); “middle zone” (extension of staining into the middle third); “top zone” (extension into the upper third); and “surface” (extension into the surface epithelium).

Two variables of p53 immunostaining were assessed: location of staining (as for Ki67), and intensity of staining - weak, moderate, strong. Weak p53 intensity staining was defined as staining which could only be detected at × 10 objective magnification and greater, strong intensity staining was defined as an equal intensity to that seen in the colorectal carcinoma used as the p53 positive control and moderate intensity staining was defined as any intensity in between.