

**PROLIFERATIVE ACTIVITY (Ki67) AND EARLY APOPTOSIS (M30)  
ASSOCIATED WITH CASPASE-3 (CPP32) IN ADENOMAS,  
ADENOCARCINOMAS AND NORMAL EPITHELIAL COLON CELLS**

BARBARA ŚLESAK<sup>1</sup>, ANTONINA HARŁOZIŃSKA<sup>1</sup>, JADWIGA  
ŁAPIŃSKA<sup>2</sup>, TOMASZ BOJAROWSKI<sup>3</sup>, JOLANTA ADAMIAK<sup>1</sup>  
and MARCIN KOTLAREK<sup>1</sup>

<sup>1</sup>Department of Clinical Immunology, <sup>2</sup>Clinic of Gastroenterology, <sup>3</sup>Department  
of Oncology, Medical Academy of Wrocław, 50-368 Wrocław, Poland

One of the earliest events occurring in colon cancer arising from the adenoma-carcinoma sequence is the alteration of the proliferation of epithelial cells in colonic crypts. Programmed cell death is the process which modulates the quality and degree of cell proliferation. The family of cysteine proteases – caspases – are responsible for the cleavage of intermediate cytoskeletal filaments (ICF). One of the major components of the ICF of epithelial cells and tumors originating from such cells is a cytokeratin 18 (CK18). In the early phases of apoptosis, CK 18 is cleaved by a caspase-3/ CPP32 liberating a neo-epitope which is specifically recognized by the M30 monoclonal antibody. The aim of our study was to evaluate the degree of proliferation and caspase-3 mediated apoptotic activity by immunohistochemical analysis in adenoma, adenocarcinoma and normal epithelial colon cells. The expression of Ki67 antigen, caspase-3/ CPP32 and M30 were examined prospectively using the EnVision method in 21 and 15 cases of colon adenomas and adenocarcinomas, respectively, and in 13 cases of colon normal epithelium taken a 7-10 cm distance from malignant lesions. In normal colonic epithelial cells, a balance between proliferative and apoptotic activity was observed. In adenomas and adenocarcinomas the growth fraction activity was higher than apoptosis. The expression of caspase-3/ CPP32 was the highest in adenomas (39.3% positive cells), lower in adenocarcinomas (15.5%) and the lowest in normal epithelium (9.2%). A comparative analysis of CPP32 and M30 expression in all locations allowed us to show the different phenotypes. In normal colon epithelium and in adenomas, CPP32+/M30+ immunostaining dominated (85%, 86%, respectively), by contrast to adenocarcinomas, in which only 47% of cases showed this phenotype. In 15%, 9% and 7% of adenocarcinomas, adenomas and normal colon epithelium, the activation of the caspase-3-dependent mechanism was not sufficient to cause early apoptosis. Phenotype CPP32-/M30+ in 40% of adenocarcinomas, and in 5% of adenomas was found, and was undetectable in normal colonic crypts. In the adenoma-carcinoma sequence, the degree of apoptotic malfunction increased. Our data define caspase-3 as an important integrator of apoptosis and reveal an essential role of caspase-3 in apoptotic pathways.

This study was partially supported by grant GU 302.