

# Evaluation of apoptosis, proliferation intensity and metallothionein (MT) expression in comparison with selected clinicopathological variables in primary adenocarcinomas of the large intestine

Małgorzata Dumańska<sup>1</sup>, Piotr Dzięgiel<sup>1, 3</sup>, Mirosław Sopol<sup>1</sup>, Andrzej Wojnar<sup>3</sup>,  
Maciej Zabel<sup>1, 2</sup>

<sup>1</sup>Department of Histology and Embryology, University School of Medicine, Wrocław; Poland

<sup>2</sup>Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland

<sup>3</sup>Department of Pathomorphology, Lower Silesia Centre of Oncology, Wrocław, Poland

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*Tumour growth and expansion are the result of proliferative activity and the capacity to eliminate cells by apoptosis and/or necrosis. The present study was aimed at comparing the apoptosis and proliferation intensity in cells of adenocarcinomas of the large intestine with the expression of metallothionein (MT), the grade of the tumour and the depth to which the tumour infiltrated the intestinal wall. The TUNEL technique and immunocytochemical reactions (expression of caspase-3, Ki-67, MT) were used to detect apoptosis. The results demonstrated augmented levels of all the variables examined, positively correlated with grade of malignancy, G, and with the depth of intestinal wall infiltration by the tumour cells. The testing of apoptosis, proliferation and MT expression may prove useful in the appraisal of the growth and progression of primary adenocarcinomas in the large intestine.*

**Key words:** large intestine adenocarcinoma, apoptosis, metallothionein, Ki-67 antigen

## INTRODUCTION

Adenocarcinomas of the large intestine are among the most frequently developing malignant tumours. For prolonged periods of time, the tumours may not manifest any specific symptoms, developing in a latent manner until the process is markedly advanced. The late diagnosis, as well as the lack of independent prognostic markers, results in unsatisfactory therapeutic results [8].

Genetic defects play a significant role in the pathogenesis of tumours. The mutations affect not

only the genes responsible for cell proliferation but also those for coding the normal course of the cell cycle. The target genes include suppressor genes (APC, p53) and proto-oncogenes (e.g., K-ras) [2].

The growth and expansion of adenocarcinomas in the large intestine represent the outcome of proliferative activity and the capacity to eliminate tumour cells by apoptosis and necrosis. Several sources on the participation of proliferation and apoptosis in large intestinal tumours document no unequivocal links between various clinicopathological variables [7].

In recent years an increasing number of reports have pointed to the important role of metallothioneins (MT) in carcinogenesis and to the involvement of the proteins in the control of cell proliferation and in the apoptotic process [3, 5].

The present study aimed at comparing the intensities of apoptosis, proliferation and MT expression in the cells of primary adenocarcinomas of the large intestine with the grade of histological immaturity (G) and the depth of intestinal wall infiltration by the tumour cells.

## MATERIAL AND METHODS

The material for the studies was obtained from 39 patients with primary adenocarcinoma of the large intestine treated in the Lower Silesia Centre of Oncology in Wrocław (DCO) in the period 1993–1994. Clinicopathological data on the patients was obtained from the DCO archive and made use of case histories and histopathology reports. Tumour samples were fixed in 4% buffered formaline and embedded in paraffin. Apoptosis was detected by the TUNEL technique using ApopTag<sup>®</sup>Plus Peroxidase *In situ* Apoptosis Detection Kit (Intergen, Norcross, USA). Expression of MT (isoforms MT-I and MT-II), Ki-67 antigen and the active form of caspase-3 were examined by conducting appropriate immunocytochemical reactions with specific monoclonal antibodies (clones E9, Ki-S5, CPP32 — DAKO, Denmark). Colour reactions were developed using the *EnVision* (DAKO, Denmark) system. Intensity of apoptosis, caspase-3 and Ki-67 antigen expression was evaluated using a 3-point scale: 3–5% positive cells corre-

sponded to 1 point, 6–10% positive cells to 2 points and over 10% positive cells to 3 points. Expression of MT was appraised employing the scale (0–12 points) of Remmele and Stegner [6]. Selected clinicopathological variables, including grade of histological immaturity (G) and the depth to which neoplastic cells infiltrated the intestinal wall [1], were also used. Statistical analysis employed the chi-square test, performed using Statistica 5.1 PL software (StatSoft, Cracow, Poland). Differences were accepted as significant at  $p < 0.05$ .

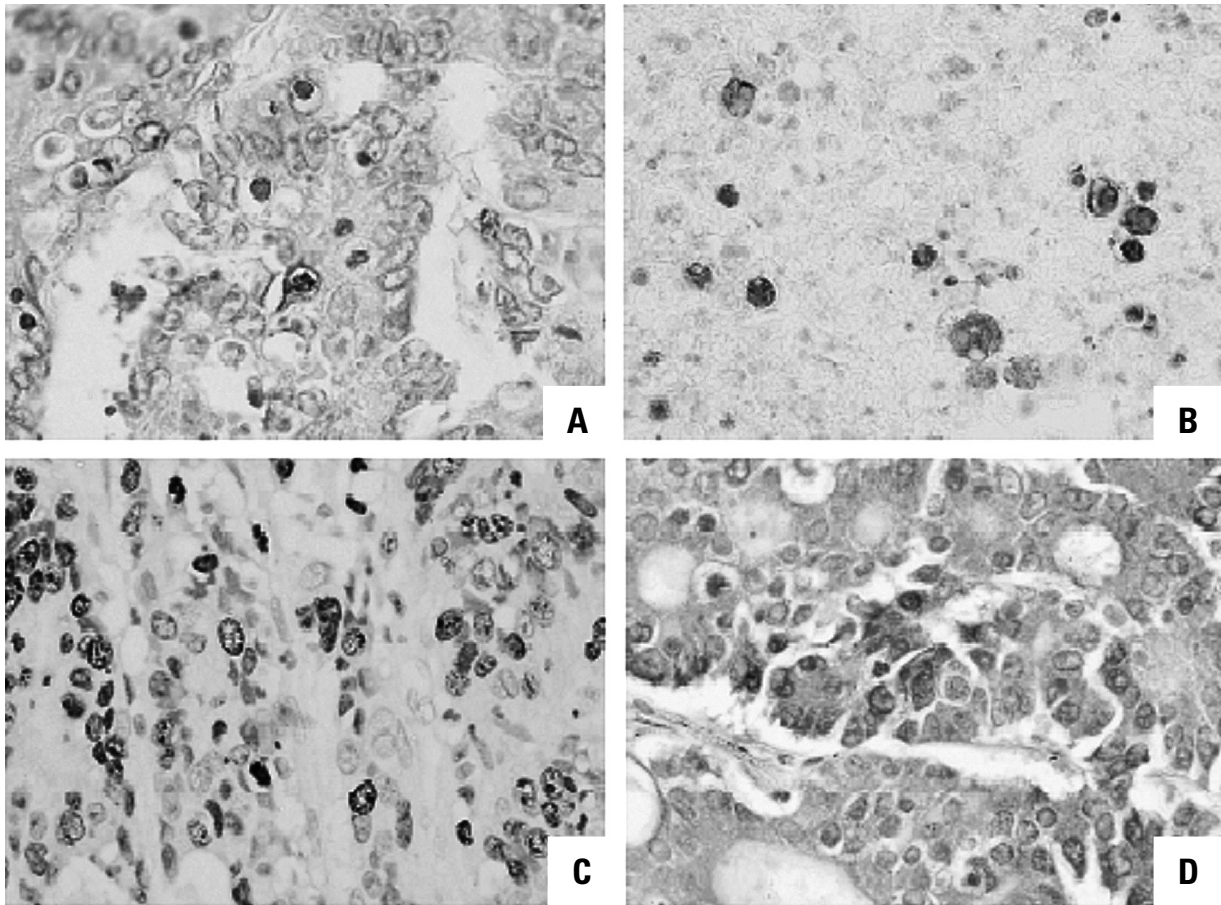
## RESULTS AND DISCUSSION

The tumours studied demonstrated similar mean levels of apoptosis whether the TUNEL technique was performed or expression of caspase-3 was measured. In the least mature (most malignant, G2, G3) adenocarcinomas the numbers of apoptotic cells and of cells demonstrating expression of caspase-3 were significantly higher than in G1 cases (Table 1, Fig. 1A, B). The results have proved consistent with those obtained by other authors and point to a relationship between intensity of apoptosis and grade of histological immaturity (G) [7]. Similar tendencies were documented in studies on proliferation (intensity of expression of Ki-67 antigen) in large intestine adenocarcinoma cells (Table 1, Fig. 1C). MT expression was also significantly more pronounced in less mature (more malignant, G2, G3) tumours (Table 1, Fig. 1D). The data confirmed our earlier results on the prognostic significance of MT expression in this type of tumour [1]. The results enable the conclusion to be drawn that less mature adenocarcinomas

**Table 1.** Mean intensities of apoptosis (TUNEL) and metallothionein (MT) expression, expression of Ki-67 antigen and that of caspase-3 as related to grade of histological immaturity (G) and depth of intestinal wall infiltration in primary adenocarcinoma of the large intestine. A — mucosa and submucosa; B — mucosa, submucosa and muscle layer; C — mucosa, submucosa, muscle layer and adipose tissue

Grade of histological immaturity (malignancy), G	MT	Ki-67	Caspase-3	TUNEL
G1	2.33 ± 1.53	1.33 ± 0.58	1.0 ± 0.0	1.33 ± 0.58
G2	3.18 ± 1.61*	1.86 ± 0.47*	1.6 ± 0.72*	1.59 ± 0.66
G3	4.5 ± 1.99**	2.21 ± 0.58**	1.85 ± 0.86*	1.86 ± 0.86*
Depth of intestinal wall infiltration	MT	Ki-67	Caspase-3	TUNEL
A	1.72 ± 0.64	1.56 ± 0.76	1.37 ± 0.54	1.41 ± 0.48
B	2.86 ± 0.74*	1.73 ± 0.48	1.44 ± 0.68	1.45 ± 0.57
C	4.2 ± 0.47**	2.41 ± 0.72**	1.83 ± 0.76*	1.89 ± 0.62*

Significant differences: \* $p < 0.05$  as compared to G1 or A; \*\* $p < 0.05$  as compared to G1 and G2 or A and B



**Figure 1.** Adenocarcinoma of the large intestine. TUNEL technique (A) and immunocytochemical reactions (B, C, D). Staining with haematoxylin.  $\times 200$ . **A.** Apoptosis in tumour cells; **B.** Nuclear expression of caspase-3 in some tumour cells; **C.** Nuclear expression of Ki-67 antigen in numerous tumour cells; **D.** Nuclear and cytoplasmic expression of metallothionein (MT) in tumour cells.

(G2, G3) exhibit more intense proliferation and a higher intensity of apoptosis. This indicates that the processes of proliferation and apoptosis are reciprocally linked. The relation may reflect the fact that intense uncontrolled proliferation leads to replication errors and the accumulating mutations direct the cells to apoptotic pathways.

Apart from histological maturity, our analysis also involved indices of apoptosis and proliferation and MT expression in relation to the depth to which the intestinal wall was infiltrated by the neoplastic cells. This last represents one of the most significant variables which determine the therapeutic potential and course of the disease [8]. In tumours which infiltrated the intestinal wall more deeply (the more advanced ones) the intensity of apoptosis and proliferation as well as of MT expression was higher. Our results, related to proliferation intensity as compared to depth of infiltration, have corroborated our earlier data [1]. On the other hand, the few other reports on the rela-

tionship between intensity of apoptosis and depth of intestinal wall infiltration have frequently brought divergent and equivocal results [4, 7].

To sum up, our data on the intensities of apoptosis and proliferation in large intestine adenocarcinomas appear consistent and positively correlated with MT expression. Augmented levels of all the variables also correlate with the grade of local advancement of the tumour (depth of intestinal wall infiltration) and with its histological immaturity (grade). The testing of apoptosis, proliferation and MT expression intensities may prove helpful in evaluation of the growth and progression of primary adenocarcinomas in the large intestine.

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