Intensity of apoptosis as related to the expression of metallothionein (MT), caspase-3 (cas-3) and Ki-67 antigen and the survival time of patients with primary colorectal adenocarcinomas

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Abstract

The present study aimed at analysing the intensity of apoptosis, as related to the expression of pro- and anti-apoptotic cellular markers (cas-3, MT, Ki-67 antigen), and at evaluating their expression in relation to the survival time of patients with colorectal adenocarcinoma. Material for the studies was obtained from 40 patients with primary colorectal adenocarcinomas (G2, T3N0M0), treated at the Lower Silesia Centre of Oncology in Wrocław. Tumour samples were fixed in 4% buffered formalin and embedded in paraffin blocks. In obtained paraffin sections, TUNEL reaction (detection of apoptosis) and immunocytochemical reactions were performed (detection of cas-3, MT and Ki-67 antigen expression). The results disclosed a weak correlation between the intensity of apoptosis and the expression of MT, cas-3 and Ki-67 antigen ($r = 0.18$; $r = 0.33$; $r = 0.15$, respectively) in cells of colorectal adenocarcinomas. The survival time of the patients was shorter, when the apoptosis and expression of Ki-67 antigen were highly pronounced. The time periods of the patients' survival showed no correlation with the expression of cas-3 or MT. The obtained results point to the key role of apoptosis and proliferation processes in the clinical course of colorectal adenocarcinomas.

Key words: colorectal adenocarcinoma, metallothionein, apoptosis.

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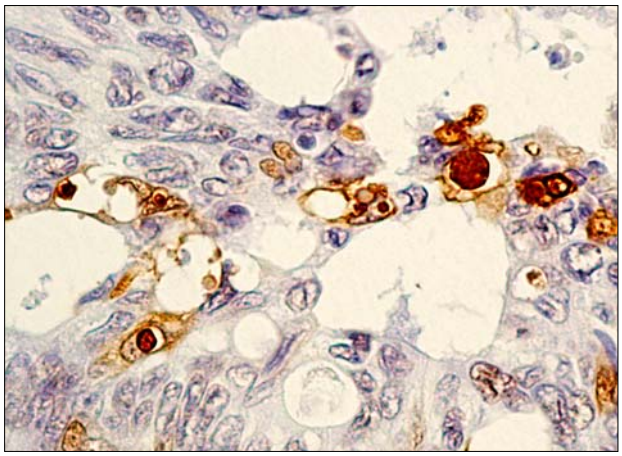
Introduction

The time of survival of patients with neoplastic disease provides the best estimate of aggressiveness, advancement of the disease, as well as of the effectiveness of treatment. Also, in cases of primary colorectal adenocarcinoma, this criterion permits to undertake appropriate therapeutic decisions (chemo- and/or radiotherapy before and/or after surgery) [1]. In colorectal adenocarcinomas, the most important prognostic factors include the morphological traits of the tumours, including its size, histological type, the grade of malignancy G (the extent of cancer cell differentiation, the frequency of mitoses, necrosis) and the depth of infiltration of the intestinal wall [2]. Immunocytochemical indices of tumour growth dynamics include, i.a., proliferation-associated antigens (e.g. Ki-67, PCNA) and, recently, also metallothionein (MT) [3, 4], a low molecular weight (7kDa) protein of a polypeptide chain which consists, depending on the isoform, of 61-68 amino acids [5]. During the recent years, several studies have demonstrated an anti-apoptotic activity of MT, both in normal and in tumour cells [6]. Tumour growth and expansion are known to reflect the proliferative activity and the capacity to eliminate cells by apoptosis and/or necrosis. Therefore, it seemed interesting to examine reciprocal correlations between the intensity of apoptosis and the expression of such pro- and anti-apoptotic cell markers as caspase-3 (cas-3), MT and Ki-67 antigen. The present study aimed at comparing the expression of the above markers and the intensity of apoptosis in cells of primary colorectal adenocarcinomas with the time periods of survival of the affected patients.

Material and Methods

The material for the studies was obtained from 40 patients with primary colorectal adenocarcinoma, treated at the Lower Silesia Centre of Oncology (DCO) in Wrocław between 1993-1994. Clinico-pathological data on the patients, in form of histories of the disease and histopathological diagnoses, were

Figure 1. Apoptosis in cells of primary colorectal carcinoma. x400; background staining with haematoxylin.



recovered from the DCO archive. Tumour samples were fixed in 4% buffered formalin and embedded in paraffin blocks. Apoptosis was detected, using the TUNEL method and the ApopTag®Plus Peroxidase In situ Apoptosis Detection Kit (Intergen, USA). The expression of MT (isoforms I and II), Ki-67 antigen and the active form of cas-3 were documented by performing immunocytochemical reactions with monoclonal antibodies (respective clones: E9, Ki-S5, CPP32, DakoCytomation, Denmark). Colour reaction was developed, using the EnVision system (DakoCytomation, Denmark). The intensity of apoptosis, the expression of cas-3 and of Ki-67 antigen were quantified, using a three-point scale: 3-5% positive cells - 1 pt, 6-10% positive cells - 2 pts, over 10% positive cells - 3 pts. The expression of MT was quantified, using the IRS scale, according to Remmele (0-12 pts), taking into account both the intensity of the colour reaction and the proportion of positive cells [7]. All the studied tumours showed G2 grade and B2 advancement stage, according to Duke (as modified by Astler and Coller), which corresponded to the clinical classification of T3N0M0 (the 2nd stage of clinical advancement) [2]. The obtained results were subjected to statistical analysis, using the STATISTICA PL software (StatSoft, Poland) and employing Spearman's correlation tests and Kaplan-Meier's survival analysis.

Results

In all the examined tumours, the expression of individual markers was variable as to its site. In the case of MT, a nuclear-cytoplasmatic expression, for cas-3 a cytoplasmatic expression and for Ki-67 antigen - a nuclear expression were disclosed. The intensity of apoptosis in cancer cells was variable in individual tumours (Fig. 1). The analysis of correlation between apoptosis and MT, cas-3 and Ki-67 antigen expression demonstrated that the parameters were poorly related to one another ($r = 0.18$; $r = 0.33$; $r = 0.15$; $p < 0.05$). In turn, the survival times of the patients with either high or moderate intensity of apoptosis were significantly shorter ($p < 0.05$) than those in the patients with a low extent of apoptosis in cancer cells (Fig. 2). Similar results were obtained in the analysis of the patients' survival times, as related to the expression of Ki-67 antigen. The longest survival time was noted at low expression of the antigen, while the shortest survival time was associated with a high and moderate expression of the antigen (Fig. 3). No significant differences in the survival time were associated with varying expressions of cas-3 or MT.

Figure 2. Survival times in patients with primary colorectal adenocarcinoma, the cells of which demonstrated high (d = 3 pts), moderate (s = 2 pts) or low (m = 1 pt) intensity of apoptosis.

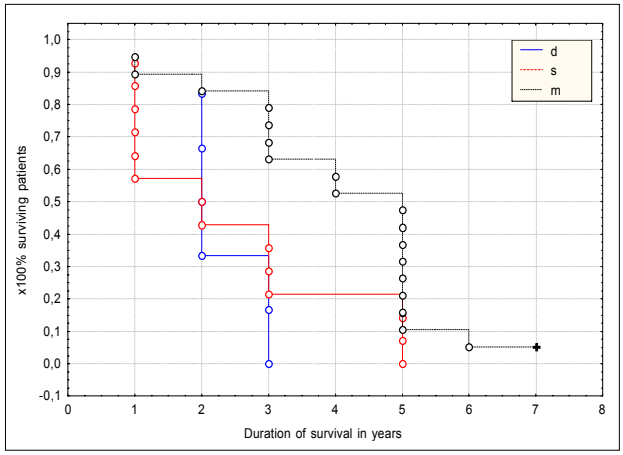
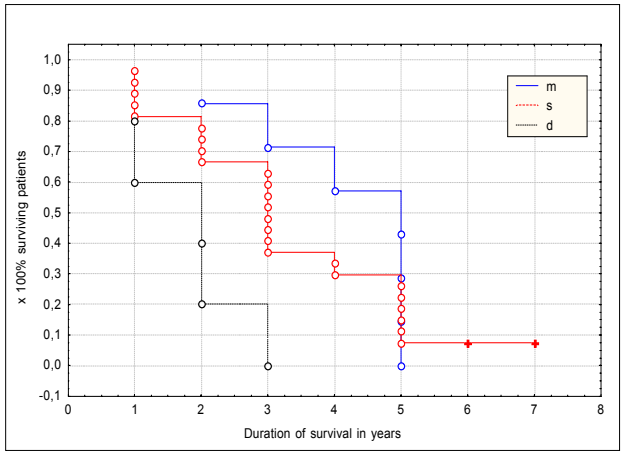


Figure 3. Survival times in patients with primary colorectal adenocarcinoma, the cells of which demonstrated high (d = 3 pts), moderate (s = 2 pts) or low (m = 1 pt) expression of Ki-67 antigen.



Discussion

The growth and expansion of a tumour are associated with several mechanisms which control cell processes, including cell proliferation and apoptosis. Among the recognized immunocytochemical markers, which reflect the activity of the processes, the expression of MT was also taken into account. The expression of the protein points to an augmented proliferative activity of neoplastic cells and to a less favourable prognosis [3]. Some data are also available, which indicate that MT may act as an anti-apoptotic factor, both in normal and tumour cells [6]. In our studies, an attempt was made to define reciprocal relations between the expression of MT, Ki-67 antigen, the intensity of apoptosis, measured by the TUNEL technique and the expression of cas-3. The significance of the markers was analyzed for the time of survival in patients with primary colorectal adenocarcinoma. In our earlier studies, and in investigations of other

authors, the expression of Ki-67 antigen was found to positively correlate with the expression of MT and with the advancement stage (the depth of intestinal wall infiltration) of colorectal adenocarcinomas [8, 9, 10]. Moreover, augmented expressions of Ki-67 antigen and of MT were observed in patients with tumours of G2 or G3 grade of malignancy and in patients with shorter survival times [8]. In the present studies, a group of patients was selected, uniform in respect to the clinical advancement and G grade. No reciprocal relations were noted between the intensities of expression of the parameters, that pointing out to an extensive heterogeneity of the studied tumours. The analysis of the patients' survival time periods, as related to the expression of Ki-67 antigen, confirmed our earlier reports, indicating a significant prognostic role of the parameter [8]. The marginally positive correlation between the expression of Ki-67 antigen and the intensity of apoptosis demonstrates that proliferation and apoptosis in tumours are not always strictly linked to each other. The principal aim of our studies involved a demonstration of the prognostic significance of apoptosis in cells of colorectal adenocarcinomas. The obtained results are inconsistent with the few earlier results of other authors [11] and indicate higher aggressiveness of colorectal carcinomas in cases with higher intensity of apoptosis. This suggests the need for further studies on apoptosis as a prognostic factor in primary colorectal adenocarcinoma.

References

1. Nowacki MP. Colorectal carcinoma (in Polish). In: Krzakowski M, editor. Onkologia kliniczna. 1st ed. Warszawa: Borgis® Wydawnictwo Medyczne; 2001, p 235-53.

2. Jass JR. Tumors of the small and large intestines (including the anal region). In: Fletcher CDM, editor. Diagnostic histopathology of tumors. 2nd ed. London: Churchill Livingstone; 2000, p 369-409.

3. Dzięgiel P. Expression of metallothioneins in tumor cells. Pol J Pathol, 2004; 55: 3-12.

4. Ioachim EE, Goussia AC, Agnantis NJ, Machera M, Tsianos EV, Kappas AM. Prognostic evaluation of metallothionein expression in human colorectal neoplasms. J Clin Pathol, 1999; 52: 876-9.

5. Coyle P, Philox JC, Carem LC, Rofe AM. Metallothionein: The multipurpose protein. Cell Mol Life Sci, 2002; 59: 627-47.

6. Shimoda R, Achanzar WE, Qu W, Nagamine T, Takagi H, Mori M, Waalkes M. Metallothionein is potential negative regulator of apoptosis. Toxicol Sci, 2003; 73: 294-300.

7. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe, 1987; 8: 138-40.

8. Dzięgiel P, Forgacz J, Suder E, Surowiak P, Kornafel J, Zabel M. Prognostic significance of metallothionein expression in correlation with Ki-67 expression in adenocarcinomas of large intestine. Histol Histopathol, 2003; 18: 401-7.

9. Dumańska M, Dzięgiel P, Sopel M, Wojnar A, Zabel M. Evaluation of apoptosis, proliferation intensity and metallothionein (MT) expression in comparison with selected clinicopathological variables in primary adenocarcinomas of the large intestine. Folia Morphol, 2004; 63: 107-10.

10. Kuroda K, Aoyama N, Tamura T, Sakashita M, Maekawa S, Inoue T, Wambura C, Shirasaka D, Minami R, Maeda S, Kuroda Y, Kasuga M. Variation in MT expression in early-stage depressed-type and polypoid-type colorectal tunours. Eur J Cancer, 2002; 38: 1879-87.

11. Sinicrope FA, Hart J, Hsu HA, Lemoine M, Michelassi F, Stephens LC. Apoptotic and mitotic indices predict survival rates in lymph node-negative colon carcinomas. Clin Cancer Res, 1999; 5: 1793-1804.