Superoxide dismutases in relation to the overall survival of colorectal cancer patients

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Summary Reactive oxygen metabolites are implicated in the initiation and promotion of cancer. In addition, oxidant scavengers, such as manganese – (Mn-SOD) and copper/zinc – superoxide dismutase (Cu/Zn-SOD), are thought to contribute to colorectal cancer treatment response. In the present study, the prognostic significance of the Mn- and Cu/Zn-SOD antigen content of normal mucosa and carcinomas of 163 patients with colorectal cancer was evaluated in comparison with major clinicopathological parameters, with respect to the 5-year overall survival. The Mn-SOD content of carcinomas was found to be significantly higher than that of normal mucosa, whereas there was no difference in the Cu/Zn-SOD content between the normal mucosa and carcinomas. No association was demonstrable between the Mn-SOD and Cu/Zn-SOD content of the tissues and the assessed clinicopathological parameters (gender, age, localization, differentiation grade, diameter and Dukes' stage), with the exception of the Cu/Zn-SOD and the differentiation grade of the carcinomas. Univariate analysis showed that a high Mn-SOD content of carcinomas was associated with a poor 5-year overall survival of the patients with colorectal cancer. Multivariate analysis including all clinicopathological parameters revealed that this Mn-SOD parameter was prognostically independent. The Mn- and Cu/Zn-SOD content of normal mucosa and the Cu/Zn-SOD content of carcinomas were not associated with the overall survival of the patients. In conclusion, this study demonstrates that for patients with colorectal cancer the Mn-SOD content of colorectal carcinomas has a significant prognostic value that is independent from major clinicopathological parameters, including Dukes' stage.

Keywords: colorectal cancer; survival; superoxide dismutases

Reactive oxygen metabolites (ROMs), i.e. hydrogen peroxide, superoxide anion (O_2^-) and hydroxyl radical (OH·), are inevitable (by)products of aerobic metabolism and are formed continuously in vivo (Sahu, 1991). A delicate balance between the generation of these toxic and unstable metabolites and the levels of endogenous antioxidants is of critical importance for normal cell functioning. When produced excessively or during deficient antioxidant defences, these ROMs can mediate DNA damage, lipid peroxidation, enzyme oxidation, etc., leading to cellular destruction, chromosomal aberrations and finally to cancer (Slater, 1984; Borek, 1987; Farber et al, 1990; Sahu, 1991; Guyton and Kensler, 1993; Wiseman and Halliwell, 1996). Paradoxically, chemotherapy, radiation therapy, photodynamic therapy and some cytokine therapies, for example tumour necrosis factor α (TNF-α), have been shown to exert part of their therapeutic efficacy by generating large amounts of these noxious radicals to kill tumour cells (Oberley and Buettner, 1979; Petkau, 1987; Oberley, 1990; Sangeetha et al, 1990). Therefore, endogenous antioxidant proteins, as a primary defence against these ROM-generating anti-cancer therapies, might play an important role in colorectal cancer therapy resistance.

One of the most important enzymes involved in the primary cellular defence against these ROMs is superoxide dismutase (SOD), which detoxifies superoxide anion to hydrogen peroxide.

Studies with cell lines and animal models revealed the relevance of antioxidants, for example SOD, with respect to the effectiveness and side-effects of colorectal cancer therapies (Petkau, 1987; Hauser et al, 1990; Eastgate et al, 1993; Hirose et al, 1993; Kizaki et al, 1993; Urano et al, 1995; Wong, 1995). In humans, SOD is known to be present in at least two forms, a constitutive cytoplasmic copper/zinc (Cu/Zn)-SOD and an inducible mitochondrial manganese (Mn)-SOD (McCord and Fridovich, 1969; Fridovich, 1975; Beyer et al, 1991; Farber, 1994). We recently showed that colorectal adenomas, carcinomas and liver metastases are characterized by a significantly increased antigen and activity level of Mn-SOD compared with normal colorectal mucosa. In contrast, no major differences were found in the Cu/Zn-SOD levels (Janssen et al, 1997). Advances in the early diagnosis, screening procedures of high-risk individuals, the surgical approach and adjuvant therapy, have hardly changed the prognosis of colorectal cancer in the last few decades (Sinnige and Mulder, 1991; Greenwald, 1992; Van Triest et al, 1995; Winawer, 1995). Until recently, only Dukes' and related stage classifications have been accepted as the most important prognostic parameters for the survival of these patients (Jass et al, 1986; Beahrs, 1992; Deans et al, 1992; Ponz de Léon et al, 1992; Bosman, 1995). However, additional functionally relevant prognostic factors that predict clinical outcome and support treatment planning for subgroups of patients with colorectal cancer might be very useful.

In the present study, we determined the Cu/Zn-SOD and Mn-SOD antigen content of normal mucosa and carcinomas of 163 patients with colorectal cancer, and evaluated their relation with clinicopathological parameters and their prognostic value for the 5-year overall survival of the patients.

MATERIALS AND METHODS

Patients and study design

All 163 patients (69 women and 96 men) were operated on for a histologically proven adenocarcinoma of the colorectum at the Department of Oncologic Surgery of the Leiden University Medical Center. Immediately after resection, fresh samples from the mid-central non-necrotic part of the carcinoma and/or from normal mucosa, taken approximately 10 cm from the tumour, were frozen and stored at $-70°C$ until extraction, when available for research purposes. From this group of patients, several clinical and pathological data were evaluated and registered or retrieved from their data files. The tumours were histologically classified according to Dukes' stage, as modified by Astler and Coller (1954). There were seven patients with Dukes' stage A, 21 with B_1, 61 with B_2, 17 with C_1, 37 with C_2, and 20 patients with Dukes' stage D cancer, corresponding to UICC (Hermanek and Sobin, 1992) TNM stages O (Dukes' A), I (Dukes' B_1), II (Dukes' B_2), III (Dukes' C_1 and C_2), and IV (Dukes' D). Thirty-seven patients with Dukes' stage B or C tumours received additional radio- ($n = 27$) or chemotherapy ($n = 4$) or both ($n = 2$), after the primary resection or during follow-up, or had to have a second resection ($n = 9$), based on clinicopathological indications. All patients entered the study at operation date and follow-up was at least 5 years, or shorter in the event of death.

Ninety-five patients (58.3%, 38 women and 57 men) died during follow-up and 68 (41.7%, 31 women and 37 men) were still alive at the common closing date of the follow-up. The overall survival of the patients gradually decreased from those with carcinomas classified as Dukes' A (85.7%), Dukes' B_1 (71.4%), Dukes' B_2 (49.2%), Dukes' C_1 (41.2%), Dukes' C_2 (27.0%) to those with Dukes' D carcinomas (0.0%), indicating a representative population of colorectal cancer patients.

Tissue extraction and protein concentration

Extractions were prepared from 50–100 mg wet tissue samples. The samples were wet weighed, and 1 ml of 0.1 M Tris-HCl, pH 7.5, with 0.1% (v/v) Tween 80 per 60 mg of sample was added. The tissue was homogenized for 2 min on ice in a Potter S (B Braun). The homogenates were centrifuged twice at 8000 g for 2.5 min at 4°C and the final supernatants were stored at $-70°C$. The protein concentration of the supernatants was determined using the method of Lowry et al (1951). The intra-tumour coefficient of variation of the procedure was assessed by processing six adjacent tissue parts from six different tumours and was found to be 10% (range 4–15%) for the protein extraction.

Standards and antibodies

The standards used were human recombinant (hr) Mn- and Cu/Zn-SOD, kindly provided by Dr Z Yavin from the Kyriat Weizmann Institute, Rehovot, Israel. The monospecific antibodies raised in rabbits showed no cross reactivity between the two SOD forms (Mn vs Cu/Zn) and provided no signal with other proteins of tissue homogenates on Western blotting.

Enzyme-linked immunosorbent assay (ELISA) for Cu/Zn-SOD

The Cu/Zn-SOD antigen level was determined by a modified ELISA, as described previously (Mulder et al. 1990; Götz et al.

1996). Microtitre plates (Dynatech Laboratories. USA: M129A) were coated with affinopurified goat α-human Cu/Zn-SOD (10 µg ml⁻¹ in 0.05 M carbonate buffer. pH 9.6) overnight at 4°C. followed by PBST/gelatine [phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20 and 0.2% (v/v) gelatine] for 30 min. After washing, 100 µl of each homogenate diluted 1:400 in PBST/gelatin was added in duplicate followed by incubation for 2 h. The plates were then washed and rabbit α-(hr)Cu/Zn-SOD polyclonal antiserum (1:2500 dilution in PBST) was added to the wells and incubated for 1 h. The final antibody, a polyclonal goat α-rabbit IgG conjugated to horseradish peroxidase (Dakopatts. P448. 1:5000 dilution in PBST) was preincubated before use with 0.2% preimmune goat serum for 30 min. After an incubation period of 1 h with the final antibody, the plates were coloured with a solution of 40 mg orthophenylenediamine and 40 µl hydrogen peroxide in 100 ml citric acid/phosphate buffer. pH 5.0. for 30 min. The reaction was terminated using 50 µl 2.5 M sulphuric acid. The optical density was read at 492 nm on a Titertek Multiscan (Flow Laboratories. UK) plate reader. The Cu/Zn-SOD concentration was calculated from a standard curve between 1.25 and 30 ng ml⁻¹ (hr)Cu/Zn-SOD and expressed per mg protein of the homogenate. The intra- and inter-assay coefficients of variation of this ELISA were 4% and 6% respectively. The intra-tumour coefficient of variation of the Cu/Zn-SOD level was found to be 17% (range 5–32%).

ELISA for Mn-SOD

This procedure is similar to the Cu/Zn-SOD ELISA described previously (Götz et al. 1996). The plates were incubated overnight at 4°C with an affinopurified rabbit α-(hr)Mn-SOD polyclonal antibody (10 µg ml⁻¹ in 0.05 M carbonate buffer. pH 9.6). The homogenates were diluted 1:150 and incubated in duplicate for 2 h at room temperature with PBST as assay diluent. The standard line of (hr)Mn-SOD ranged from 1.25 to 40 ng ml⁻¹. After incubation with the tissue homogenates. the plates were washed and incubated for 90 min with a rabbit α-(hr)Mn-SOD coupled with horseradish peroxidase (1:250 dilution in PBST). After a final wash. bound antibodies were detected as described for Cu/Zn-SOD. The intra- and inter-assay coefficients of variation of this ELISA were 5% and 10% respectively. The intra-tumour coefficient of variation of the Mn-SOD level was 21% (range 9–35%).

Statistical analyses

The significance of the differences in the mean superoxide dismutase antigen levels between different patient and sample groups was assessed by ANOVA and the unpaired Student's t-test, with separate variance estimates if the standard deviations were significantly different according to the F-test. For the statistical survival analyses of this group of patients, the clinicopathological parameters were dichotomized as follows: Dukes' stage was divided into Dukes A/B vs C/D; tumour localization in the colon into right-sided (from caecum to splenic flexure) and left-sided (from splenic flexure to the end of the rectum); diameter of the tumour into < 4 cm vs ≥ 4 cm; tumour differentiation into well/moderately vs poorly differentiated; and gender into men vs women. The cut-off points of the age and the significant SOD parameters were determined by slowly increasing the level until the point of best discrimination was found. i.e. the optimal dichotomization.

Univariate survival analysis was performed with the Cox proportional hazard model (Cox. 1972), using the SPSS 6.0 statistical
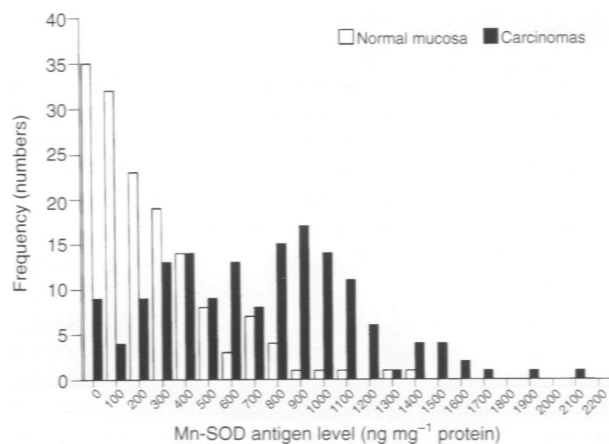
Figure 1 Frequency distribution of the normal colorectal mucosa (*n* = 150, open bars) and the colorectal carcinomas (*n* = 156, black filled bars) according to their Mn-SOD antigen level

software package (SPSS, Chicago, IL, USA), resulting in identification of covariates which significantly correlated with the overall survival of the patients.

Multivariate survival analyses were performed using the Cox proportional hazards method by separately adding the significant SOD variables to the six dichotomized clinicopathological parameters (i.e. age and gender of the patients, and Dukes' stage, diameter, differentiation and localization of the tumours). Overall survival curves were constructed using the method of Kaplan and Meier (1958). The statistical significance of the difference in survival of the groups was calculated using the log-rank test.

Differences were considered significant when the *P*-value was ≤ 0.05.

RESULTS

Mn-SOD and Cu/Zn-SOD concentrations

The Mn-SOD content of the carcinomas (714 ± 34 ng mg^{-1} protein, *n* = 156) was found to be significantly ($P < 0.0005$) higher than that of the normal mucosa (257 ± 22 ng mg^{-1} protein, *n* = 150). Despite an overlap in the absolute Mn-SOD level between carcinomas and normal mucosa (Figure 1), a large majority (82.5%) of the tumours had a higher level than their corresponding normal mucosa. Concerning the Cu/Zn-SOD content, there was no significant difference between the mean level of the carcinomas (527 ± 19 ng mg^{-1} potein, *n* = 155) and that of the normal mucosa (535 ± 17 ng mg^{-1} protein, *n* = 149).

When the normal mucosa and carcinomas were divided into two subgroups according to the survival or the dichotomized clinicopathological parameters, no significant differences in Mn-SOD concentration were noticed. There were also no significant differences in the Cu/Zn-SOD level except for the concentration of the poorly differentiated carcinomas, which was significantly lower than that of the well/moderately differentiated carcinomas (Table 1).

SOD concentrations and survival

Optimal dichotomization of the Mn-SOD concentration of the carcinomas resulted in two cut-off points, at 330 and 975 ng mg^{-1} protein. At both cut-off points, a high Mn-SOD level of the carcinomas was associated with a relatively poor survival of the patients in the

Table 1 Mn-SOD and Cu/Zn-SOD antigen level (ng mg^{-1} protein) in normal mucosa and colorectal carcinomas dichotomized according to various clinicopathological parameters. Results shown are mean values ± s.e.

Parameter dichotomized	Normal mucosa		Carcinoma	
	Mn-SOD (*n*)	Cu/Zn-SOD (*n*)	Mn-SOD (*n*)	Cu/Zn-SOD (*n*)
Patients				
Alive	288 ± 42 (62)	554 ± 26 (62)	664 ± 52 (67)	537 ± 29 (67)
Deceased	236 ± 24 (88)	521 ± 23 (87)	752 ± 45 (89)	519 ± 27 (88)
Gender				
Women	283 ± 36 (65)	532 ± 22 (65)	728 ± 49 (67)	529 ± 28 (66)
Men	237 ± 28 (85)	538 ± 25 (84)	704 ± 47 (89)	525 ± 27 (89)
Age (years)				
< 66.1	247 ± 37 (60)	524 ± 29 (60)	684 ± 47 (66)	513 ± 26 (65)
≥ 66.1	265 ± 28 (90)	543 ± 21 (89)	736 ± 48 (90)	537 ± 28 (90)
Localization				
Right colon	244 ± 30 (55)	559 ± 29 (55)	747 ± 59 (57)	516 ± 36 (57)
Left colon	265 ± 31 (95)	521 ± 21 (94)	695 ± 41 (99)	532 ± 23 (98)
Differentiation				
Well/moderate	275 ± 30 (76)	539 ± 24 (75)	653 ± 44 (80)	571 ± 30 (80)
Poor	240 ± 33 (74)	532 ± 25 (74)	779 ± 51 (76)	479 ± 24 (75)[a]
Diameter				
< 4 cm	263 ± 55 (32)	511 ± 37 (32)	744 ± 67 (35)	494 ± 35 (34)
≥ 4 cm	256 ± 24 (118)	542 ± 19 (117)	706 ± 39 (121)	536 ± 23 (121)
Dukes' stage				
AB	273 ± 32 (82)	545 ± 22 (81)	674 ± 43 (84)	548 ± 29 (81)
CD	239 ± 31 (68)	524 ± 27 (68)	761 ± 53 (72)	501 ± 25 (71)

[a]Compared with well/moderate (*P* = 0.02).

Table 2 Univariate and multivariate analysis of the categorized Mn-SOD concentration of the carcinomas in relation to overall survival of patients with colorectal cancer

Mn-SOD antigen[a]	Survivors/total n/n (%)	Median survival (months)	Cox hazard ratio	(95% CI, P-value)
			univariate	multivariate
≤ 330	21/34 (61.8)	64.5		
> 330	46/122 (37.7)	35.5	2.0 (1.1–3.7. 0.02)	1.9 (1.1–3.5. 0.03)
< 975	55/116 (47.4)	59.0		
≥ 975	12/40 (30.0)	30.5	1.5 (1.0–2.4.0.07)	1.2 (0.7–1.8. NS)
≤ 330	21/34 (61.8)	64.5		
330–975	34/82 (41.5)	45.0	1.9 (1.0–3.5. 0.05)	1.9 (1.0–3.6. 0.04)
≥ 975	12/40 (30.0)	30.5	2.4 (1.2–4.6. 0.01)	1.9 (1.0–3.7. 0.07)

[a]ng mg^{-1} protein; CI. confidence interval; NS. not significant. Multivariate analysis was performed by adjusting the Mn-SOD parameter to the clinicopathological parameters (gender. age. localization, differentiation grade. diameter, and Dukes' stage).
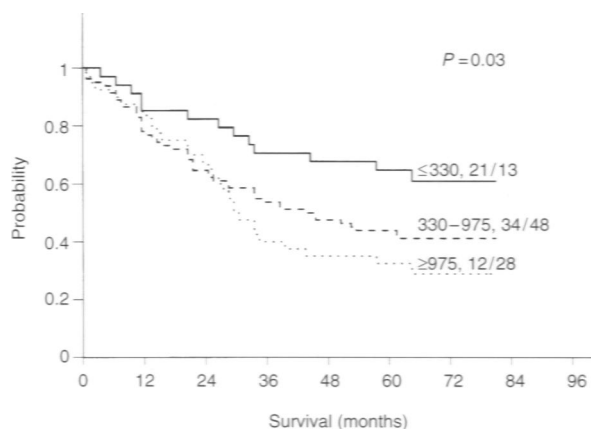

Figure 2 Overall survival curves according to high (≥975 ng mg^{-1} protein). intermediate (330–975 ng mg^{-1} protein) and low (≤330 ng mg^{-1} protein) Mn-SOD level in carcinomas of patients with colorectal cancer. Values are the number of patients alive/deceased at the end of the follow-up. Statistical significance according to the log-rank test

univariate analysis (Table 2). Based on these two cut-off points. the Mn-SOD concentration was stratified into three subgroups with sufficient numbers in each group: the first group included carcinomas with a Mn-SOD level ≤ 330 ng mg^{-1} protein. the second group contained between 330 and 975 ng mg^{-1} protein. and the third group had ≥ 975 ng mg^{-1} protein. Univariate Cox analysis. evaluating the prognostic value of Mn-SOD as a stratified variable. showed that patients with carcinomas containing ≤ 330 ng Mn-SOD per mg protein had a significantly longer survival time than those with a level of ≥ 975. Those patients with carcinomas containing a

Mn-SOD antigen level between 330 and 975 ng mg^{-1} protein showed an intermediate survival (Figure 2 and Table 2).

The multivariate Cox analyses of the dichotomized clinicopathological parameters revealed that only Dukes' stage of the tumour [hazard ratio 2.8 (95% confidence interval 1.8–4.3). P < 0.00005] and age of the patient [hazard ratio 2.1 (95% confidence interval 1.4–3.4). P = 0.001] were independently associated with survival. The dichotomized and stratified Mn-SOD variables were finally also adjusted to all the assessed clinicopathological parameters. In this multivariate analysis. a high Mn-SOD concentration (i.e. > 330 ng mg^{-1} protein) remained associated (0.03 ≤ P ≤ 0.07) with a relatively poor 5-year survival of the patients. indicating its independent prognostic value (Table 2). Concerning the Mn-SOD antigen level in normal mucosa and the Cu/Zn-SOD antigen concentration of the carcinomas and normal mucosa. no cut-off point. discrimating between survivors and non-survivors. could be identified.

Table 3 shows the multivariate analyses of the Mn-SOD parameter within subgroups of patients according to their Dukes' stage. With regard to the patients with Dukes' stage B or C carcinomas. all tested levels had a prognostic value with the exception of the higher cut-off point (≥ 975 vs < 975 ng mg^{-1} protein). Furthermore. this table indicates that within the subgroup of patients with Dukes' stage B the higher cut-off point (≥ 975 vs < 975 ng mg^{-1} protein and ≥ 975 vs ≤ 330 ng mg^{-1} protein) had a prognostic impact. whereas within the subgroup of patients with Dukes' stage C the lower cut-off point (330–975 vs ≤ 330 ng mg^{-1} protein and to a lesser extent > 330 vs ≤ 330 ng mg^{-1} protein) was of prognostic value. Within the subgroup of patients with Dukes' stage B or C. the survival of those who received additional treatment after the primary surgical resection was significantly (P = 0.01) poorer than the survival of those patients

Table 3 Multivariate analysis based on the Mn-SOD concentration of the carcinomas within Dukes' stage subgroups of patients with colorectal cancer in relation to the overall survival

	Mn-SOD (ng mg^{-1} protein)			
	> 330 vs ≤ 330	≥ 975 vs < 975	330–975 vs ≤ 330	≥975 vs ≤ 330
Dukes				
C vs B	2.0 (1.0–3.8. 0.04)[a]	1.3 (0.8–2.2. NS)	1.9 (1.0–3.8. 0.07)	2.1 (1.0–4.5. 0.05)
B₂ vs B₁	1.5 (0.6–3.5. NS)	2.4 (1.1–5.4. 0.03)	1.1 (0.4–2.8. NS)	2.6 (1.0–6.9. 0.06)
C₂ vs C₁	2.5 (0.8–7.7. 0.1)	1.0 (0.4–2.2. NS)	2.7 (0.8–8.8. 0.009)	2.1 (0.6–7.5. NS)

[a]Cox hazard ratio (95%₀ confidence interval. P-value): NS. not significant. Multivariate analysis was performed by adjusting the Mn-SOD parameter to the clinicopathological parameters (gender. age. localization. differentiation grade. diameter and Dukes' stage).

who did not receive an additional therapy (27.0% vs 52.5%). However, there was no difference in the Mn-SOD content of the carcinomas between these two groups of patients [751 ± 70 (n = 36) vs 699 ± 44 ng mg^{-1} protein (n = 96) respectively]. From the 32 patients who had had additional radio- and/or chemotherapy, nine were still alive and 23 had died at the end of follow-up. Between the carcinomas of these patients, there was a remarkable, though not statistically significant (P = 0.14), difference in the Mn-SOD content, respectively 591 ± 139 and 841 ± 86 ng mg^{-1} protein.

DISCUSSION

Colorectal carcinomas were found to be associated with a significant increase in the Mn-SOD content compared with the normal mucosa, whereas there was no significant difference in the Cu/Zn-SOD content between the normal mucosa and carcinomas. In addition, the Mn-SOD level in the tumours was found to be an independent prognostic indicator for the overall survival of the patients.

An increased level of ROMs in colorectal cancer development and/or stimulation by cytokines, such as TNF or interleukin 1, produced by the cancerous cells themselves or by infiltrating macrophages, probably act as autocrine factors to induce Mn-SOD in the carcinomas (Wong and Goeddel, 1988; Salim, 1992; Valentine and Nick, 1992; Molmenti et al, 1993; Qureshi et al, 1994, Yoshimi et al, 1994; Warner et al, 1996). Although the exact mechanisms that cause the alterations in the antioxidant enzyme levels, particularly Mn-SOD, in cancer are not yet known (as reviewed by Oberley and Oberley, 1997), they may be clinically highly relevant with regard to patient selection and the administration and development of (neo-)adjuvant therapy in colorectal cancer.

Except for the Cu/Zn-SOD content of poorly differentiated carcinomas, which appeared to be lower than that of well-differentiated or moderately differentiated carcinomas, no association between the Mn-SOD and Cu/Zn-SOD content of the carcinomas and any of the six assessed clinicopathological parameters was noticed, indicating their independent regulation. Optimal dichitomization of the Mn-SOD content of the carcinomas in relation to survival resulted in two cut-off points. The Mn-SOD parameter could thus be analysed either as a dichotomized parameter or as a variable comprising three categories. Univariate analysis of this Mn-SOD parameter revealed a significant association between a high Mn-SOD antigen content of the colorectal carcinomas and a relatively poor 5-year overall survival. In contrast, for the Cu/Zn-SOD content of the carcinomas, it was not possible to identify a cut-off point discriminating between survivors and non-survivors. Multivariate Cox's proportional hazard analysis with the six dichotomized clinicopathological parameters (gender, age, localization, differentiation grade, diameter and Dukes' stage) revealed only staging and patient age as independent prognostic variables. Adding the Mn-SOD content of the carcinomas as a parameter to this multivariate model revealed, for the first time, the independent prognostic value of Mn-SOD for the overall 5-year survival of patients with colorectal cancer.

Recognition of prognostic factors is of great significance for outcome prediction and treatment planning in colorectal cancer, which is the second leading cause of cancer-related death in the Western world. The Dukes' pathological staging system, either in its original or in its modified form, is still the most powerful predictor of final outcome in colorectal cancer patients against which all other prognostic factors in colorectal cancer should be assessed. This pathological staging system, based upon tumour invasiveness,

lymph node involvement and distant metastases, is also the basis for advocating additional therapy after radical surgery, offering adjuvant chemo-, immuno- and/or radiotherapy only to those patients with prognostically less favourable disease (Jass et al, 1986; Beahrs, 1992; Deans et al, 1992; Ponz de Léon et al, 1992). However, until now these therapeutic modalities have been successful only in a minority of the cases, and the overall survival rate of colorectal cancer has not improved dramatically in the last decade (Bosman, 1995). Therefore, it is of crucial importance to identify additional prognostic parameters that also relate to treatment response, enabling better patient selection for adjuvant therapy.

Little is known from the literature about the prognostic value of endogenous antioxidants, including SOD, to the survival of (colorectal) cancer patients. Öfner et al (1994) evaluated the prognostic significance of immunohistochemical expression of metallothionein (MT), a hydroxyl radical-scavenging metalloprotein, in colorectal adenocarcinomas. They found a statistically significant correlation of high MT expression and favourable clinical outcome in a univariate analysis but not in a multivariate analysis with Dukes' stage as a stratification factor. There have been several reports about the clinical significance of the Mn-SOD serum level as a tumour marker for cancer patients. Ishikawa et al (1990) found elevated Mn-SOD levels in the serum of patients with epithelial ovarian cancer and these correlated with the clinical stage of the disease and with the response to treatment. Similarly, Schadendorf et al (1995) reported elevated serum Mn-SOD levels in patients with malignant melanoma compared with normal controls, and these elevated Mn-SOD concentrations corresponded to tumour load and correlated with progression of malignant melanoma. Furthermore, an increased SOD activity level of carcinomas was found to be associated with the malignant intensity of colorectal carcinomas (Satomi et al, 1995). In our present study, however, we could not demonstrate any significant association between the Mn-SOD content of the carcinomas, primarily localized in the malignant epithelial cells (unpublished observation), and the malignancy parameters of the tumour (i.e. Dukes' stage, differentiation grade, diameter, etc.). Very recently, Landriscina et al (1996) reported enhanced expression of Mn-SOD, evaluated by Western blotting and immunohistology, in neuroepithelial brain tumours which correlated with the grade of differentiation. Furthermore, they indicated that a low Mn-SOD level in glioblastomas was associated with a longer survival and a high level with a shorter survival of the patients. Interestingly, one of these brain tumours was found to be a metastasis of colon cancer which expressed a high level Mn-SOD. Our study extends these observations, indicating that colorectal tumours are not only characterized by increased Mn-SOD levels but that this SOD isoform also acts as a functionally relevant and independent prognostic parameter to the overall survival of these patients.

Because ROMs may be involved in the mechanism(s) by which several anti-cancer treatments, including chemotherapy, immunotherapy, photodynamic therapy and radiotherapy, exert their therapeutic effect (Oberley and Buettner, 1979; Petkau, 1987; Oberley, 1990; Sangeetha et al, 1990), it can be hypothesized that a relatively high Mn-SOD level of the colorectal carcinomas in our study contributes to tumour cell resistance and therapy insensitivity resulting in a poor clinical outcome. In support of this hypothesis, Nakano et al (1996) recently demonstrated that the Mn-SOD level of cancer cells was an important prognostic factor in radiation therapy sensitivity for patients with cervical carcinoma, i.e. cervical tumours expressing Mn-SOD were associated

with a significantly poorer survival than those negative for Mn-SOD. Also, other studies have shown that SOD protects cells in tissue and laboratory animals against the harmful (side-)effects of ionizing radiation (Petkau, 1987; Eastgate et al, 1993; Hirose et al, 1993; Wong, 1995), cytokines (Wong et al, 1989; Hauser et al, 1990; Hirose et al, 1993; Kizaki et al, 1993), and several anti-cancer drugs (Hirose et al, 1993; Zyad et al, 1994). It is supposed that Mn-SOD removes toxic superoxide radicals and protects against the damaging effects of these oxygen radical-mediated treatments. In our study, there was no significant difference in the Mn-SOD content of the carcinomas between those patients who received additional treatment and those who did not after the primary surgical resection, within the subgroup of patients with Dukes' stage B or C. The relatively poor survival of the patients who did receive additional treatment, because of the clinicopathological indication of incomplete resection and/or tumour recurrence, was to be expected. The observation that the patients who were given additional radio- and/or chemotherapy and were still alive at the end of follow-up had considerable lower tumour Mn-SOD levels than those who had died, however, conveys the impression that this SOD isoform contributes to therapy resistance in colorectal cancer.

In conclusion, the increased Mn-SOD content of colorectal carcinomas can be regarded as an independent prognostic parameter for overall survival in colorectal cancer. Further larger studies, for example in which the Mn-SOD content of carcinomas or cell lines will be related to (adjuvant) therapy sensitivity and efficacy, are necessary to elucidate the underlying mechanism.

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