Prognostic Significance of Metallothionein in Human Gastrointestinal Cancer


ABSTRACT

Purpose: Metallothionein (MT) is a small protein with a high affinity for divalent heavy metal ions. This metalloprotein is involved in many (patho)physiological processes, like metal homeostasis and detoxification, cell proliferation, apoptosis, therapy resistance, and protection against oxidative damage. Alterations in the immunohistochemical expression of MT have been reported for various human tumors, and a high expression has been found to be associated with a poor clinical outcome. We showed previously that gastrointestinal cancer is accompanied by a decrease in MT expression, but the most malignant phenotypes had the highest MT levels. The purpose of the present study was to assess the clinical relevance of MT in gastrointestinal cancer.

Experimental Design: In this study, we determined the MT levels, by radioimmunoassay, in intestinal tissue of 251 patients with colorectal cancer and 81 patients with gastric cancer and assessed the relation with the overall survival of these patients.

Results: More than 74% of the carcinomas were found to have a lower MT level than their corresponding normal mucosa. In colorectal cancer patients, but not in gastric cancer patients, a high MT level in both the carcinomas and normal mucosa was, however, significantly associated with a poor overall survival, independently from clinicopathological features.

Conclusions: Overexpression of MT in intestinal tissue of colorectal cancer patients is a prognostic marker for a poor overall survival. In gastric cancer, however, MT expression in the gastric mucosa is not of prognostic significance. This observation emphasizes the clinical relevance of this multifunctional metalloprotein in colorectal carcinogenesis and therapy.

INTRODUCTION

MT is a ubiquitous small intracellular metalloprotein with a high content of cysteins exhibiting a selective affinity for divalent heavy metal ions, such as zinc and cadmium (1). In humans, the synthesis of MT is regulated by polymorphic genes and induced by many factors like metals, hormones, cytokines, drugs, and physical and oxidative stresses (2). Besides playing a homeostatic role in the control and detoxification of the heavy metals, several lines of evidence indicate that MT has the capacity to scavenge ROMs, particularly the hydroxyl radical (3). These substances, which are produced continuously during normal aerobic metabolism, may become noxious in situations of dysbalance with endogenous antioxidants and then can induce DNA damage, lipid peroxidation, enzyme oxidation, etc., leading to cellular destruction, chromosomal aberrations, and finally to cancer (4–7). Paradoxically, anticancer treatments such as radio, chemo, and photodynamic therapy, kill tumor cells by generating toxic amounts of ROM (8–10).

By scavenging ROM, antioxidant proteins like MT may play an important role in carcinogenesis, including tumor cell pathobiology and drug resistance (2, 11, 12). In this context, we showed previously that colorectal and gastric carcinogenesis was associated with a significant increase in the level of Mn-SOD, an antioxidant enzyme that detoxifies superoxide to hydrogen peroxide. Furthermore, a high Mn-SOD content of gastric and colorectal cancers was associated with a relatively poor overall survival of the patients (13–15). Concerning MT, conflicting results have been reported concerning its immunohistochemical expression in tumor tissue, as compared with normal tissue and its association with clinicopathological parameters and survival (16–26). We showed previously that colorectal and gastric carcinogenesis was associated with a significant decrease in MT content as measured by both RIA and immunohistochemistry (27), which was confirmed recently by others in colorectal cancer using gene expression analysis by DNA array techniques (28). In the present study, we evaluated the prognostic significance of the MT content in colorectal and gastric cancer patients.

The abbreviations used are: MT, metallothionein; ROM, reactive oxygen metabolite; TNM, Tumor-Node-Metastasis; Mn-SOD, manganese containing superoxide dismutase.
MATERIALS AND METHODS

Patients and Study Design. Fresh tissue could be collected prospectively from 251 patients (111 females and 140 males, median age 68.8 years, range 37.4–89.6) operated on for a histologically proven adenocarcinoma of the colorectum and from 81 patients (21 females and 60 males, median age 66.2 years, range 35.1–91.3) who underwent resection for primary gastric adenocarcinoma, 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 distant normal mucosa, taken ~10 cm from the tumor, were frozen and stored at ~70°C until extraction, when available for research purposes. From these two groups of patients, several clinical and pathological data were evaluated and registered or retrieved from their data files. All carcinomas were histologically classified according to the TNM classification (UICC 1992). In addition, macroscopic pathological features as localization and diameter of the carcinomas were assessed. Microscopic histological parameters, including the differentiation grade of the colorectal carcinomas and the WHO and Laurén (29) classification, as well as the absence/presence of intestinal metaplasia in the normal gastric mucosa, were revised by one pathologist.

All patients entered the study at operation date, and a patient’s time experience ended in the event of death or, when still alive, at the common closing date. For patients with colorectal cancer, the minimal follow-up was 5 years. The 5-year overall survival decreased gradually with TNM stage, from TNM I (77.8%, n = 45), to TNM II (51.5%, n = 99), to TNM III (30.6%, n = 72), and to TNM IV (0%, n = 35), indicative of a representative population of patients. Forty-eight of these patients received additional radiotherapy (n = 39), chemotherapy (n = 8), or both (n = 1), most of them with a TNM II (n = 18) or TNM III (n = 27) stage tumor. For the colorectal cancer patients, the minimal follow-up was 33 months and similar to the colorectal cancer patients with a decreasing overall survival according to TNM stage, i.e., from TNM I (52.2%, n = 23), to TNM II (26.9%, n = 26), to TNM III (28%, n = 25), and to TNM IV (0%, n = 7).

Tissue Preparation and Protein Concentration. From 50–100 mg of wet tissue samples, homogenates were prepared. The samples were wet weighed, and 1 ml of 0.1 M Tris-HCl (pH 7.5) with 0.1% (volume for volume) Tween 80 extraction buffer/60 mg sample was added as described previously (13–15, 27, 30). 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, 4°C, and the final supernatants were stored at −70°C. The protein concentration of all homogenates was determined using the method of Lowry et al. (31).

RIA for MT. MT was determined using an RIA as described previously (27, 30). In brief, MT was isolated from the liver of a patient with primary biliary cirrhosis using a combination of gel filtration and anion exchange chromatography; antisera were raised in rabbits, and the MT was labeled with 125Iodine (32). Assay buffer was 0.05 M Tris-HCl (pH 8.0), degassed, and nitrogen gas saturated with 0.1% (w/v) hydrolyzed gelatin and 0.01% (w/v) sodium azide (NaN3). Incubation consisted of 0.5 ml of appropriately diluted standard or sample, 0.1 ml of rabbit-α-MT serum (1/3000 final dilution), and 0.1 ml of diluted 125I-MT providing ~5000 counts/min.

The standard line ranged from 0.4 to 100 ng of MT/ml, and tissue samples were diluted 1/50. After incubating for 4 days at 4°C, the antibody-bound fraction was precipitated using 0.5 ml of sheep-α-rabbit antibodies coupled to microsephorose beads, and the pellets were counted in a gamma counter. Intrassay and interassay coefficients of variation were 3% and 7%, respectively. The intratumor variation was assessed in six colorectal tumors in sextuplicate, which resulted in a coefficient of variation of 19% (range 9–34%).

Statistical Analyses. Data obtained by the RIA are expressed as mean ng MT/mg protein ± SE. The differences in the MT antigen levels between different patient and sample groups were assessed by ANOVA and the unpaired Student t test, with separate variance estimates if the SDs were significantly different according to Levene’s F test, and by the paired Student t test where appropriate. For the statistical overall survival analyses of the group of colorectal cancer patients, the clinicopathological parameters were optimally dichotomized as described previously (14), i.e., gender into males versus females, age in years into ~66.1 versus ≤66.1, TNM stage was divided into TNM I/II versus III/IV, tumor localization in the colon into right sided (from cecum to splenic flexure) and left sided (from splenic flexure to the end of the rectum), diameter of the tumor into <4 cm versus ≥4 cm, and tumor differentiation into well/moderate versus poor differentiated. Similarly, the clinicopathological parameters of the gastric cancer patients were dichotomized (15); gender into males versus females, age in years into <66.2 versus ≥66.2, TNM stage in stage I/II versus stage III/IV, tumor localization in antrum versus corpus, fundus and cardia, diameter of the tumor into <5 cm versus ≥5 cm, Laurén classification in diffuse/mixed versus intestinal, WHO classification in differentiated (papillar, tubular, mucinous, and adenosquamous) versus undifferentiated (signet cell and undifferentiated), and intestinal metaplasia in normal mucosa in present versus absent. The optimal cutoff points of the MT parameters in carcinoma and normal mucosa were determined by slowly increasing the level until the point of best discrimination was found, i.e., optional dichotomization.

Univariate survival analyses were performed with the Cox proportional hazards model (33), using the SPSS 8.0 statistical software package (SPSS, Inc., Chicago IL), resulting in the identification of covariates that 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 MT variables to the dichotomized clinicopathological parameters. Overall survival curves were constructed using the method of Kaplan and Meier (34), and the statistical significance of the difference in survival of the groups was calculated using the Log-rank test. Differences were considered significant when: P ≤ 0.05.

RESULTS

MT Concentration. The MT content of colorectal carcinomas (187 ± 5.6 ng of MT/mg protein, n = 235) was found to be significantly (P < 0.0005) lower than that of normal colorectal mucosa (303 ± 8.1 ng of MT/mg protein, n = 238). Gastric carcinomas (118 ± 7 ng of MT/mg protein, n = 81)
Table 1 MT antigen level (ng/mg protein) in normal mucosa and carcinomas according to some clinicopathological parameters. Results shown are mean values ±SE.

<table>
<thead>
<tr>
<th>Parameter dichotomized</th>
<th>Normal mucosa MT (ng/mg)</th>
<th>Carcinoma MT (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>290 ± 13 (82)</td>
<td>176 ± 7.1 (80)</td>
</tr>
<tr>
<td>Deceased</td>
<td>311 ± 10 (156)</td>
<td>193 ± 7.6 (155)</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>328 ± 13 (85)</td>
<td>204 ± 11 (83)</td>
</tr>
<tr>
<td>Left colon</td>
<td>290 ± 10 (153)</td>
<td>178 ± 6.3 (152)</td>
</tr>
<tr>
<td>Gastric cancer patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>128 ± 8.6 (15)</td>
<td>119 ± 14 (15)</td>
</tr>
<tr>
<td>Deceased</td>
<td>166 ± 9.3 (66)</td>
<td>118 ± 8.1 (66)</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>134 ± 7.6 (34)</td>
<td>110 ± 8.5 (34)</td>
</tr>
<tr>
<td>Other</td>
<td>177 ± 12 (47)</td>
<td>123 ± 10 (47)</td>
</tr>
</tbody>
</table>

a vs. alive (P = 0.02); b vs. right (P ≤ 0.03); c vs. alive (P = 0.004); d vs. antrum (P = 0.003).

contained also significantly (P < 0.0005) less MT as compared with their corresponding normal mucosa (159 ± 7.9 ng of MT/mg protein, n = 81). In 84.2% (187 of 222) of the colorectal carcinomas, the MT content was lower compared with that of their corresponding normal counterpart; similarly of the gastric carcinomas, 74.1% (60 of 81) were found to have less MT than their corresponding normal gastric mucosa.

MT Levels in Relation to Clinicopathological Parameters. Divided into two subgroups according to the survival of the patients, colorectal carcinomas of patients who had died during follow-up contained significantly (P = 0.02) more MT than colorectal carcinomas of patients who were still alive, whereas in the normal mucosa, no difference was found. In contrast, in gastric cancer patients, the MT content of the normal mucosa was significantly higher (P = 0.004) in the patients who had died as compared with those still alive at the end of the follow-up, but the levels in the carcinomas were similar (Table 1).

Analysis of the MT levels in relation to the clinicopathological parameters revealed that in colorectal cancer patients, there was a significant (P ≤ 0.03) association between a rightsided localization and a high MT content of both the normal mucosa and carcinoma tissue, as compared with those located in the left side of the colon (Table 1). There were no associations discernible between the other clinicopathological parameters, i.e., gender, age, TNM stage, diameter, tumor differentiation grade, and the MT level.

In gastric cancer patients, there were also no significant associations found between the MT level and the clinicopathological parameters, i.e., gender, age, TNM stage, tumor localization, Lauren’s and WHO classification, diameter, and intestinal metaplasia in normal mucosa, except for a significantly (P = 0.003) lower MT level of the normal gastric mucosa in the antrum (Table 1).

Survival Data. Overall, 164 patients with colorectal cancer (65.3%, 64 females and 100 males) had died during follow-up, with a median survival time of 1.8 years (range: 0.5 month to 11.7 years), and 87 patients (34.7%, 47 females and 40 males) were still alive at the common closing date of the follow-up, with a median survival period of 9.3 years (range: 5.3–13.1 years). Patients who had died were significantly older (70 ± 0.9 years) than those still alive (63.7 ± 1.2 years, P ≤ 0.0005). Furthermore, at this common closing date, the survival of patients with the advanced TNM stage III or IV carcinomas (17.8%) was lower than the survival of patients with TNM stage I or II carcinomas (47.2%).

Within the population of gastric cancer patients, 66 (81.5%, 19 females and 47 males) had died during follow-up, with a median survival time of 11.5 months (range: 0.5 month to 6.2 years). Only 15 (18.5%, 2 females and 13 males) were still alive at the end of the study, with a median follow-up time of 4.2 years (range: 2.8–9.8 years). The gastric cancer patients who had died were slightly older (66.3 ± 1.4 years) compared with those still alive (63.9 ± 4.1), and at the common closing date, there was a lower survival rate of the patients with TNM stage III or IV carcinomas (15.6%) compared with patients with TNM stage I or II disease (20.4%).

MT and Survival. Optimal dichotomization of the MT concentration identified two cutoff points in the colorectal carcinomas, at 202 and 315 ng/mg protein, that were associated with the survival of the patients. Univariate Cox analyses revealed that at both cutoff points, the survival time of those patients with a relatively high carcinoma MT level was significantly shorter than those with a low MT content, and concomitantly, the percentage survival was lower (Table 2). Moreover, when colorectal carcinomas were stratified into three subgroups based on these two cutoff points (≤202, 202–315, and >315 ng of MT/mg protein), we found by univariate Cox analysis that the survival of the group of patients with the highest carcinoma MT level (11.1%, at >315 ng/mg protein) was significantly poorer than the survival of the group with the lowest MT content (38%, at ≤202 ng/mg protein). The survival of patients with carcinomas containing an intermediate MT level was also significantly (29.6%, P = 0.04) better than the survival of those patients with a carcinoma MT content >315 ng/mg protein (Fig. 1A and Table 2). Assessing the MT level of the carcinomas as a continuous variable revealed that an increase of 100 ng of MT/mg protein was associated with a hazard ratio of 1.22 (95% confidence interval 1.02–1.46, P = 0.03).

In the normal colorectal mucosa, one cutoff point was identified also, at 163 ng/mg protein. Univariate Cox analysis with this cutoff point showed that the survival of patients with a MT content >163 ng/mg protein was significantly poorer compared with the survival of the relatively small group of patients with a MT content ≤163 ng/mg protein (Fig. 1B and Table 2).

We also assessed whether the carcinoma over normal mucosa ratio of the MT content within each patient was related to the overall survival of the colorectal cancer patients. At a ratio of ≤0.6, the survival was considerably poorer than above this ratio (Fig. 1C and Table 2).

All Kaplan-Meier curves at the above mentioned MT cutoff levels with their corresponding survival probabilities showed a consistent pattern over time (Figs. 1, A–C).

Univariate Cox analysis of the dichotomized clinicopathological parameters from the colorectal cancer patients revealed that male gender [hazard ratio 1.4 (95% confidence interval
1–1.9), \( P = 0.05 \) and older age [hazard ratio 2.3 (95% confidence interval 1.7–3.3), \( P < 0.00005 \)] of the patient and advanced TNM stage [hazard ratio 3.2 (95% confidence interval 2.3–4.5), \( P < 0.00005 \)] and a poor differentiation grade [hazard ratio 0.6 (95% confidence interval 0.4–0.9), \( P = 0.005 \)] of the tumor were significantly associated with survival. To assess the prognostic significance of the MT parameters relative to the clinicopathological parameters, the dichotomized and stratified MT variables were added separately to all of the assessed clinicopathological parameters in a multivariate analysis. In all cases, a high MT concentration of the carcinomas (\( \geq 202 \) and \( \geq 315 \) ng/mg protein) and as a continuous variable with a hazard ratio of 1.2 (95% confidence interval 1.01–1.42) per 100 ng of MT/mg protein and a high MT concentration of normal mucosa (\( > 163 \) ng/mg protein) remained significantly, thus independently, associated (0.01 \( \leq P \leq 0.04 \)) with a relatively poor survival of the colorectal cancer patients. In addition, a low carcinoma over normal mucosa MT ratio (\( \leq 0.60 \)) tended to be an independent prognosticator associated with poor survival.

Administration of additional radio and/or chemotherapy was found not to affect the above mentioned associations. The number of patients receiving this treatment was evenly distributed over the MT subgroups, and their carcinoma MT level (180 ± 10 ng of MT/mg protein, \( n = 48 \)) was similar to that of the total group of carcinomas. In addition, of these patients receiving radio and/or chemotherapy, those still alive at the common closing date had a similar MT level to those who had died [190 ± 22 (\( n = 11 \)) versus 177 ± 12 (\( n = 37 \)) ng of MT/mg protein]. Furthermore, radio and/or chemotherapy was not found to be significantly associated with survival, although a tendency to a poorer survival was discernable, 22.9% compared with 39.1% for those without this additional treatment. Inclusion of radio and/or chemotherapy hardly affected the multivariate analyses of the diverse clinicopathological and MT parameters, neither of all carcinomas nor of the TNM II and TNM III subgroup analyses. Only for those patients with a low MT carcinoma, i.e., \( \leq 202 \) ng/mg protein, radio and/or chemotherapy was significantly associated with a poor survival (21.2 versus 44.2%, \( P < 0.05 \), which was not found for the high MT carcinoma patients (26.7 and 26.5%, respectively).

Concerning the gastric cancer patients, no significantly discriminating cutoff point could be identified in the MT concentration of the carcinomas in relation to their survival (Table 3). In contrast, optimal dichotomization of the MT content of normal gastric mucosa did result in a cutoff point, which turned out to be identical to the point found in the normal mucosa of the colorectal cancer patients. Univariate analysis indicated that a high MT level of the normal mucosa (\( > 163 \) ng/mg protein) was also significantly associated with a relatively poor survival of the gastric cancer patients (Fig. 2A and Table 3), which was consistent over time. Analyzing the carcinoma over normal mucosa MT ratio revealed that the survival of patients with a ratio \( \leq 0.59 \) was consistently and significantly poorer compared with the survival of patients with a higher MT ratio (Fig. 2B and Table 3). The relatively best survival (33.3%, 7 of 21) was observed in the patients with a MT ratio \( \geq 1 \), but this was only borderline significant (\( P = 0.07 \)). All these dichotomized MT parameters in gastric cancer patients lost their prognostic significance in a multivariate Cox analysis (Table 3), including all assessed clinicopathological parameters of which the univariate Cox analysis is reported previously (15).

**DISCUSSION**

In the present study we showed that MT overexpression in colorectal cancer is a prognostic indicator for poor survival,
The prognostic role of MT in human cancer survival has been evaluated previously in several immunohistochemical studies in different types of tumors. In ductal breast carcinoma, which is the best studied tumor in this respect, several investigators reported a relationship between immunohistochemical MT overexpression and a significant poorer prognosis, either in terms of overall survival (20, 21, 35) or disease-free survival (20, 21, 35, 36). A significant positive association of MT expression and poor clinical outcome has also been observed in pancreatic carcinomas (18), malignant melanomas (37), astrocytoma (38), squamous cell carcinoma of the esophagus (39), renal cell carcinoma (40), and small cell carcinoma of the lung (41). Evidence in favor of a possible role of MT as a prognostic marker predicting poor clinical outcome in cancer patients has also been suggested by the fact that in various tumor types, MT overexpression is closely related to a high proliferative activity of the tumor cells (18, 19, 38, 39). In addition, in a wide variety of human cancers, it appears that advanced stage and high-grade types of tumors, exhibiting the more malignant character, frequently overexpress MT (18–20, 38, 40, 41).

In various types of human cancers, MT overexpression thus seems to be associated with progressive disease and poor prognosis. The available information on the role of MT in gastrointestinal cancer is very limited. We showed previously that colorectal and gastric carcinogenesis is characterized by a decreased MT concentration in the carcinoma tissue (27, 30). Furthermore, MT overexpression within the neoplasias appeared to be correlated with a more malignant character of the tissues. In accordance with these observations, the present study shows that the MT content of gastric and colorectal carcinomas is significantly decreased compared with the content in their corresponding normal mucosa. In addition, MT overexpression in both normal colorectal mucosa and colorectal carcinomas was found to be associated with a consistent significantly poorer overall survival of the patients over time. The carcinoma over normal mucosa MT ratio was also assessed and found to be of limited value in relation to survival prognosis. Most probably this is attributable to the fact that in both tissue types, MT overexpression was accompanied with a poorer survival. Therefore, a ratio does not strengthen the survival association as it would have when the tendencies within the tissues were in an opposite direction, as was found previously for Mn-SOD and gastric cancer survival (15). In agreement to our results, Giuffre et al. (25) reported previously that particularly in advanced carcinomas, MT positivity was encountered frequently, although no significant correlation with tumor stage was found. Moreover, in a recent study from Sutoh et al. (42), it was shown that immunohistochemical overexpression of MT in colorectal carcinomas is related to a poor disease-free survival of the patients. In contrast to these studies, Ofner et al. (16) reported that immunohistochemical MT expression of colorectal carcinomas was related inversely to tumor stage and appearance of metastatic spread. In addition, MT positivity was significantly associated with a favorable clinical outcome in a univariate analysis, but this significance was lost in a multivariate analysis with Dukes’ stage as a stratification factor. The contrasting discrepancy between our results and those reported by Ofner et al. (16) may be a consequence of the different immunoreactivity of the MT antibodies used in the two studies as reported and discussed before (27). Furthermore, our results are based on a quantitative RIA, which is considerably different from the semi-quantitative assessment of immunohistochemically detectable MT. We described previously that the immunohistochemical MT staining in neoplastic and metastatic gastrointestinal tissues shows a patchy pattern varying within the same malignant tissue. Because of this, the linear correlations between the immunohistochemical MT tissue score and the results of the RIA were only moderately strong (27).
Ioachim et al. (43) speculated recently that, because of an absence of a correlation between immunohistochemical MT expression and several clinicopathological and other tumor progression-associated factors, the MT expression in colorectal cancer might not represent an independent prognostic marker. In our present study, we do show, however, that the absence of major associations between MT expression and important clinicopathological parameters is highly relevant and, in contrast, a prerequisite for the independent prognostic character of this metalloprotein with regard to prognosis. Indeed, multivariate analysis revealed that a high MT content of colorectal carcinomas, a high MT content of normal mucosa, and a low carcinoma over normal mucosa MT ratio were all significant predictors for a relatively poor survival of the colorectal cancer patients, independent of major clinicopathological parameters, including patients’ gender and age, localization, differentiation, diameter, and TNM stage of the tumor.

Although univariate analysis revealed that also in gastric cancer patients, MT overexpression in the normal mucosa, but not in carcinoma, was associated with a poor overall survival, in the multivariate analysis, this predictor was not statistically significant, although a trend was discernable. Again the carcinoma over normal mucosa MT ratio was of no value, for similar reasons as discussed above. Our findings are in accordance with the results of a study from Monden et al. (26), who reported that although the MT expression of gastric carcinoma tissue was related to the proliferative activity of the tumor, there were no associations between MT overexpression and clinicopathological parameters and with disease-free survival. In addition, Ebert et al. (44) very recently also did not find an association between MT expression and tumor stage or grade of differentiation in gastric cancer patients. In general, the prognostic role of MT thus appears to be dependent on the origin of the tumor and related to differences in the isofrom and/or metal status of MT overexpression between different types of tumors, as discussed previously by Jasani and Schmid (45).

Although the (patho)physiological role of MT is still controversial, only in the past decade it has become clear that the function of this metalloprotein is not limited to its role in the Cu and Zn

<table>
<thead>
<tr>
<th>Parameter dichotomized</th>
<th>Survivors/total</th>
<th>Median survival in months</th>
<th>Cox hazard ratio (95% CI, P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/n (%)</td>
<td></td>
<td>Univariate</td>
</tr>
<tr>
<td>Carcinoma (CA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥98 (median)</td>
<td>8/41 (19.5)</td>
<td>17.0</td>
<td>1.0 (0.6–1.6, n.s.)</td>
</tr>
<tr>
<td>&gt;98</td>
<td>7/40 (17.5)</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>Normal mucosa (NM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥163</td>
<td>14/51 (27.5)</td>
<td>27.5</td>
<td>1.7 (1.0–2.7, 0.04)</td>
</tr>
<tr>
<td>&gt;163</td>
<td>1/30 (3.3)</td>
<td>10.5</td>
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</tr>
<tr>
<td>MT ratio</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CA over NM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.59</td>
<td>1/31 (3.2)</td>
<td>16.0</td>
<td>0.6 (0.4–1.0, 0.04)</td>
</tr>
<tr>
<td>&gt;0.59</td>
<td>14/50 (28.0)</td>
<td>18.0</td>
<td></td>
</tr>
</tbody>
</table>

* Multivariate analysis was performed by adjusting each MT parameter to the clinicopathological parameters (gender and age of the patients, TNM stage, Laurén classification, WHO classification, localization, diameter, and intestinal metaplasia of the normal mucosa).

* CI, confidence interval.

* ng/mg protein, n.s. = not significant.

Fig. 2 Overall survival curves, according to the Kaplan-Meier method (34), of patients with gastric cancer according to their MT levels within normal gastric mucosa (A) and the carcinoma:normal mucosa ratio of the MT levels (B). Common minimal follow-up time is indicated by an arrow, and censored data are indicated by the small bars. Values indicate the cutoff levels of the MT parameters, and the number of patients alive/deceased at the end of the follow-up is given. x numbers are the patients with a longer follow-up than graphically shown. Ps are according to the Log-rank test.
homeostasis and protection against heavy metal toxicity. There are several lines of evidence indicating that MT may play a role in various carcinogenic processes, including tumor cell pathobiology and drug resistance (1, 2, 11, 12). It has been reported, e.g., that MT acts as a cell cycle regulator and can be used as a marker of proliferation in adenocarcinoma of the breast and colon, as well as in a colorectal cell line (46, 47). In addition, MT antisense studies showed that MT is necessary for tumor growth and survival, and prevents apoptosis (48–51), supporting the hypothesis that MT is a growth-promoting and antiapoptotic protein. Furthermore, MT overexpression is associated with resistance to the (side) effects of a number of antineoplastic drugs, such as alkylating agents and cisplatin (52–57). A number of antineoplastic drugs, such as alkylating agents and cisplatin, illustrate the complexity of the (patho)physiological functions of this protein. The mechanisms underlying the adverse effects of MT overexpression are also not understood completely. It is hypothesized that MT by its metal chelating and antioxidant properties (60–65) can affect the intracellular disposability of Zn and disturb the oxygen balance, which can modify various cellular processes and contribute to the process of malignant transformation, to the development of oxygen-mediated treatment resistance and, as a consequence, to the progression of cancer.

In conclusion, MT overexpression in colorectal carcinomas is an independent prognosticator for a relatively poor survival of the patients, whereas in gastric cancer patients there was no relation between MT expression and overall survival. Additional studies are necessary to elucidate the underlying molecular mechanism(s) and establish a possible relationship between MT overexpression and adjuvant therapy efficacy in gastrointestinal cancer.

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