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Review Article

Piotr Dzięgiel

Expression of Metallothioneins in Tumor Cells

Chair and Department of Histology and Embryology, University School of Medicine, Wrocław

Metallothioneins (MT) are low molecular weight proteins present both in normal and in neoplastic cells. They represent the main mechanism of the cell which protects it from action of heavy metal ions and the principal zinc-binding proteins. MT act as controllers of zinc-dependent enzymes, participating, i.a., in cell proliferation processes. The latter is of basic significance also in neoplastic diseases. Despite the relatively short period of studies on the role of MT in neoplastic processes, several data confirm their prognostic significance. Such data have been obtained in studies on expression of the proteins in breast, renal, urinary bladder, ovarian, laryngeal and lung cancers. MT are thought to play certain role in carcinogenesis, as indicated by results of studies on malignant tumors of large bowel, liver and stomach. The frequently noted positive correlation between MT expression and the expression of Ki-67 and PCNA antigens points to role of the proteins in the cell proliferation mechanism. Results collected and discussed in present paper, obtained by various authors and related to the role of MT expression in cells of various malignant tumors suggest a potential for using the protein as a prognostic factor in neoplastic diseases.

Introduction

Metallothioneins (MT) are low molecular weight about 7kDa) proteins present in the entire animal world. They consist of a polypeptide chain of, depending upon their type, 61 to 68 amino acids, of which around 30% are cystein residues. The latter represent a significant component of MT structure due to their thiol (-SH) groups, through which metal ions are bound [8]. In the structure of MT two domains can be distinguished (α and β), which are linked through a lysine dimer. The domain α binds four Cd ions, while the domain β captures three metal ions, including two Zn ions and one Cd ion (Fig. 1). Regions of MT domain β, containing amino amid sequences 1 to 5 and 20 to 25, exhibit the most pronounced antigenicity. Studies on structure and function of MT permitted to distinguish four main types of the proteins: MT-I, MT-II, MT-III and MT-IV. In human cells, MT are encoded by 15 genes localized in chromosome 16. They include genes for MT-II, MT-III, MT-IV and a group of 12 genes for MT-I. Genes of MT-I and MT-II undergo expression in multiple tissues and organs as well as in cells of several tumors. On the other hand, expression of the gene for MT-III has been detected only in cerebral neurons. In turn, expression of the gene for MT-IV is restricted to stratified squamous epithelium of the skin and upper gastrointestinal tract [49]. Presence of the proteins and of their mRNAs in cells of various tissues can be detected by specific techniques (ELISA, radioimmunological techniques, immunohistochemistry, Western-blot and Northern-blot, RT-PCR and *in situ* hybridization), evaluating directly or indirectly their type and amount. The immunohistochemical technique is one of the most frequently employed approaches to MT detection in tumors, taking advantage of specific antibodies directed to specific antigenic determinants of MT. Under a light microscope, the technique allows to evaluate the site of protein expression, its distribution in the cells (cell nucleus, cytoplasm) and to estimate intensity of the color reaction. MT can be localized in cell nucleus and/or in cytoplasm. It shows a variable character, depending upon the type of normal or neoplastic tissue examined [3]. Immunohistochemical technique is routinely used in histopathological diagnosis to evaluate expression of, e.g. estrogen receptors (ER), progesterone receptors (PgR), HER-2 receptor in breast cancers as well as many other accessory markers, useful in establishing prognosis and indispensable in documenting histological type of the tumor. Using immunohistochemical reactions to detect selected tumor antigens, intensity of the reaction as well and the number of positive cells are recorded. Thus, the results favorably compare with quantitative (biochemical) data which provide no information on the site of manifestation of a given antigen.

The principal and the earliest to be recognized function of MT involves their effects on metal homeostasis in the cell. MT represent the principal protective mechanism of a cell against action of free ions of toxic metals (e.g., cadmium, lead, mercury, copper). Binding of the ions by MT leads to formation of inactive complexes [70]. Apart from their detoxifying role, MT are cellular proteins which bind zinc. Therefore, MT can act as controllers of zinc-dependent enzymes, such as enzymes which play role in DNA replication, transcription, translation and in energy turnover in the

cell. Regulatory sequences of MT genes can induce transcription of the genes. Such regulatory sequences are sensitive to augmented levels of heavy metals, glucocorticoids, interferon and other agents. Specific transcription factors interact with the regulatory sequences through mediation of the so called zinc fingers, the proteins which can function only in presence of Zn ions. Interactions within elements of the system result in augmented synthesis of MT (Fig. 2). Therefore, MT play a significant role in processes of cellular protection from actions of harmful agents (metals, free radicals, etc.) and in mechanisms which control growth, differentiation and proliferation of cells [2]. Moreover, the augmented expression of MT in tumor cells was demonstrated to be linked to their enhanced resistance to cytotoxic agents. This has been explained by the ability of MT to inactivate free radicals, generated by some anti-neoplastic drugs (anthracyclins), and to bind selected chemotherapeutics (cisplatin, carboplatin) which results in inactive forms [51, 61]. These potential effects of MT activity have induced interest in their role in a neoplastic process, in which principal disturbances in the affected cells involve defects leading to uncontrolled proliferation of the cells and to inhibition of their differentiation. In view of the above the question arises as to significance of metallothionein expression in tumor cells.

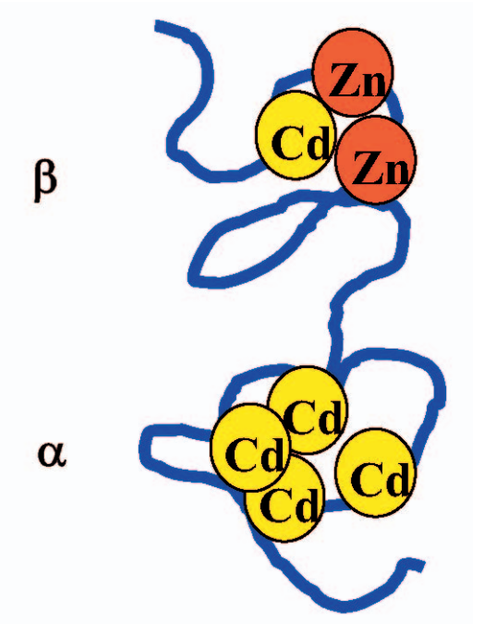

Fig. 1. Structure of metallothioneins (MT). The two domains of polypeptide chain (α and β) are linked by a lysine dimmer. Domain α binds four cadmium (Cd) ions, domain β binds two zinc (Zn) ions and one cadmium (Cd) ion.

Expression of metallothioneins in breast carcinoma cells

Breast cancer represents one of the most frequently developing malignant tumor in women worldwide. In breast carcinomas numerous prognostic variables were defined. They include size of the primary tumor, presence of metastases to axillary lymph nodes, histological type of the tumor and grade of differentiation (G). Development of molecular biology techniques permitted to distinguish several other markers, which represent protein components of tumor cells.

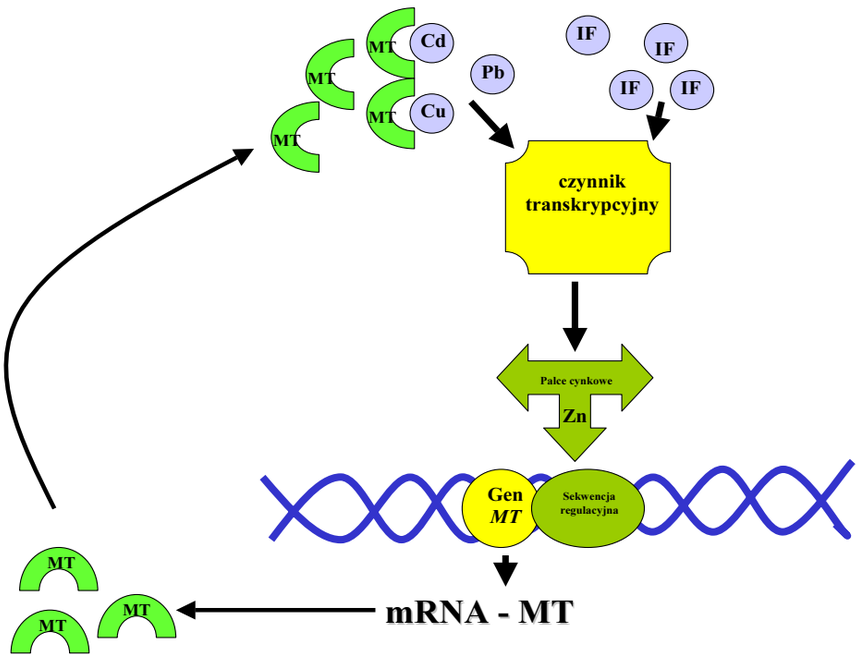

Fig. 2. Mechanism of metallothionein (MT) synthesis, induced by heavy metals and/or by interferon (IF). Metal ions and/or interferon affect the transcription factor (→ altered conformation), which through the so called zinc fingers interacts with regulatory sequences of MT gene, leading to its activation. The transcription results in MT mRNA and respective translation yields metallothionein, which binds heavy metal ions in the cell.

The most important ones include estrogen receptors (ER), progesterone receptors (PgR), markers of cell proliferation (Ki-67, PCNA) and exponents of resistance to cytostatic drugs [15]. Also MT belong to factors which are suggested to indicate proliferative potential of tumor cells as well as reflect resistance to anti-neoplastic drugs [7, 55].

Breast cancer was one of the first tumors in the cells of which MT was demonstrated using immunohistochemical techniques. Cells of ductal breast carcinoma demonstrate expression of two MT isoforms, MT-I and MT-II (Fig. 3) [1]. Eventually, also expression of MT-III isoform was demonstrated [52]. In a decisive majority of cases, augmented intensity of MT expression was observed in breast cancer cells, correlating with a more rapid progression of the diseases, i.e., with worse prognosis [52, 75]. Jin et al. [36, 37] demonstrated that expression of MT-I and MT-II isoforms positively correlated with the grade of differentiation (G) and expression of Ki-67 antigen [36, 37]. Also in our studies, performed on the material of ductal breast carcinoma, positive correlation was noted between MT-I and MT-II expression and intensity of Ki-67 antigen expression. Moreover, MT expression was most pronounced in the poorly differentiated cancers (G2, G3) [59]. The data have corroborated earlier hypotheses on the role of MT in processes of cell proliferation and differentiation. The observation was also interesting, confirmed by results of a few authors, that MT expression (MT-I, MT-II) was enhanced in cells of breast cancer showing no expression of estrogen receptors (ER) [26, 35]. The cases of breast cancer which manifest absence of ER expression show a more aggressive course and, thus, a less favorable prognosis.

The above listed facts on MT expression in breast cancer cells allow to conclude that manifestation of the protein is linked to a higher proliferative potential of tumor cells and, thus, faster progression of the disease and worse prognosis. The hypothesis has been confirmed by Vasquez-Ramirez et al. [71], who noted that augmented expression of MT in breast cancer cells correlated with more frequent appearance of metastases and shorter survival of the patients. Using not only the immunohistochemical but also quantitative techniques, a three-fold increase was demonstrated in MT levels in nuclear fraction of ductal breast cancer cells, as compared to cells of benign breast dysplasia (mastopathy) [16].

Another interesting fact involves MT expression (MT-I and MT-II) in myoepithelial cells composing both normal structures of the breast and neoplastic foci in the organ [34]. Several reports suggest effects of myoepithelial cells on biology of breast cancer. Absence of the cells promotes higher invasiveness and formation of metastases [10]. Jin et al. [34] using immunohistochemical techniques demonstrated augmented expression of MT in myoepithelial cells of normal breast structures as well as in such cells surrounding neoplastic foci. Also in cases of pre-invasive breast cancer MT expression was observed in myoepithelial cells. Lele et al. [41] in turn detected MT expression in myoepithelial cells in every other case of benign lesions which imitated breast cancer (radial scar) but absence of the expression in a case of well differentiated cancer (tubular carcinoma). In our studies, the highest intensity of MT expression in myoepithelial cells was detected in myoepithelial cells of well differentiated cancers (G1). The lowest intensity of MT expression in myoepithelial cells was noted in poorly differentiated cancers, highly aggressive and manifesting rapid progression (G3) [59].

The presented data indicate that MT expression in breast cancer cells and in myoepithelial cells of the tumor may represent an important prognostic variable. Intensity of MT expression in breast cancer cells positively correlates with the grade of differentiation, expression of Ki-67 antigen and with frequency of metastases to axillary lymph nodes. Correlation of recognized prognostic parameters (G, expression of Ki-67, metastases to lymph nodes) with MT expression has been confirmed by shorter survival in breast cancer cases with pronounced MT expression.

Expression of metallothioneins in tumors of alimentary tract

Tumors of the large bowel

Colorectal carcinoma represents the most frequently manifested malignant tumor of alimentary tract. Due to the absence of specific signs and symptoms, in a vast fraction of cases tumor is detected relatively late when the advanced process can not longer be effectively cured. Also, no independent specific markers are available which would permit precise estimation of prognosis in patients with the tumor. In this part of gastrointestinal tract adenocarcinoma is the main histological type of the tumors. Pathogenesis of the neoplasm involves a significant role of genetic defects and various inflammatory lesions, such as ulcerative colitis and Crohn's disease. In sequels of either of the latter diseases, the chronic inflammatory process results in dysplastic lesions in intestinal epithelium and may lead to development of adenocarcinoma [74].

Bruewer et al. [4, 5] demonstrated augmented expression of MT in the inflammatory bowel disease, which positively correlated with intensity of the inflammation. They studied also MT expression at individual stages of dysplasia in intestinal mucosal cells in ulcerative colitis, which precede development of colorectal carcinoma. The most extensive MT expression was noted in cases with the lowest grade of dysplasia. The lowest expression of MT was noted in cells of the cancer while in the control cells of normal intestinal mucosa practically no MT expression was detected. The authors evaluated also expression of p53 antigen as a potential marker of carcinogenesis, the expression intensity of

which was strictly reciprocal to that of MT (negative correlation) and reached peak values in neoplastic cells. In cases of ulcerative colitis, expression intensities of the two markers were also negatively correlated with each other and expression of MT was most pronounced. The results may point to MT role as an early marker of colorectal carcinoma risk. Reports on MT expression in cells of colorectal carcinoma have yielded contradictory data. Several authors claim that MT expression fails to correlate with stage of the disease or survival. Ioachim et al. [25] demonstrated a reciprocal relation between MT expression and expression of CD44 antigen, the index of invasiveness and metastatic potential of the tumor. Other authors noted a strongly positive relation between MT expression in cells of primary colorectal adenocarcinoma on the one hand and duration of survival/frequency of metastases to lymph nodes on the other [21]. Sutoch et al. [60] examined effect of multidrug resistance (MDR) proteins and of MT expression on survival of patients with colorectal carcinoma. They noted a significantly shorter survival in the patient group with high expression of the markers. In our studies on 81 cases of primary colorectal adenocarcinoma we compared expression of MT in cells of the cancer (Fig. 4) with several clinico-pathological variables [11] . Expression of MT was found to be significantly higher in tumors of the poorest differentiation (G3). Moreover, expression of the protein strongly correlated with manifestation of Ki-67 antigen, indicating high proliferative activity of cells. In subsequent studies we noted increase in MT expression in cancer at most advanced stages of the disease, considering the depth of intestinal wall infiltration. No significant differences were detected in survival of the patients, which could be related to intensity of MT expression. Nevertheless, patients with the most pronounced MT expression in cells of primary colorectal adenocarcinoma were found to die earlier [11].

The data presented above do not allow to draw unequivocal conclusions as to the role and significance of MT expression in cells of primary colorectal adenocarcinomas. The contradictory data on relations between MT manifestation and various clinico-pathological variables may reflect the lack of uniform investigated groups and comparisons involving various indices. Undoubtedly, MT expression in cells of colorectal cancer plays certain role both in the carcinogenesis process which results in development of the tumor and in the course of the disease.

Cancer of the liver and pancreatic tumors

Few reports are available on MT expression in tumor cells in parenchymatic organs of alimentary tract. Deng et al. [9] evaluated expression of MT and apoptosis in primary liver carcinomas and in metastases of colorectal adenocarcinoma to the organ. Using immunohistochemical technique they demonstrated MT expression in 11 out of 13 cases of primary hepatocellular carcinomas. On the other hand, no such a protein could be demonstrated in cells of metastatic tumors. In turn, the fraction of apoptotic cells was significantly lower in MT(+) cancers as compared to cells of MT(-) metastases of adenocarcinomas. Other authors compared expression of MT in primary liver carcinoma with expression of the protein in pseudoneoplastic lesions and in normal cells. They detected a lowered expression intensity of MT in tumor cells as compared to expression in normal cells and in pseudoneoplastic lesions [23]. Similarly to Deng et al. [9], Stenram et al. [57] in a large group of cases (37 primary liver carcinomas and 117 metastases of colorectal adenocarcinoma) detected lowered intensity of MT expression or its absence in the metastases.

The pancreas was the other parenchymatic organ of alimentary tract, the tumors of which were tested for MT presence. Tomita [66] examined expression of MT-I in primary tumors developing from various types of pancreatic island cells. He noted variability in MT-I manifestation. MT-I expression was observed in almost 100% of insulinomas. Total absence of the expression was observed in glucagonoma-type tumors while the intensity of MT expression in gastrinoma was very low.

Cancers of upper alimentary tract

Mainly squamous cell carcinomas of the upper alimentary tract represent still another group of tumors. Cardoso et al. [6] examined expression of MT in 60 cases of squamous cell carcinoma in oral cavity as compared to clinico-pathological variables, including TNM classification, grade of differentiation (G), expression of Ki-67 antigen, and to survival of the patients. They found a complete lack of correlation between MT expression on the one hand and TNM classification, G or Ki-67 antigen expression on the other. The patients in whom over 76% of cancer cells manifested MT expression showed a significantly shorter survival. The data indicate that MT expression is an independent prognostic factor in primary cancers of oral cavity, not linked to other clinico-pathological variables.

Similarly to tumors of oral cavity, MT manifestation was examined also in esophageal tumors. In course of the studies relatively consistent results were obtained, pointing to prognostic significance of the proteins. Hishikawa et al. [22] analyzed MT expression in 57 cases of squamous cell carcinomas using the immunohistochemical technique and RT-PCR (detecting mRNA for MT). The results were compared to clinico-pathological variables and to patient survival time. The authors noted positive correlation between intensity of MT and MT mRNA expression on the one hand and metastases of cancer cells to regional and distant lymph nodes on the other. Survival analysis demonstrated shorter survival of patients in whom tumor cells showed more pronounced MT expression. Moreover, the authors exam-

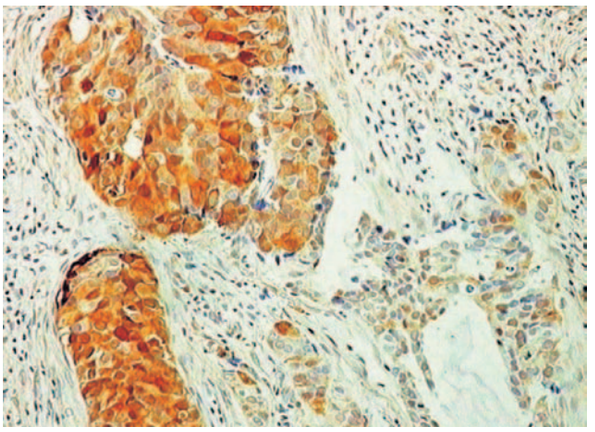

Fig. 3. Expression of metallothionein (isoforms MT-I and MT-II), obtained by immunohistochemical reaction in the paraffin section of ductal breast carcinoma. The brown color reaction can be noted both in the cytoplasm and in cell nuclei of cancer cells. Hematoxylin counterstaining. Magn. 100x.

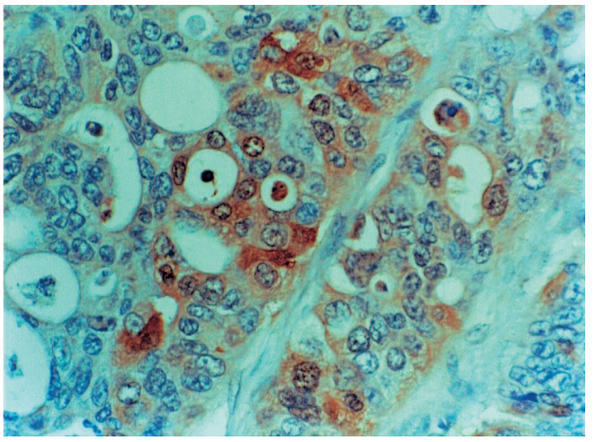

Fig. 4. Expression of metallothionein (isoforms MT-I and MT-II), obtained by immunohistochemical reaction in the paraffin section of primary colorectal carcinoma. The brown color reaction can be noted both in the cytoplasm and in cell nuclei of cancer cells. Hematoxylin counterstaining. Magn. 200x.

ined correlation between MT expression and expression of PCNA antigen documenting that it was positive and highly significant. Once again, this points to a role of MT in mechanisms of cell proliferation and suggests potential for using MT expression as a prognostic index in esophageal tumors. Kishi et al. [39] studied expression of MT in the course of chemo- and radiotherapy. In samples of esophageal tumors (squamous cell carcinoma) obtained before the treatment they estimated MT and p53 protein using immunohistochemical techniques. Patients with tumors in which cells were positive for MT and p53 were less responsive to treatment and had shorter survival. Thus, MT expression in

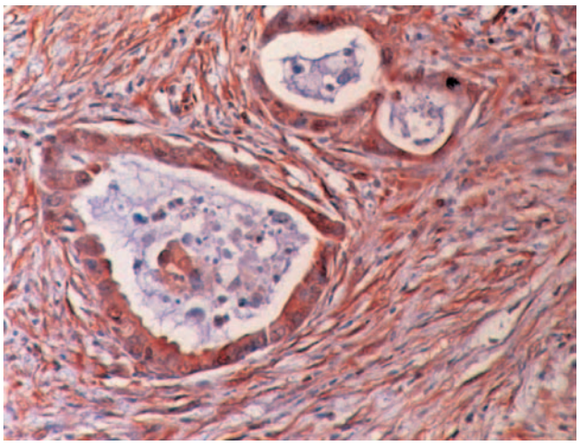

Fig. 5. Expression of metallothionein (isoforms MT-I and MT-II), obtained by immunohistochemical reaction in the paraffin section of synovial sarcoma (biphasic type). The brown color reaction can be noted in cell nuclei and in cytoplasm of epithelioid and fusiform cells of the tumor. Counterstaining with hematoxylin. Magn. 200x.

esophageal cancers may not only affect prognosis but also may influence results of treatment.

Gastric carcinoma

Relatively few reports have been published on MT expression in gastric tumors. The studies which appeared till now provided highly consistent results. Tuccari et al. [68] evaluated 112 cases of primary gastric carcinoma at the early or late stage and noted a significantly higher expression of MT in less advanced tumors. The parameter did not correlate with various clinico-pathological variables or with patient survival time. Also Ebert et al. [13] in a rather scanty material (35 cases of gastric carcinoma) obtained similar results in studies on MT expression in the tumors. Interestingly, the expression proved markedly higher in dysplastic and metaplastic lesions which accompanied the tumor. The observation has confirmed the hypothesis on potential role of MT in neoplastic transformation in gastric mucosa. Janssen et al. [31] evaluated manifestation of MT in tumor cells of the stomach using two independent approaches, radioimmunological technique and immunohistochemical one. The two techniques provided consistent results: the content and expression of the protein was significantly higher in cells of normal gastric mucosa as compared to tumor cells. They observed no correlation between MT content in cells of gastric carcinoma and clinico-pathological variables or patient survival time [30]. The data allow to assume that MT expression in cells of gastric cancer does not seem to be a prognostic factor. On the other hand, the expression may be an exponent of the tumor risk (similarly to pre-neoplastic conditions in the large bowel [4, 5]), which is manifested by increased intensity of the protein expression in metaplastic

and dysplastic lesions of the mucosa, which predispose the cells to neoplastic transformation.

Gallbladder carcinoma

Till today only one report has been published on MT expression in the cancer of gallbladder. In 27 cases of the cancer MT expression was immunohistochemically examined and compared to results obtained in control cases (8 cases of chronic cholecystitis, 7 samples of normal mucosa). The highest intensity of MT expression was noted in tumor cells, much lower was the expression in inflammatory conditions and complete absence of the protein was disclosed in samples of normal gallbladder mucosa. Moreover, maximum intensity of MT expression was observed in poorly differentiated cancers (G3). The results encourage further investigative attempts since they point to a potential prognostic value of MT expression [53].

As noted above, only in few primary tumors of various parts of alimentary tract intensity of MT expression may correlate with aggressiveness of the pathological process, effects of applied treatment and may represent a significant prognostic factor.

Expression of metallothioneins in tumors of urogenital system

Renal carcinoma

In cells of clear cell carcinoma of the kidney presence of two basic MT isoforms was documented, including MT-I and MT-II [47]. In recently published studies the results on expression of the proteins in the cancer are highly consistent. In each case an augmented intensity of MT expression was observed, negatively correlated with grade of differentiation (G). In cases of poorly differentiated tumors (G3), expression of the protein was most pronounced. Some authors performed also a survival analysis in patients with clear cell carcinoma of the kidney and demonstrated much shorter survival in MT(+) cases [48, 69]. Although the hypotheses of MT prognostic significance in primary cancers of the kidney are based on just few reports, the results are highly compatible. Moreover, Zhang et al. [76] detected increased frequency of apoptosis in cells of renal clear cell carcinoma, which was directly related to intensity of MT expression. The results may point to a significant role of MT in cell proliferation processes, of which apoptosis is an important element.

Urothelial carcinoma

Urothelial carcinoma represents another tumor of urogenital system in which MT expression was examined. It is characterized by an ill-defined prognosis. A number of studies were performed in the recent four years, in which MT expression was confirmed in tumors of this type. Moreover, the increased expression of MT paralleled the tumor grade and stage of the disease. Also the analysis of survival demonstrated that MT(+) cases of urothelial carcinoma were associated with shorter survival of the patients [27, 50, 56]. Other authors described also augmented resistance of MT(+) urothelial and prostate carcinomas to cytostatic drugs used in treatment of the tumors [42, 54].

Neoplasms of testes

Results completely different than the above ones were presented by Eid et al. [14] who examined immunohistochemically MT expression in 77 cases of primary germ cell tumors of testes. The cases in which MT expression in tumor cells showed higher intensity responded better to chemotherapy. The results contradict those pertaining tumors of other organs. This indicates how restricted knowledge on MT is available at present and how variable role may MT play in the mechanism of multidrug resistance to cytostatic drugs in various tumors.

Ovarian cancer

Relatively multiple studies were performed on MT expression in primary tumors of the ovary. Interesting results were presented, i.a., by Tan et al. [63], who compared intensity of MT expression in serous tumors of the organ, including benign, borderline and malignant forms. The results demonstrated that the highest MT expression was seen in cancers, a lower one in borderline tumors and the lowest one in benign tumors. The same authors analyzed MT expression in mucinous tumors of the ovary. In this histological type the most intense MT expression was observed in borderline tumors and the lowest in benign neoplasms [62]. This points to the potential of using MT as a helpful marker in diagnosis and in the appraisal whether a given tumor already represents a malignant form, which is linked to appropriate therapeutic decisions. In turn, Wirigley et al. [73] used radioimmunological techniques to evaluate MT content in ovarian tumors before chemotherapy. They detected no relationship between patient survival and MT content in the tumors. Hengstler et al. [20] analyzed MT expression by immunohistochemical techniques in 151 primary and 38 relapsing malignant tumors of the ovary and correlated the expression with patient survival, grade, stage, presence of estrogen receptors (ER) and progesterone receptors (PgR). They found that MT expression was least pronounced in G1 and it was higher in G2 and G3 tumors. A relation to stage was only partial. MT expression was found independent of the receptor (ER, PgR) status. Worse prognosis (shorter survival) was found for patients with MT(+) cases. Mc Cluggage et al. [45] examined 139 various types of ovarian tumors and detected MT expression in 56% of cases. They noted that

intensity of MT expression was directly related to grade (G) of the tumor.

Manifestation of MT in cells of primary ovarian tumors not always confirms the hypothesis on its prognostic role. However, most of the published papers evaluating presence of MT in ovarian cancer cells indicate usefulness of the variable for appraisal of malignancy and of prognosis.

Uterine carcinoma

Up to now, only two studies have been published on MT expression in endometrioid adenocarcinomas of uterine corpus [28, 44]. In the tumors a positive correlation was detected between intensity of MT expression on the one hand and expression of Ki-67 antigen, p53 and grade of the tumor (G) on the other. A reverse relation was observed between MT expression and presence of ER and PgR. The data may indicate also a reciprocal relationship and effects of receptor proteins (ER, PgR) and of p53 protein on synthesis of MT [28, 44].

Expression of metallothioneins in tumors of the respiratory tract

Epidemiological data indicate that lung cancer is the most frequent malignant tumor worldwide, both when morbidity and mortality are considered. According to the World Health Organization, four basic histological types of the tumor can be distinguished, including squamous cell carcinoma, adenocarcinoma, giant cell carcinoma and small cell lung carcinoma. When biology of the lesion is taken into account, the first three types decisively differ from the latter one (which exhibits a more aggressive course and worse prognosis) and, therefore, the first three types together used to be termed the non-small cell lung carcinoma [46].

For a long time attempts have been made to detect independent predictive markers useful in prognostic evaluation and in therapeutic practice. In the recent two years a few studies were published which pertained MT expression and its role in lung tumors. Joseph et al. [38] examined immunohistochemically 58 cases of small cell lung carcinoma and detected expression of MT (isoforms I and II) in 45% of cases. Patients with MT(+) tumors demonstrated shorter survival and intensity of MT expression in cells of the tumors was directly related to frequency of PCNA(+) cells. Theocharis et al. [65] studied a group of non-small cell lung carcinomas and evaluated immunohistochemically expression of, i.a., MT. The highest proportion of MT(+) tumors was noted in the group of squamous cell carcinomas (74%), followed by adenocarcinomas (34%) while the protein was completely absent in small cell lung carcinomas. The authors detected no correlation of MT expression with other clinico-pathological variables. Volm et al. [72] as well as Mattern et al. [43] examined non-small cell lung carcinomas in respect to their resistance to cytostatic drugs. Both in *in vitro* and *in vivo* experiments they noted higher resistance to doxorubicin of MT(+) cells. Moreover, intensity of MT expression was directly related to the grade (G) and expression of another protein responsible for multidrug resistance to cytostatic drugs, glutathione-s-transferase-π (GST-π).

At present it seems too early to draw convincing conclusions on the role of MT expression in malignant tumors of lungs. Nevertheless, preliminary results point to certain prognostic significance of MT, also in respect to applied treatment.

Still other authors undertook to examine MT manifestation in tumors of the upper respiratory tract. Ioachim et al. [24] compared expression of MT with those of PCNA and p53 protein in 44 cases of laryngeal squamous cell carcinoma, few dozens of pre-invasive cancer, dysplasia, papilloma and leukoplakia. They found that intensity of MT expression was directly related to the number of PCNA(+) cells. On the other hand, they found no relationship between manifestation of MT in cells of the above lesions and the expression of p53 protein or other clinico-pathological variables. Jayasura et al. [32, 33] in two reports evaluated the role of MT in cancers of nasopharynx. They compared expression of MT in tumor cells with intensity of apoptosis (TUNEL technique), expression of Ki-67 antigen and content of Zn in nuclei of the cells. The results demonstrated negative correlation between MT expression and intensity of apoptosis. On the other hand, intensity of MT expression correlated with expression of Ki-67 antigen in tumor cells. Zn content in cell nuclei, evaluated by atomic mass spectrometry, was directly related to intensity of MT expression in the tumors.

The observations prompt investigators to confirm the hypothesis on effects of MT on cell proliferation. In addition, MT may represent factors which protect cells from action of pro-apoptotic stimuli (e.g., from free radicals). Restricted amount of experimental evidence as well as the narrow range of studies on manifestation and significance of MT in lung tumors do not permit yet to draw final conclusions on the topic.

Expression of metallothioneins in primary tumors of the skin

Few reports were published on MT expression in various tumors of the skin. Sugita et al. [58] evaluated 44 cases of malignant melanoma and immunohistochemically examined manifestation of MT (isoforms I and II). MT expression was detected in 56% of melanomas and it was positively related to the depth of skin infiltration. Analysis of survival demonstrated a significantly shorter survival of patients with MT(+) tumors. In squamous cell carcinoma of the skin Goldman et al. [18] examined expression of MT and detected no correlations with other clinico-pathological variables. On

the other hand, Han et al. [19] demonstrated low intensity of MT expression in basal cell carcinoma and in squamous hyperplasia concomitant with dermatofibromas.

Expression of metallothioneins in cells of cerebral tumors

Expression of MT in cerebral tumors was evaluated in the respect to their role in multidrug resistance to cytostatic drugs. Korshunov et al. [40] analyzed MT expression using immunohistochemical techniques in 168 cases of multiform glioblastomas, subjected to chemotherapy. They compared manifestation of the protein as compared to other markers of resistance to cytostatic drugs: glutathione-s-transferase-π (GST-π) and P-glycoprotein (P-GP). Cases with MT expression in over 50% of tumor cells exhibited a significantly longer survival as compared to patients carrying tumors in which less than 50% of cells were MT(+). Inverse results were obtained in studies on expression of P-GP and GST-π in cells of the tumors.

Tews et al. [64] evaluated by the same technique expression of MT in various types of meningiomas. The highest number of MT(+) cells was noted in benign tumors and malignant MT(+) tumors were very rare. Thus, also in brain tumors MT expression not always points to worse prognosis and may be more pronounced in benign that in malignant tumors.

Expression of metallothioneins in tumors of mesenchymal origin (sarcomas)

In our studies on 20 cases of synovial sarcoma we detected immunohistochemically expression of MT-I and MT-II in 19 tumors of a variable histological structure (Fig. 5). We revealed the presence of the proteins in type A synovial cells, which might point to histogenesis of the tumor. Significantly, MT expression was demonstrated in most cases (4 out of 5 cases) of poorly differentiated tumors what, apart from cytokeratin expression, may be helpful in differential diagnosis of synovial sarcoma [12]. No correlation was noted between MT manifestation in cells of osteosarcoma or leiomyosarcoma and patients' survival [17, 67]. Isik et al. [29] examined MT expression in cells of 67 cases of malignant mesothelioma. Fifty-two per cent of the tumors were MT(+) but prognostic significance of MT expression could not be confirmed.

Summary

Metallothioneins are proteins which have been recognized 50 years ago. Despite the continuous studies, little is known about their physiology and pathology, particularly in neoplastic diseases. However, their effects on cell proliferation and their anti-oxidative activity turned attention of investigators to their role in neoplastic processes. In this report, results of studies performed till now have been presented, frequently contradictory and difficult to interpret. Standardization of research material and of investigative techniques will certainly cause that the result obtained will be more objective. Considering the available data one may cautiously suggest that MT play certain role in mechanisms of carcinogenesis and of tumor cell proliferation. This has been indicated by augmented expression of MT in cells of pre-neoplastic lesions and in early stages of cancers (colorectal carcinoma, liver carcinoma, gastric carcinoma) and the frequent positive correlation between their expression and manifestation of PCNA and Ki-67 antigens. The higher proliferative activity of MT(+) tumor cells results in an accelerated course of the disease and in shortened survival of the patients. Such a significance of MT expression has been documented in primary tumors of various organs, such as the breast, kidney, urinary bladder, ovary, larynx and, in part, lungs. This points to a potential significance of MT expression in establishing prognosis. Nevertheless, despite numerous confirmations of the results on MT expression in tumor cells, contradictory reports have also been published, which put in doubt prognostic significance of the proteins (gastric cancer, testicular tumors, cerebral tumors). This, most probably, reflects insufficient knowledge on functions of MT in our body.

References

1. *Barnes NL, Ackland ML, Cornish EJ:* Metallothionein isoform expression by breast cancer cells. Int J Biochem Cell Biol 2000, 32, 895-903.
2. *Beyersmann D, Haase H:* Functions of zinc in signaling, proliferation and differentiation of mammalian cells. BioMetals 2001, 14, 331-341.
3. *Boon-Huat B, Jin R, Jayasurya A:* Analysis of metallothionein expression in human cancers. Acta Histochem Cytochem 2001, 34, 171-176.
4. *Bruewer M, Schmid KW, Krieglstein CF, Senninger N, Schuermann G:* Metallothionein: early marker in the carcinogenesis of ulcerative colitis-associated colorectal carcinoma. World J Surg 2002, 26, 726-731.
5. *Bruewer M, Schmid KW, Metz KA, Krieglstein CF, Senninger N, Schuermann G:* Increased expression of metallothionein in inflammatory bowel disease. Inflamm Res 2001, 50, 289-293.
6. *Cardoso SV, Barbosa HM, Candelloriim IM, Loyola AM, Aguiar MCF:* Prognostic impact of metallothionein on oral squamous cell carcinoma. Virchows Arch 2002, 441, 174-178.
7. *Cherian MG, Apostolova MD:* Nuclear localization of metallothionein during cell proliferation and differentiation. Cell Mol Biol 2000, 46, 347-356.
8. *Coyle P, Philcox JC, Carey LC, Rofe AM:* Metallothionein: The multipurpose protein. Cell Mol Life Sci 2002, 59, 627-647.
9. *Deng DX, Chakrabarti S, Waalkes M, Cherian MG:* Metallothionein and apoptosis in primary human hepatocellular carcinoma and metastatic adenocarcinoma. Histopathology 1998, 32, 340-347.

10. *Deugnier MA, Teuliere J, Faraldo MM, Thiery JP, Glukhova MA:* The importance of being a myoepithelial cell. Breast Cancer Res 2002, 4, 224-230.
11. *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, 401-407.
12. *Dzięgiel P, Suder E, Surowiak P, Kornafel J, Zabel M:* Expression of metallothionein in synovial sarcoma cells. Appl Immunohistochem Mol Morphol 2002, 10, 357-363.
13. *Ebert MPA, Gnther T, Hoffmann J, YU J, Miehlke S, Schulz HU, Roessner A, Korc M, Malfertheiner P:* Expression of metallothionein II in intestinal metaplasia, dysplasia, and gastric cancer. Cancer Res 2000, 60, 1995-2001.
14. *Eid H, Geczi L, Bodrogi I, Institoris E, Bak M:* Do metallothioneins affect the response to treatment in testis cancers? J Cancer Res Clin Oncol 1998, 124, 31-36.
15. *Elis O, Pinder SE, Lee AHS, Elston CW:* Tumors of the breast. In: Diagnostic Histopathology of Tumors. Fletcher CDM, ed. Churchill Livingstone, London, Edinburgh, New York, Philadelphia, St Louis, Sydney, Toronto 2000, 865-930.
16. *Floriańczyk B, Grzybowska L:* Metallothionein levels in cell fraction from breast cancer tissues. Acta Oncol 2000, 39, 141-143.
17. *Gaumann A, Tews DS, Mentzel T, Petrow PK, Mayer E, Otto M, Kirkpatrick CJ, Kriegsmann J:* Expression of drug resistance related proteins in sarcomas of the pulmonary artery and poorly differentiated leiomyosarcomas of the other origin. Virchows Arch 2003, 442, 529-537.
18. *Goldmann T, Moorkamp A, Wiedorn KH, Suter L, Otto F:* The prognostic value of the expression of collagenase IV, cathepsin D and metallothionein in squamous cell carcinomas of the skin determined by immunohistochemistry. Arch Dermatol Res 2001, 293, 115-120.
19. *Han KH, Huh CH, Cho KH:* Proliferation and differentiation of the keratinocytes in hyperplastic epidermis overlying dermatofibroma. Am J Dermatopathol 2001, 23, 90-98.
20. *Hengstler JG, Pilch H, Schmidt M, Dahlenburg H, Sagemüller J, Schiffer I, Oesch F, Knapstein PG, Kaina B, Tanner B:* Metallothionein expression in ovarian cancer in relation to histopathological parameters and molecular markers of prognosis. Int J Cancer 2001, 95, 121-127.
21. *Hishikawa Y, Kohno H, Ueda S, Kimoto T, Dhar DK, Kubota H, Tachibana M, Koji T, Nagasue N:* Expression of metallothionein in colorectal cancers and synchronous liver metastases. Oncology 2001, 61, 162-167.
22. *Hishikawa Y, Koji T, Dhar DK, Kinugasa S, Yamaguchi M, Nagasue N:* Metallothionein expression correlates with metastatic and proliferative potential in squamous cell carcinoma of the oesophagus. Br J Cancer 1999, 81, 712-720.
23. *Huang GW, Yang LY:* Metallothionein expression in hepatocellular carcinoma. World J Gastroenterol 2002, 8, 650-653.
24. *Ioachim E, Assimakopoulos D, Peschos D, Zissi A, Skevas A, Agnantis NJ:* Immunohistochemical expression of metallothionein in benign premalignant and malignant epithelium of the larynx: correlation with p53 and proliferative cell nuclear antigen. Pathol Res Pract 1999, 195, 809-814.
25. *Ioachim E, Goussia AC, Agnantis NJ, Machera M, Tsianos EV, Kappas AM:* Prognostic evaluation of metallothionein expression in human colorectal neoplasms. J Clin Pathol 1999, 52, 876-879.
26. *Ioachim E, Kamina S, Demou A, Kontostolis M, Lolis D, Agnantisn N:* Immunohistochemical localization of metallothionein in human breast cancer in comparision with cathepsin D, stromelysin-1, CD44, extracellular matrix components, p53, Rb, C-erbB-2, EGFR, steroid receptor content and proliferation. Anticancer Res 1999, 19, 2133-2140.
27. *Ioachim EE, Charchanti AV, Stavropoulos NE, Athanassiou ED, Michael MC, Agnantis NJ:* Localization of metallothionein in urothelial carcinoma of the human urinary bladder: an immunohistochemical study including correlation with HLA-DR antigen, p53, and proliferation indices. Anticancer Res 2001, 21, 1757-1762.
28. *Ioachim EE, Kitsiou E, Carassavoglou C, Stefanaki S, Agnantis NJ:* Immunohistochemical localization of metallothionein in endometrial lesions. J Pathol 2000, 191, 269-273.
29. *Isik R, Metintas M, Gibbs AR, Metintas S, Jasani B, Oner U, Harmanci E, Demircan S, Isiksoy S:* p53, p21 and metallothionein immunoreactivities in patients with malignant pleural mesothelioma: correlations with the epidemiological features and prognosis of mesotheliomas with environmental asbestos exposure. Respir Med 2001, 95, 588-593.
30. *Janssen AML, Van Dujin W, Kubben FJ, Griffioen G, Lamers CBHW, Van Krieken JHJM, Van De Velde CJH, Verspaget HW:* Prognostic significance of metallothionein in human gastrointestinal cancer. Clin Cancer Res 2002, 8, 1889-1896.
31. *Janssen AML, Van Dujin W, Oostendorp-Van De Ruit MM, Kruidenier L, Bosman CB, Griffioen G, Lamers CBHW, Van Krieken JHJM, Van De Velde CJH, Verspaget HW:* Metallothionein in human gastrointestinal cancer. J Pathol 2000, 192, 293-300.
32. *Jayasurya A, Bay BH, Yap WM, Tan NG, Tan BKH:* Proliferative potential in nasopharyngeal carcinoma: correlations with metallothionein expression and tissue zinc levels. Carcinogenesis 2000, 21, 1809-1812.
33. *Jayasurya A, Bay BH, Yap WM, Tan NG:* Correlation of metallothionein expression with apoptosis in nasopharyngeal carcinoma. Br J Cancer 2000, 82, 1198-1203.
34. *Jin R, Bay BH, Chow VT, Tan PH, Dheen T:* Significance of metallothionein expression in breast myoepithelial cells. Cell Tissue Res 2001, 303, 221-226.
35. *Jin R, Bay BH, Chow VT, Tan PH, Lin VC:* Metallothionein 1E mRNA is highly expressed in estrogen receptor-negative human invasive ductal breast cancer. Br J Cancer 2000, 83, 319-323.
36. *Jin R, Bay BH, Chow VT, Tan PH:* Metallothionein 1F mRNA expression correlates with histological grade in breast carcinoma. Breast Cancer Res Treat 2001, 66, 265-272.
37. *Jin R, Chow VT, Tan PH, Dheen ST, Duan W, Bay BH:* Metallothionein 2A expression is associated with cell proliferation in breast cancer. Carcinogenesis 2002, 23, 81-86.
38. *Joseph MG, Banerjee D, Kocha W, Feld R, Stitt LW, Cherian MG:* Metallothionein expression in patients with small cell carcinoma of the lung. Cancer 2001, 92, 836-842.
39. *Kishi K, Doki Y, Miyata H, Yano M, Yasuda T, Monden M:* Prediction of the response to chemoradiation and prognosis in esophageal squamous cancer. Br J Surg 2002, 89, 597-603.
40. *Korshunov A, Golanov A, Sycheva R, Pronin I, Fadeeva L:* Prognostic value of the immunoexpression of chemoresistance-related proteins in cerebral glioblastomas: an analysis of 168 cases. Neuropathol 1999, 19, 143-149.
41. *Lele SM, Graves KMT, Gatalica Z:* Immunohistochemical detection of maspin is a useful adjunct in distinguishing radial sclerosing lesion from tubular carcinoma of the breast. Appl Immunohistochem Mol Morphol 2000, 8, 32-36.
42. *Lynn NNK, Howe MC, Have RJ, Collins GN, O'Reilly PH:* Overexpression of metallothionein predicts resistance of transitional cell carcinoma of bladder to intravesical mitomycin therapy. J Urol 2003, 169, 721-723.
43. *Mattern J, Koomägi R, Volm M:* Expression of drug resistance gene products during progression of lung carcinomas. Oncol Rep 2002, 9, 1181-1184.
44. *McCluggage WG, Maxwell P, Hamilton PW, Jasani B:* High metallothionein expression is associated with features predictive of aggressive behaviour in endometrial carcinoma. Histopathology 1999, 34, 51-55.

45. *McCluggage WG, Strand K, Abdulkadir A:* Immunohistochemical localization of metallothionein in benign and malignant epithelial ovarian tumors. Int J Gynecol Cancer 2002, 12, 62-65.
46. *Moran CA, Suster S:* Tumors of the lung and pleura. In: Diagnostic Histopathology of Tumors. Fletcher CDM, ed. Churchill Livingstone, London, Edinburgh, New York, Philadelphia, St Louis, Sydney, Toronto 2000, 171-208.
47. *Naguyen A, Jing Z, Mahoney PS, Davis R., Sikka SC, Agrawal KC, Abdel-Mageed AB:* In vivo gene expression profile analysis of metallothionein in renal cell carcinoma. Cancer Lett 2000, 160, 133-140.
48. *Paraskevakou H, Theocharis SE, Tseleni S, Athanasiades A, Davaris PS:* Expression of metallothionein in renal cell carcinoma: an immunohistochemical study. Lab Invest 1999, 79, 103.
49. *Romero-Isart N, Vak M:* Advances in the structure and chemistry of metallothioneins. J Inorg Biochem 2002, 88, 388-396.
50. *Saga Y, Hashimoto H, Yachiku S, Tokumitsu M, Kaneko S:* Immunohistochemical expression of metallothionein in human bladder cancer: correlation with histopathological parameters and patient survival. J Urol 2002, 168, 2227-2231.
51. *Satoh M, Naganuma A, Imura N:* Modulation of adriamycin toxicity by tissue-specific induction of metallothionein synthesis in mice. Life Sci 2000, 67, 627-634.
52. *Sens MA, Somji S, Garrett SH, Beall CL, Sens D:* Metallothionein isoform 3 overexpression is associated with breast cancers having a poor prognosis. Am J Pathol 2001, 159, 21-26.
53. *Shukla VK, Aryya NC, Pitale A, Pandey M, Dixit VK, Reddy CD, Gautam A:* Metallothionein expression in carcinoma of the gallbladder. Histopathology 1998, 33, 154-157.
54. *Siegsmud MJ, Marx C, Seemann O, Schummer B, Steidler A, Toktomambetova L, Kohrmann KU, Rassweiler J, Alken P:* Cisplatin-resistant bladder carcinoma cells: enhanced expression of metallothionein. Urol Res 1999, 27, 157-163.
55. *Simpkins CO:* Metallothionein in human disease. Cell Mol Biol 2000, 46, 465-488.
56. *Somji S, Sens MA, Lamm DL, Garrett SH, Sens DA:* Metallothionein isoform 1 and 2 gene expression in the human bladder: evidence for upregulation of MT-1X mRNA in bladder cancer. Cancer Detect Prev 2001, 25, 62-75.
57. *Stenram U, Ohlsson B, Tranberg KG:* Immunohistochemical expression of metallothionein in resected hepatic primary tumors and colorectal carcinoma metastases. APMIS 1999, 107, 420-424.
58. *Sugita K, Yamamoto O, Asahi M:* Immunohistochemical analysis of metallothionein expression in malignant melanoma in Japanese patients. Am J Dermatopathol 2001, 23, 29-35.
59. *Surowiak P, Dzięgiel P, Matkowski R, Wojnar A, Kornafel J, Zabel M:* Significance of metallothionein (MT) expression in tumor and myoepithelial cells in structures of ductal breast carcinoma. (submitted).
60. *Sutoh I, Kohno H, Nakashima Y, Hishikawa Y, Tabara H, Tachibana M, Kubota H, Nagasue N*: Concurrent expressions of metallothionein, glutathion s-transferase-[pi], and P-glycoprotein in colorectal cancers. Dis Colon Rectum 2000, 43, 221-232.
61. *Szilgyi Z, Fenselau C:* Molecular dynamics simulation of metallothionein-drug complexes. Drug Metab Dispos 2000, 2, 174-179.
62. *Tan YI, Sinniah R, Bay BH, Singh G:* Expression of metallothionein and nuclear size in discrimination of malignancy in mucinous ovarian tumors. Int. J Gynecol Pathol 1999, 18, 344-350.
63. *Tan YI, Sinniah R, Bay BH, Singh G:* Metallothionein expression and nuclear size in benign, bordeline, and malignant serous ovarian tumors. J. Pathol 1999, 189, 60-65.
64. *Tews DS, Fleissner C, Tiziani B, Gaumann AKA:* Intrinsic expression of drug resistance-associated factors in meningiomas. Appl Immunohistochem Mol Morphol 2001, 9, 242-249.
65. *Theocharis S, Karkantaris C, Philipides T, Agapitose A, Gika A, Margeli A, Kittas C, Koutselinis A:* Expression of metallothionein in lung carcinoma: correlation with histological type and grade. Histopathology 2002, 40, 143-151.
66. *Tomita T:* Metallothionein in pancreatic endocrine neoplasms. Mod Pathol 2000, 13, 389-395.
67. *Trieb K, Kotz R:* Proteins expressed in osteosarcoma and serum levels as prognostic factors. Int J Biochem Cell Biol 2001, 33, 11-17.
68. *Tuccari G, Giuffre G, Arena F, Baresi G:* Immunohistochemical detection of metallothionein in carcinomatous and normal human gastric mucosa. Histol Histopathol 2000, 15, 1035-1041.
69. *Tüzel E, Kirkali Z, Yorukoglu K, Mungan MU, Sade M:* Metallothionein expression in renal cell carcinoma: subcellular localization and prognostic significance. J Urol 2001, 165, 1710-1713.
70. *Vak M, Hasler DW:* Metallothioneins: new functional and structural insights. Curr Opin Chem Biol 2000, 4, 177-183.
71. *Vazquez-Ramirez FJ, Gonzalez-Campora JJ, Hevia-Alvarez E, Fernadez-Santos JM, Rios-Martin JJ, Otal-Salaverri C, Gonzalez-Campora R:* P-glycoprotein, metallothionein and NM23 protein expression in breast carcinoma. Pathol Res Pract 2000, 196, 553-559.
72. *Volm M, Koomägi R, Mattern J, Efferth T:* Protein expression profiles indicative for drug resistance of non-small cell lung cancer. Br J Cancer 2002, 87, 251-257.
73. *Wirgley E, Verspaget HW, Jayson GC, McGown AT:* Metallothionein expression in epithelial ovarian cancer: effect of chemotherapy and prognostic significance. J Cancer Res Clin Oncol 2000, 126, 717-721.
74. *Wronkowski Z, Zwierko M:* Epidemiology, risk factors and potential for bypassing them (in Polish). In: Nowotwory jelita grubego. Nowacki MP, ed. Wydawnictwo Wiedza i Życie, Warszawa 1996, 24-50.
75. *Zhang R, Zhang H, Wei H, Luo X:* Expression of metallothionein in invasive ductal breast cancer in relation to prognosis. J Environ Pathol Toxicol Oncol 2000, 19, 95-97.
76. *Zhang XH, Takenaka I:* Incidence of apoptosis and metallothionein expression in renal cell carcinoma. Br J Urol 1998, 81, 9-13.

Address for correspondence and reprint requests to:
P. Dzięgiel M.D.
Chair and Department of Histology and Embryology
University School of Medicine
ul. Chałubińskiego 6a, 50-368 Wrocław
tel. (071) 784-00-81
fax (071) 784-00-82
e-mail: piotr@hist.am.wroc.pl