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ORIGINAL ARTICLE

Metallothionein stroma reaction in tumor adjacent healthy tissue in head and neck squamous cell carcinoma and breast adenocarcinoma

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Abstract

INTRODUCTION: An accumulation of genetic alterations forming the field of cancerization is an important event for the transformation from normal to cancer cell in multistep carcinogenesis. Histopathologically healthy tumor adjacent tissue might be considered as a cancerization field which is typified by genetic changes required for the development of cancer. Metallothionein (MT) is considered to be a protective and anti-apoptotic protein. The aim of our study was to evaluate the MT expression in head and neck squamous cells carcinoma and breast adenocarcinoma and their histologically healthy adjacent tissue. Materials and Methods: We have sampled 29 tissue samples in total derived from head and neck cancers and 29 samples of their clear surgical margins, 33 breast adenocarcinomas and 33 clear surgical margins. Antibody recognizing MT-1 was used for immunohistochemical analysis.

RESULTS: MT expression was revealed in 85,7% of head and neck cancers and 94% of breast adenocarcinomas. It was found in all tumor adjacent tissue. MT expression was statistically significantly higher in tumor adjacent tissue than in cancer tissue in cases with the presence of lymph node metastases in both, breast adenocarcinoma and head and neck squamous cell carcinoma. Generally stroma seems to respond to the presence of cancer by the expression of MT, even in tissues which normally do not express MT.

CONCLUSIONS: MT might be a normal or protective reaction of healthy adjacent tissue to the presence of tumor.

Introduction

An accumulation of genetic alterations forming the field of cancerization is an important event for the transformation from normal to cancer cell in multistep carcinogenesis. Genetically altered cells from expanding fields are identified both in head and neck and breast cancers [1,2,3]. Molecular factors generated in this contaminated tissue might be responsible for the elevated risk of cancer recurrence [4]. The potential of molecular diagnosis using mutations in the p53 gene as a tumor specific marker has been proven for the detection of occult cells clonally related to the tumor in surgical margins [5]. Head and neck cancer is the sixth most common cancer in the world. It is characterized by high recurrence rate or the occurrence of second primary tumors within upper respiratory tract mucosa, which is estimated at about 10–30% of surgically dissected tumors, independently of the histopathologically clear surgical margins [4,6]. Breast cancer is the most common cancer in women in developed countries. It was suggested that each breast cancer seems to have its own, unique pattern of genetic changes caused by a complicated interaction of accumulating genetic predisposing factors with somatic changes [7]. Some of the invasive breast cancers may have developed directly from morphologically normal epithelium, not only from ductal carcinoma in situ. Thus, at least some of the genetic changes found in the invasive cancers may be present in the tumor-adjacent normal tissue [5,8,9].

Histopathologically healthy tumor adjacent tissue might be considered as a cancerization field which possesses genetic alterations required for the development of cancer [10]. It is also suspected that single tumor cells clonally originating from primary cancer might migrate creating micrometastasis [5]. Occult tumor cells can also be found in healthy tumor adjacent tissue, and are not detected by current diagnostic procedures. Brennan and Partridge identified mutated p53 in healthy margins of head and neck cancer, and the p53 presence was related to more frequent recurrence of the disease and lower survival rate [5,10].

Metallothionein (MT) is a low-molecular weight (61 amino acids) cysteine rich metal-binding protein with functional roles in cell growth, repair and differentiation [11]. The MT-1 and MT-2 isoforms have been extensively studied, and are believed to serve an important role in the homeostasis of essential metals such as Zn^{+2} or Cu^{+2}, during growth and development, as well as in the detoxification of heavy metals such as Cd^{+2} and Hg^{+2}. MT could cause the deregulation of zinc finger transcription factors as well as other zinc--requiring proteins. The resulting of zinc-depleted state of the cell, can be implicated in the generation of genetic instability necessary for breast tumor progression [12]. The pro or anti apoptotic role of MT is somehow conflicting depending on studies but in most cases MT is considered as a protective and an anti-apoptotic protein [13,14]. MT is mainly a cytoplasmic protein although it is also detected in nucleus of cells in fetal or early neonatal period [14]. MT immunoreactivity was prominent in nasopharyngeal cancer tissue, presenting a nuclear pattern of staining being inversely correlated with apoptotic index [15]. It is also known that perinuclear MT localization is important for protective function of MT against DNA damage and apoptosis induced by external stress stimuli [11,14]. According to this study it was suggested that MT overexpression might protect tumor cells from entering apoptotic process and therefore to contribute to tumor expansion.

Cancer cells and clear surgical margin exert an effect on each other, and the environmental response probably participates in the process of tumor growth.

The aim of our study was to evaluate the MT expression in two histologically different tumors, head and neck squamous cell carcinomas and breast adenocarcinomas, and their histologically healthy adjacent tissue.

Materials and Methods

Group of patients

In all cases patients' consent was obtained. The approval for the research program from the Ethical Committee of the Jagiellonian University in Krakow: KBET/379/13/2003 was also granted. The patients in this study were randomly selected. Surgically removed material was evaluated to determine histological type and metastases of the lymph nodes using histological methods in the Department of Pathomorphology of the Jagiellonian University. The clear surgical margin was defined as $1cm^2$ area of tumor adjacent tissue microscopically free of neoplasmatic texture. The surgical resection line was macroscopically and histological free from cancer texture. All patients with head and neck and breast cancer in this study had undergone surgery between March 2003 and December 2003 at the Department of Otolaryngology and Head and Neck Surgery and in First Surgery Department of the Jagiellonian University in Krakow.

2.1 Head and neck cancer

We have sampled the total of 58 tissue samples: 29 samples derived from head and neck squamous cell carcinoma, 29 samples from clear surgical margins of these tumors and 20 healthy salivary glands. Squamous cell carcinoma was recognized in 29 cases of examined patients. Men constituted 79.3% (23 patients) of examined group, while women 20.7% (6 patients). The mean age of all patients was 55.3 years, ranging 42–74 years, the mean age of men was 59.6 years, the mean age of women was 53.8 years. The disease was recognized most commonly in 5^{th} decade of life (11 patients), 4^{th} decade (9 patients), 6^{th} decade (7 patients) and 7^{th} decade (2 patients). Clinical staging of 29 invasive tumors was as follows: 7 cases in stage II, 7 cases in stage III and 15 cases in stage IV. The cases were staged according to the TNM Classification of Malignant Tumors 5th edition 1997. Eleven radical laryngectomies were performed, 7 laryngectomies with partial pharyngectomy and 11 partial pharyngectomies. Twenty-one patients received radiation therapy after operation because of lymph node metastases.

2.2 Breast cancer

The study group consisted of 33 patients with breast cancer. All women underwent mastectomy with axillary lymphadenectomy in the First General Surgery Department of the Jagiellonian University. The women's age ranged between 34–79 years (mean age – 61 years). In each case invasive carcinoma was identified. In all cases surgical material was obtained after modified radical amputation of the breast with the use of Patey's method with simultaneous removal of axillary lymph nodes. Surgical material was fixed in 10% buffered formalin. Then tissue was embedded in paraffin and stained with hematoxylin and eosin. Each specimen was inspected to specify tumor size and a number of lymph nodes obtained for the study. Microscopic examination was performed to identify histological type and grade of invasive ductal carcinoma, the presence of vessel invasion and tumor metastases to the lymph nodes. Histological grades of invasive carcinoma were diagnosed according to Bloom and Richardson classification modified by Elston and Ellis, recommended by the National Coordinating Group for Breast Pathology [18,19].

2.3 Immunohistochemistry

Immunohistochemical analysis was performed in the Pathomorphology Department of the Jagiellonian University. In all immunohistochemical stainings Envision method was applied using Dako Autostainer, so the procedure was the same besides single steps (antigen unmasking, where appropriate, antibody dilutions, incubation period). Five-μm slides were deparaffinized, rehydrated and rinsed 5 times in distilled water. Endogenous peroxidase activity was blocked by 8-min-incubation in 3% H_2O_2 at room temperature. The slides were then rinsed and immersed in boiling citrate buffer (pH 6.0) in a microwave oven with three changes of buffer for 5 minutes each. For Metallothionein immunostaining the monoclonal mouse antibody ImmunO^{TM} (MP Biomedicals, Inc., clone 1A12 in dilution 1:1000) was used, without any previous unmasking procedure, with 60-min-incubation at room temperature. In both cases the same specimens were used as a negative control with omission of the primary antibody. This antibody recognized human MT-1 izoforms. The slides were subsequently rinsed in TBS buffer (pH 7.6) and incubated with secondary antibody (DAKO Envision TM+ System Labelled Polymer HRP – anti mouse (DAKO, Denmark) for 30 minutes at room temperature. Visualization was performed using AEC (3-amino-9-ethyl-carbazole) as a chromogen (AEC Substrate Chromogen ready-to-use, DAKO, Denmark) for 10 minutes at room temperature. Sections were counterstained with hematoxylin and mounted in glycergel. Immunohistochemical expression of MT was evaluated without any knowledge on the clinicopathologic data. The degree of Metallothionein positivity was quantified as the percentage of MT-positive cells in the tissue section, done in at least 5 high power fields (usually at least 150 cells were counted). Only cytoplasmic staining was evaluated as: 0 – lack of any positivity; 1+ – weak staining in less than 5% of the cells; 2+ – moderate – various staining intensity but in <50% of the cells, 3+ – strong – staining of more than 50% of the cells.

2.4 Statistical analysis

The distribution of subjects was analyzed using Shapiro-Wilk's test. Obtained data and control data were compared using Student's t-test and Mann-Whitney test. Significance was accepted at $p<0.05$.

Results

3.1. Head and neck cancer- squamous cell carcinoma

MT expression was identified as a brown, granular staining pattern. In cancer cells it was found both in their cytoplasm and nuclei (*Fig. 1*). Stromal cells as well as cartilage cells represented an evident cytoplasmic staining pattern (*Fig. 2*). Basal cells of stratified epithelium represented a nuclear staining pattern (*Fig. 3*). Lymph nodes and tumor infiltrating lymphocytes were MT expression negative (*Fig.1*). Infiltrating inflammatory cells were MT expression positive. They represented a cytoplasmic staining pattern.

3.1.1 MT expression in malignant tissues

MT expression was identified in 85,7% squamous cell carcinomas, while 4 squamous cell carcinoma specimens were MT expression negative. Cancer cells in the center of the nests were MT expression negative, while peripherally located cells strongly expressed MT. Most of them represented an evident cytoplasmic staining pattern but single cells demonstrated nuclear pattern of staining (*Fig. 4*).

3.1.2 MT expression in healthy tissues

Basal cells of stratified squamous cell epithelium expressed MT in 44,8% of cases. MT expression was identified in healthy stromal cells of tumor adjacent tissues. All stromal specimens, mainly composed of spindle-shaped fibroblasts, expressed MT. MT stromal expression was detected even in cases in which cancer specimens did not show MT expression. All salivary glands were MT expression negative. We observed an intense MT expression at the border of cancer and

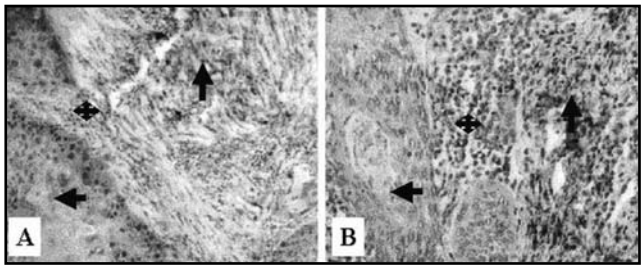

Figure 1. Demonstrates MT expression in head and neck cancer and clear surgical margin. Cancer cells (vertical arrow), were MT expression positive (A) or negative (B), represented a nuclear and cytoplasmic staining pattern. Stroma expressed MT in both cases (horizontal arrow). Lymphocytes (double arrow) were MT immunoreactivity negative.

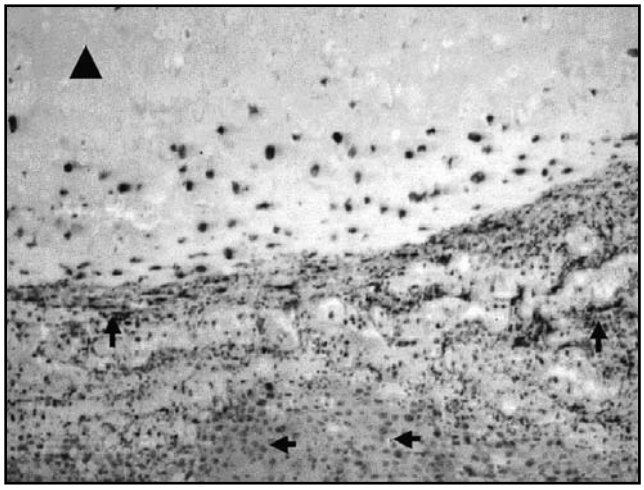

Figure 2. Presents head and neck cancer (vertical arrow) with healthy adjacent tissue, stroma and cartilage. Healthy cartilage expressed MT (triangle) when it adjusted to tumor tissue, in other regions, which were distant from tumor cartilage did not express MT. Healthy tumor adjacent tissue represented an evident cytoplasmic staining pattern (horizontal arrow).

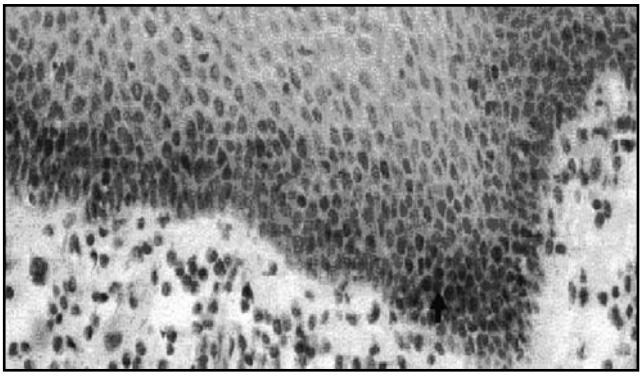

Figure 3. Demonstrates MT expression in normal head and neck mucosa (stratified squamous epithelium), where it is expressed by basal and parabasal dividing cells.

stroma, both in cancer and stroma. It appeared that tumor induced MT expression in adjacent healthy stroma (*Fig. 5*). Strong MT immunoreactivity was also identified in healthy cartilage in the direct cancer vicinity, while cartilages in other regions of tissue slides, distant from cancer were MT expression negative (*Fig. 2*).

3.1.3a MT expression in cancer and tumor adjacent tissue and clinicopathological features

MT expression in head and neck cancer and its clear surgical margin with respect to the presence of lymph node metastases is presented in *Table 1*.

MT immunoreactivity was significantly higher in tumor adjacent healthy tissue than in cancer tissue, in cases with the presence of lymph node metastases. Higher MT immunoreactivity in tumor adjacent healthy tissue than in cancer was identified in poorly differentiated tumors than in moderately differentiated ones.

3.1.3b Independent analysis of MT expression in cancer and adjacent healthy tissue

No significant differences were found in MT immunoreactivity in tumor cells according to cancer differentiation in moderately (G2) and poorly (G3) differentiated tumors, although MT expression was higher in G2 tumors (p=0.7). Similarly, no significant differences in MT immunoreactivity in tumor adjacent healthy tissue were observed with respect to cancer cells differentiation. Still MT immunoreactivity in tumor adjacent tissue was higher in G2 tumors stroma than in G3 (p=0.4).

Significantly higher MT immunoreactivity in cancer samples was found in cases with the presence of lymph node metastases than in cases without metastases (p=0.03). Also higher MT immunoreactivity was identified in tumor adjacent healthy tissue in cases with the presence of lymph node metastases than in cases without metastases (p=0.1).

Table 1. Demonstrates the changes of MT expression in head and neck cancer and its clear surgical margin with respect to the presence of lymph node metastases.

	Metallothionein expression	0*	1*	2*	3*	p-value
Head and neck cancer tissue, N0 status (n=13)	Cancer expression (n=13)	15(2)	39(5)	39(5)	7(1)	0.32
	Clear surgical margin expression (n=13)	-	93(12)	7(1)	-	
Head and neck cancer tissue, N1,N2 status (n=16)	Cancer expression (n=16)	19(3)	68(11)	13(2)	-	0.046
	Clear surgical margin expression (n=16)	-	56(9)	31(5)	13(2)	

Table 2. Immunohistochemical analysis of Metallothionein expression in breast cancer tissue

	Metallothionein expression	0*	1*	2*	3*	p-value
Breast cancer tissue, N0 status (n=15)	Cancer expression (n=15)	6 (1)	33 (5)	49 (7)	12 (2)	0.01
	Clear surgical margin expression (n=15)	-	12 (2)	33 (5)	55 (8)	
Breast cancer tissue N1,N2 status (n=18)	Cancer expression (n=18)	5.5 (1)	27.5 (5)	44 (8)	23 (4)	0.0003
	Clear surgical margin expression (n=18)	-	5.5 (1)	11 (2)	83.5 (15)	

*All figures refer to percentage of samples

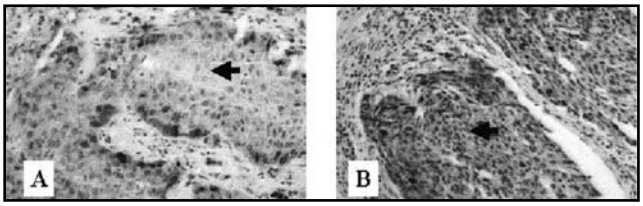

Figure 4. Presents MT immunoreactivity in head and neck cancer nests. Immunoreactivity is growing from the center to the peripheral part.

A - The intensity of immunoreactivity is negative in the center and positive in the peripheral part.
B - The intensity of staining is growing from moderate in the center to strong in the peripheral part of the nest; staining pattern is cytoplasmic and nuclear.

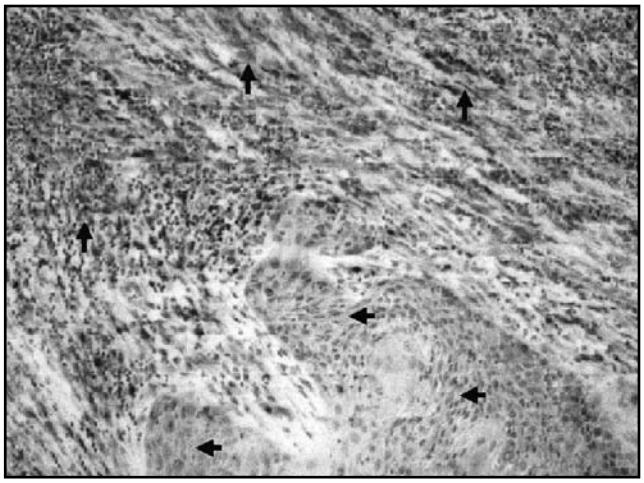

Figure 5. Positive MT reactivity in stroma (vertical arrow) and weak MT reactivity in head and neck cancer nests (horizontal arrow).

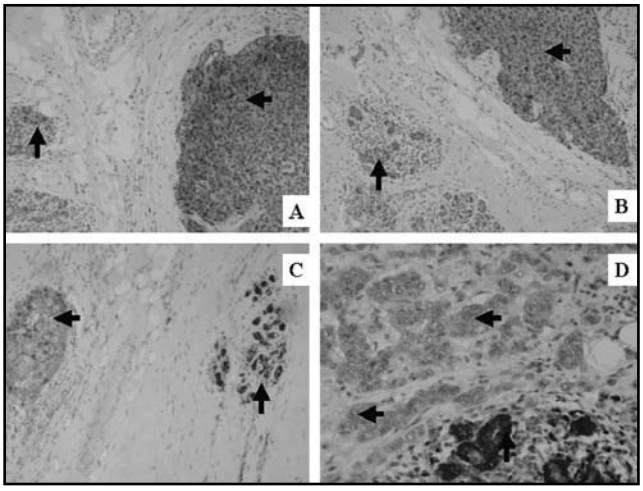

Figure 6. MT expression in breast cancer cells (horizontal arrow) represented brown, granular staining pattern, and in stromal cells as well as myoepithelial and normal glandular epithelium cells both nuclear and cytoplasmic (vertical arrow). MT expression was higher in breast cancer tissue than in healthy tumor adjacent tissue (A, B) and in others MT expression was higher in healthy adjacent tissue than in cancer (C,D). MT immunoreactivity was higher in peripherally located cells of cancer nests than in the center of the nests (A) and higher in the neighborhood of MT positive normal glands (B).

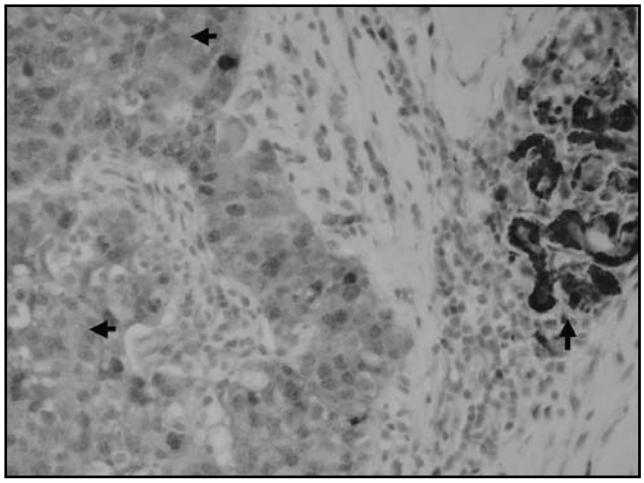

Figure 7. MT expression in breast cancer (horizontal arrow) and in normal breast glands (vertical arrow): myoepithelial cells and glandular epithelium represented both nuclear and cytoplasmic staining pattern.

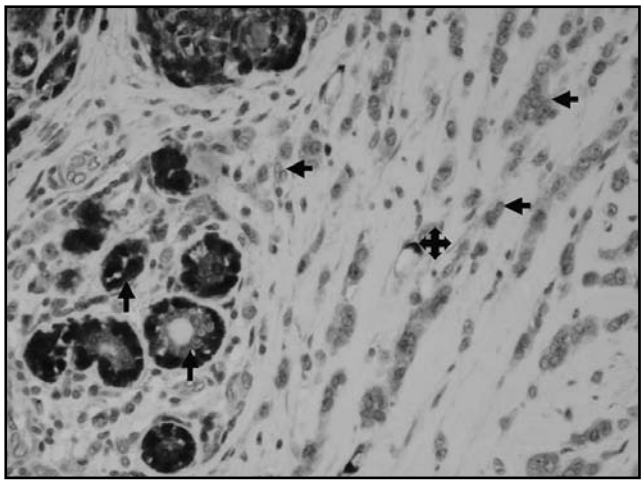

Figure 8. MT expression in breast cancer (horizontal arrow) and in normal breast glands (vertical arrow) and myoepithelial cells (vertical arrow). Myoepithelial MT positive cells were detected in tumor adjacent healthy vascular wall (double arrow). Healthy gland cells in the tumor neighborhood express MT, the immunoreactivity decreases from the tumor adjacent cells to the gland's lumen.

3.2 Breast cancer-adenocarcinoma

MT expression represented brown, granular staining pattern, and it was present in cancer and in stromal cells as well as in myoepithelial and normal glandular epithelium cells, both nuclear and cytoplasmic ones. Tumor infiltrating lymphocytes were MT expression negative (*Fig. 6*).

3.2.1 MT expression in malignant tissues

MT expression was revealed in 94% of cancer tissue samples. The MT staining pattern differed according to the intensity of MT expression. Cytoplasmic staining pattern was predominantly detected in cases of weak (+1) intensity of MT expression. In cases of moderate or strong intensity of expression, nuclear staining pattern was more frequently recognized than cytoplasmic. In cancer nests MT expression was stronger in peripher-

ally located cells than in cells in the center of the nest (*Fig. 6*).

3.2.2 MT expression in healthy tissues

MT was identified in all samples derived from tumor adjacent healthy tissue. MT immunoreactivity decreased with the growing distance from tumor. The immunoreactivity of MT in tumor adjacent healthy tissue was represented predominantly by myoepithelial and epithelial cells of the glands. In myoepithelial cells both cytoplasmic and nuclear type of staining was observed, but cytoplasmic staining dominated, whereas glandular epithelium showed nuclear staining rather than cytoplasmic staining pattern (*Fig. 7*).

3.2.3 MT expression in cancer with respect to clinicopathological features

MT expression was evaluated in cancer and healthy tumor adjacent tissue and analyzed as regards the presence of lymph node metastases and tumor grading.

3.2.3a Comparison of MT expression in cancer and adjacent healthy tissue

The expression of MT with respect to the presence of lymph node metastases is presented in *Table 2*.

MT immunoreactivity was statistically significantly higher in tumor adjacent healthy tissue than in cancer tissue in cases with the presence of lymph node metastases. Also significantly higher MT immunoreactivity was observed in cases without the presence of lymph node metastases, but the difference was not that pronounced, as in the group of the patients with the presence of lymph node metastases.

The differentiation of breast cancer was evaluated according to Bloom classification: 13% of cases represented Bloom-1 stage, in 54% Bloom-2 stage, and in 33% Blooom-3 stage were recognized. Higher MT immunoreactivity in tumor adjacent healthy tissue than in cancer was identified in all types of tumor differentiation. Breast cancer tumors classified as Bloom-2 group represented significantly higher MT expression in tumor adjacent tissue than in cancer (p=0.017). In Bloom-1 group MT immunoreactivity was a little higher, while in Bloom-3 it was on the verge of statistical significance (p=0.08).

3.2.3b Independent analysis of MT expression in cancer and adjacent healthy tissue

No differences were found in MT immunoreactivity in tumor cells according to the cancer differentiation in Bloom-2 and Bloom-3 cancers. No differences in MT immunoreactivity in tumor adjacent healthy tissue were observed according to cancer cells differentiation. Higher MT immunoreactivity in cancer samples was found in cases with the presence of lymph node metastases than in cases without metastases (p=0.68). Also higher MT expression was identified in tumor adjacent healthy tissue in cases with the presence of lymph node metastases than in cases without metastases (p=0.08).

The analysis of correlation between the presence and number of lymph node metastases and the expression of MT correlation coefficient was 0.3 and revealed statistically significant correlation (p=0.045). MT expression in cancer did not correlate with the presence of lymph node metastases.

Discussion

MT expression in tumor adjacent tissue of examined cancers (breast adenocarcinoma and head and neck squamous cells carcinoma) in comparison to MT expression in cancer tissue was significantly higher in cases with the presence of lymph node metastases in comparison to cancers without the presence of lymph node metastases (*Table 1,2*).

MT expression is recognized as a useful prognostic tool, especially in invasive ductal carcinoma [20]. In head and neck cancer high MT-1 and MT-2 immunoreactivity correlated with increased local and regional recurrence, resulting in poor prognosis [21]. Contrary to these findings, the analysis of MT-1 and MT-2 expression performed in oesophageal cancer revealed little impact on the outcome [22]. Although MT-3 expression analysis in infiltrating ductal breast carcinoma indicated that MT-3 positive cells were more frequent in patients with bad outcome [12]. Both in the studied head and neck and breast cancers the connection between MT expression in healthy tumor adjacent tissue and the presence of lymph node metastases was noted. The presence of lymph node metastases is a poor prognostic factor. Consequently MT expression in clear surgical margin indicates a worse prognosis.

Immunohistochemical studies have demonstrated MT to be present in the cytoplasm and nuclei of several physiological tissues and, besides, to be expressed at elevated levels in a variety of tumor cells. In normal oral squamous cell epithelium MT was located only in the basal-parabasal dividing cells [23]. In our study MT expression was localized in the nuclei of basal cells of normal epithelium of head and neck (*Fig. 3*), which is understandable because these cells are responsible for proliferation and renewal of the epithelium. Normal stromal cells and inflammatory cells represented an evident cytoplasmic staining pattern. Healthy salivary glands were chosen as a control tissue. MT expression was reported to be intense in pleomorphic adenoma and adenoid cystic carcinoma, and was present in duct epithelial and myoepithelial cells [14,24]. In our study healthy salivary glands in tumor adjacent tissue of head and neck cancer were MT expression negative. However, normal breast glands adjacent to breast cancer were MT positive and nuclear type of staining dominated over the cytoplasmic staining pattern in these cells. MT cytoplasmic staining pattern was also observed in normal myoepithelial cells adjacent to breast cancer (*Fig. 7*).

Both breast cancer and head and neck cancer represented a cytoplasmic and nuclear staining pattern. This remains in concordance with oral cavity squamous cell carcinomas, in which MT positivity was observed in the nests of well-differentiated carcinomas in the peripherally located tumoral cells, but not in the center of the nests, where increased rate of apoptosis was found [15].

Less differentiated areas presented MT positivity, but little apoptosis, suggesting that MT, possibly due to its chelating properties, might contribute to delaying cells entering apoptosis both in normal epithelium near the base and in less differentiated regions of tongue squamous cell carcinoma [23]. Similarly, in our research the cells located in the center of head and neck squamous cell carcinomas nests did not express MT, while peripherally located cells strongly expressed MT (*Fig. 4*).

Although Jin et al. demonstrated both nuclear and cytoplasmic staining patterns type of MT-1 and MT-2 in breast cancer [24,25,26], in our study predominant nuclear MT staining pattern was observed in cancers of strong and moderate MT immunoreactivity, while predominant cytoplasmic staining pattern was observed in weak MT tumors [25,26]. In breast cancer, as well as in head and neck cancer, MT expression was higher in peripherally located cells in cancer nests (*Fig. 4, 6*). MT-1 and MT-2 immunohistochemical analysis in breast cancer was significantly higher in poorly differentiated tumors in comparison to moderately differentiated tumors [25,26]. In the present study, we did not observe any differences in MT expression with respect to cancer grade. However, when the comparison of MT expression in cancer and tumor adjacent tissue of each case was considered, it turned out that significantly higher MT expression was detected in tumor adjacent healthy tissue in comparison to breast cancer classified as Bloom-2 tumors and Bloom-3 tumors. However, the difference was lower in Bloom-3 tumors. This might indicate that cancer cells which are less differentiated in Bloom-3 are not properly recognized and exert weak effect on the adjacent tissue, which might let the single occult tumor cell spread without a significant trace in healthy adjacent tissue.

In the current study it appeared that healthy tumor adjacent tissue responded to tumor growth by the expression of MT. The presence of MT expression was revealed in all healthy tumor adjacent samples, even in those in which cancer cells did not express MT (*Fig. 5*). We also observed that healthy cartilage in the direct head and neck tumor vicinity expressed MT while healthy cartilage distant from tumor did not express MT. In addition, in breast cancer MT positive cells were detected in tumor adjacent healthy vascular wall. Healthy gland cells in the tumor vicinity also expressed MT. The immunoreactivity decreased from the tumor adjacent cells to the gland's lumen (*Fig. 8*).This might suggest that cancer cell migrating from the tumor evokes the MT expression in adjacent cells. It might therefore be speculated that healthy adjacent tissue responded to cancer growth by MT expression, which high representation is related to accepted poor prognostic factors like the presence of lymph node metastases. Myoepithelial cells in tumor adjacent healthy tissue might be responsible for the proliferation of adjacent tumor cells by, for example secretion of growth factors [27]. MT overexpression in tumor adjacent healthy tissue in our study might confirm this possible participation of MT in maturation and proliferation. This could be also a reaction to the factors secreted by tumor cells, or tumor cell fragments which might be represented by apoptotic bodies, or tumor cell derived microvesicles (TMV). It was demonstrated that TMV might modulate biological function of tumor infiltrating monocytes and lymphocytes. In 1972 JF Kerr described apoptosis and indicated the possible usage of apoptotic cell fragments by normal epithelial cells and neoplasmatic cells [28]. This phenomenon seems also to be very important in the light of multistep genetic carcinogenesis [3]. Apoptotic bodies include normal cell organelles, mRNA and DNA. It was also demonstrated that mRNA and DNA are separately packed in apoptotic bodies during apoptosis [29]. DNA amplification derived from apoptotic body was also confirmed [30,31]. Garcia showed that mutated DNA originating from apoptotic body may create a genometastasis [32,33]. MT expression might also result from the normal cell exposure to cancer and cancer derived factors like DNA or cell fragments. Klimek's theory concerning cancerogenesis posits the existence of an area adjacent to tumor exposed to cancerous transformation and defines this area as a dysypatogenic structure comprising the cells that have already entered the neoplastic transformation [34,35]. Our findings remain in compliance with this theory, the expression of Metallothionein in tumor adjacent histopathologically healthy tissue might be the manifestation of already present alterations in these cells, which have not yet developed phenotypic changes.

In conclusion, this finding might suggest, that MT expression is a normal or protective reaction of healthy adjacent tissue. We posit that MT expression in healthy stroma is induced by spreading tumor cells leaving a "snailmark" delaying apoptosis and inducing immortalization of these cells, which might presage the development of cancer.

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