[Frontiers in Bioscience 13, 4029-4038, May 1, 2008]

The possible biological role of metallothionein in apoptosis

Magdalena Dutsch-Wicherek[1], Jerzy Sikora[2], Romana Tomaszewska[3]

[1]Department of Otolaryngology, Head and Neck Surgery of the Jagiellonian University, Krakow, Poland,[2]Department of Gynecology and Obstetrics of the Medical University, Katowice, Poland,[3]Department of Pathomorphology of the Jagiellonian University, Krakow, Poland

TABLE OF CONTENTS

1. ABSTRACT

Metallothioneins (MTs) are a family of low molecular weight proteins with a high affinity for divalent metals. Metallothionein has been shown to have a protective role in apoptosis. Specifically, it controls the cellular zinc ion levels. The proper intracellular Zn^{2+} level maintains the fragmentation of DNA associated with caspase-3 activity. In cancer nests, MT has been identified in the peripheral regions and associated with higher cell proliferation rates and fewer positive apoptotic cells. The expression of MT in the stroma has been linked with tumor spread. The tumor stroma may respond to tumor growth and aggressiveness by the expression of MT, thus protecting itself from and developing a resistance to apoptosis. Multistep carcinogenesis is related to a breakdown in immune response that enables tumor progression and dissemination. This breakdown is a crucial for tumor survival. The evaluation of MT expression in cancer and its stroma seems to correlate with the level of immune system inhibition in cancer-adjacent stroma.

2. INTRODUCTION

The development of cancer is a multistep process. First, a cell acquires a malignant phenotype. In this stage, the accumulation of genetic alterations forms a molecular basis for the transformation from normal cell to cancer cell. For example, in a proposed model, ovarian cancer was demonstrated to arise from definite genetic alterations (mutations of TP53, hTERT, HRAS)(1). But genetic alterations were also identified in histopathologically healthy mucous membranes from tumor-adjacent tissue. Initially, the histological examinations performed on oral epithelium in 1953 by Slaughter *et. al* revealed that oral cancer developed from the multifocal areas of precancerous alterations that surrounded the tumor and in which second primary tumors would develop following surgical treatment. This abnormal tissue was defined as "field cancerization" (2). Later, genetic changes were discovered in this "field cancerization," also called "field effect". These genetic alterations were found in organs such as the oral cavity, oropharynx, larynx (3), lung (4), esophagus (5),

vulva (6), cervix (7), colon (8), breast and bladder (9), and skin (10). A concept of "field cancerization" and a model of biological multistep carcinogenesis were thus proposed (11), (12). In this model, a stem cell acquires one or more genetic alterations and forms a patch with genetically altered daughter cells; these escape from normal growth control and form a field, thus displacing normal epithelium. It is this field that develops into cancer (11) (12). Further progression of the disease will depend upon the interaction between immune and cancer cells and is enabled by the phenomenon of tumor escape from host immunological surveillance. In patients with cancer, immune system function is disrupted. For example, in head and neck cancer patients, a decrease in the number and activity of TIL cells correlates with poor survival rates (13,14,15). TILs were found to undergo spontaneous apoptosis via the Fas/FasL pathway and other death molecules (16,17,18). Moreover, a significantly higher number of peripheral blood mononuclear cells undergoing apoptosis were found in head and neck cancer patients in comparison to healthy controls. It has been shown that these lymphocytes (19) were programmed to undergo apoptosis even though the symptoms of the disease were clinically absent in the patients from whom the lymphocytes were obtained (following surgery, and chemo- and radiotherapy). This effect was even more pronounced in patients prior to treatment. A tumor suppresses immune system activity not only locally, but also systemically, and this suppression has a long-term effect (19). The final progression of the tumor is associated with the development of resistance to anti-cancer treatment.

Metallothioneins (MTs) are a family of low molecular weight proteins that have a high cystein content and a tri- dimensional structure together with a high affinity for divalent metals—both essential metals, such as zinc and copper, and toxic metals, such as cadmium and mercury (20,21). Mammalian metallothioneins include four isoforms. MT-1 and MT-2 are widely distributed in the tissues, while MT-3, MT-4 are found in specialized cells (21). This metal-binding property is linked with MT's biological roles which include protection against metal toxicity, the donation of zinc and copper to metallo-enzymes involved in apoptosis, production of gene transcription factors, and protection against oxidative stress (21). MT may also play a role in cell proliferation and differentiation (21,22). The expression of MT in the cytoplasm is thought to be indicative of its protective role against cytotoxicity, while its nuclear expression is related to its ability to protect against genotoxicity (21-23). Genotoxicity has to do with the cell's acquisition of the malignant phenotype, especially certain mutations that play a critical role in carcinogenesis. Cell cytotoxicity is important in the interactions that occur between immune cells and cancer cells. Both kinds of interactions are involved in the resistance to chemo- and radiotherapy. In sum, although MT was discovered more than 50 years ago, its many biological roles are not yet fully understood.

The MT antioxidant properties may include the interception of free radicals, complexation of redox-sensitive transition metals, and the alteration of zinc homeostasis or interaction with glutathione (GSH) (24). The redox properties of MT are essential for buffering zinc ions in cellular signaling. MT is also an efficient scavenger of hydroxyl radicals. Yeast and mammalian MTs can functionally substitute for superoxide dysmutase in protecting yeast from oxidative stress. Expression of the mouse MT-I gene in response to zinc is regulated by the zinc-finger transcription factors (MTF-I) (25). MTF-I is a metalloregulatory protein whose DNA-binding activity is reversibly activated in response to changes in free zinc concentration levels in the cell (25). Cells which contain higher levels of MT expression are protected against heavy metal toxicity and oxidative stress, whereas under-expression in cell lines or in mice with null mutations of the MT-I and MT-II genes leads to increased susceptibility to cadmium toxicity and oxidative stress (25). The redox status of the cell, measured by the ratio of reduced to oxidized glutathione and energy metabolism together, affects the binding and release of zinc from MT. An increase in oxidized glutathione in the cell can facilitate the release of zinc from MT. The metabolic enzyme activity can be transiently inhibited by redistributed zinc in the cells. Zinc may protect free thiols from oxidation and stabilize membranes, as well as restore activity to zinc-finger proteins (25).

3. METALLOTHIONEIN AND METAL IONS

Mammalian metallothionein can bind with metal ions in two distinct cluster structures: the first cluster, closer to the N-terminal, binds three metal atoms to nine cysteins; the second cluster, closer to the C-terminal, binds four metal atoms to eleven cysteins (21). Cadmium and zinc induce metallothionein synthesis and bind avidly to MT (25).

Zinc is an intracellular mediator of apoptosis which may interfere with the action of calcium (26). Zinc prevents DNA fragmentation in many cell lines, inhibiting the calcium-magnesium dependent endonuclease (27), while zinc chelation induces apoptosis in different cell lines (28). Zinc specifically inhibits caspase-3, a protease implicated in apoptosis (29). Changes in intracellular zinc levels are sufficient to alter susceptibility to apoptosis; in order to regulate tissue growth via molecular targets such as endonucleases and caspases proteases (30), zinc may serve as a coordinating regulator of mitosis and apoptosis. A model has been proposed for zinc's regulatory role according to which it protects cells from apoptosis by maintaining caspase-3 and calcium/magnesium endonuclease in an inactive form in the resting state of the cell. Changes in zinc concentration and/or localization in the cell constitute the major step that may set off the cascade of events leading to cell death (31).

Cadmium is an environmental pollutant for most organisms; in mammals it is nephrotoxic, neurotoxic, and carcinogenic. It can induce cell death by either necrotic or apoptotic mechanisms. The induction of apoptosis by cadmium seems to trigger the mitochondrial pathway of apoptosis (32). Furthermore, exposure to cadmium has been shown to increase endogenous bcl-2 protein sooner

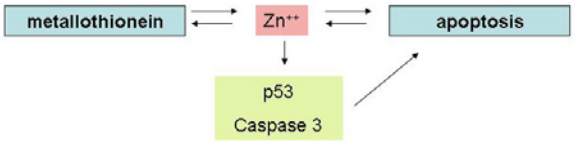

Figure 1. MT regulation of apoptosis.

than would occur through metallothionein induction. Bcl-2 protein over-expression prevented not only cadmium-induced apoptosis, but also the necrotic effects of cadmium (33).

The anti-apoptotic effects of MT are highly dependent on cellular MT levels (34). MT can provide protection from apoptosis regardless of the metal content of the protein as it is just as effective when induced by cadmium as by zinc (35).

4. METALLOTHIONEIN AND APOPTOSIS

Apoptosis is a physiological process of programmed cell death. It occurs in such physiological events as embryogenesis (36) and the selection of lymphocytes in the thymus (37). Apoptosis is responsible for the regulation of cell populations in normal tissues and cell population increases in malignant neoplasms where it participates in tumor regression and ontogenesis (38).

4.1. Apoptosis phases

Apoptosis is characterized by internucleosomal DNA fragmentation, chromatin condensation, and cytoplasmic blebbling with the formation of apoptotic bodies which are membrane-enclosed structures containing cytosol, condensed chromatin, and organelles (38). As the cell dying by apoptosis is metabolically still active, the process often depends on RNA and protein synthesis (39). DNA fragmentation is associated with the activity of DFF40 nuclease and with calcium/magnesium-dependent endonuclease which can be inhibited by zinc (40).

4.2. Apoptosis pathways

Caspases are proteolytic enzymes activated via various pathways in the early stage of apoptosis (41). Two of the major pathways will be described here. The first is the caspase-8 pathway that mediates the extracellular signal of TNFα or FasL for the mitochondria, leading to the generation of activated caspase-8, as well as to the release of cytochrome *c* and the activation of procaspase-9. Activated caspases-8 and -9 are responsible for procaspases-3 and -7 activation. The second pathway involves chemical compounds, such as etoposide, which induce apoptosis by the direct activation of caspase-9 and leads to the activation of caspase-3 (31, 42). The activation of caspase-3 seems to be an essential step in apoptosis. Casapse-3 is responsible for the activation of DNA fragmentation factor (DFF) which is composed of a 40kDa protein DFF40 with a nuclease activity and a 45 kDa protein inhibitory subunit DFF45 (43). DFF45 has been demonstrated to be a substrate for caspase-3 during apoptosis (44).

4.3. Regulation of apoptosis

The phenomenon of apoptosis is regulated by a variety of factors. Apoptosis can be induced by mutagens, genotoxic anti-cancer drugs, ionizing irradiation, and nitric oxide, as well as other factors. Its inhibition is controlled by Bcl-2 and the antioxidants superoxide dysmutase and glutathione peroxidase (45). Metallothionein has been shown to have a protective role in apoptosis. MT controls the cellular zinc ion level and thereby seems to modulate the functional activity of p53 protein (Figure 1). p53 protein is a zinc-binding transcription factor that can either inhibit cell cycle progression or induce apoptosis in response to DNA damage or stress so that the damaged DNA can then be eliminated. Various mechanisms are responsible for the attenuation of functional p53 in cancer cells (46). MT controls the intracellular distribution of zinc and acts as a potent chelator, removing zinc from p53 (Figure 1). Zinc ions are essential for the maintenance of the wild type conformation and stability of p53 protein and its affinity for a specific DNA sequence (47). Ostrakovitch *et al*. has observed that, in its apo-form (metal-free form), MT interacts with p53, and so has suggested that apo-MT/MT could be a control mechanism regulating p53 activity (48). Persistent apo-MT over-expression in tumor cells may promote their accelerated growth and enhance their survival through the induction of a p53-null state (49). MT may also protect cells against p53-mediated apoptosis (23) (Figure 1).

The cleavage of chromosomal DNA into nucleosomal units would seem to be the biochemical hallmark of apoptosis. The fragmentation of DNA is associated with caspase-3 activity, and this is maintained by the proper intracellular Zn^{2+} level. A decrease in Zn^{2+} leads to an increase in caspase-3 activity (31), and conversely, Zn^{2+} has been observed to inhibit caspase-3 activity (29). The maintenance of the proper intracellular Zn^{2+} level (34) is a function of MT (Figure 1). The relation between an increase in the cytoplasmic MT level and a decrease in caspase-3 activity has also been identified with a decrease in mitochondrium-originating cytochrome c concomitant with the rise of MT expression in the cytoplasm. Kondo *et al*. has suggested that the rise in cytoplasmic MT expression observed during chemotherapy does not result directly from drug action, but rather from hormonal activity in the tumor microenvironment (24,50). Kondo has further proposed a possible complementary anti-apoptotic interaction between MT and Bcl-2. A bilateral regulation between MT-2A and ECRG2 has been shown (51). The ECRG2 gene expression product is responsible for the inhibition of the proliferation and induction of apoptosis, while MT intensifies proliferation and restricts apoptosis (51). An increase in the spontaneous apoptosis level has been observed in mouse fetal cells deprived of MTI and MTII isoform genes (24,50).

MT has been found to be a negative regulator of NF-kappa B (nuclear factor kappa B) activity (52). NF-kappa B is a transcription factor; the activation of this factor is associated with HIAP (human inhibitor of apoptosis protein) which cuts IkB in cytoplasm. Consequently, the membrane receptors TNFR-1 (p55),

CD40, DR3, DR4, and DR5, which possess the TNF receptor associated death domain (TRADD), are activated. An increase in NFκB expression has been associated with an increase in telomerase activity, and its participation in the promotion of tumor progression in squamous cell carcinoma has been demonstrated (53). NFκB expression has also been shown to participate in the induction of drug resistance (54). NFκB regulates pro-inflammatory cytokine gene expression through TNF-α (tumor necrosis factor-alfa). NFκB also promotes tumor transformation and survival; it therefore participates in the acquisition of the malignant phenotype (53). TNF-α may simultaneously induce the caspases cascade through the activation of NF-κB, which seems to be connected with the inhibition of apoptosis (55). While MT inhibits TNF-dependent I-κB degradation affecting NF-κB activity, it may also regulate the transcription of NF-κB-related genes (52). MT therefore seems to participate in the modulation of intracellular signal transduction that occurs after the activation of TNFR-1 and of cytoplasmic death domains (TRADD, RIADD), while MT over-expression has been found to confer resistance to the cytotoxic effects of TNF (56, 57).

Bcl-2 family proteins are important regulators of apoptosis. Bcl-2 protein inhibits the apoptosis of proliferating human B lymphoma cells (58). Other proteins from this family that inhibit apoptosis are Bcl-x_L and A, while the proteins Bax and Bcl-x_S induce apoptosis (59). The latter protein plays a major role in protecting cells from inappropriate apoptosis. Bcl-2 has been detected in the normal epithelia of the bronchi and the head and neck. Here the expression was localized in the basal membrane in cells thought to stem from epithelial cells. Bcl-2 has also been identified in malignant neoplasms (prostate, lung, and breast carcinomas), where bcl-2 expression correlated with the chance for survival of the patient (60-62). MT and Bcl-2 can rescue yeast mutants null toward superoxide dismutase from death after oxidant injury (63,64). It has been suggested that MT functionally complements the anti-apoptotic protein Bcl-2 in yeast; however, there is no evidence that MT can complement Bcl-2 in higher eukaryotes (24,50).

5. METALLOTHIONEIN IN TUMORS

MT is highly expressed in many types of tumors and is involved in carcinogenesis. MT expression is considered to be cell-cycle dependent and is a marker for cell proliferation (49).

5.1. Proliferation, differentiation, and apoptosis

MT has been identified in both normal oral squamous epithelium and in tongue squamous cell carcinoma. In every case, MT was distributed in the basal-parabasal layer of epithelial cells which are proliferating cells. In squamous cell carcinomas of the tongue, the presence of MT in the periphery of tumor nests was accompanied by fewer positive apoptotic cells and higher proliferation rates (65). MT was also found in the basal layer in the normal epithelium of the nasopharynx, as well as in nasopharyngeal carcinoma. An inverse correlation between MT expression and the apoptotic rate has been demonstrated (66). Moreover, in nasopharyngeal cancer, a significant correlation has been identified between MT expression and positive Ki-67 (67). Similarly, in breast cancer, MT 2A has been positively correlated with cell proliferation (a significant correlation was found between Ki-67 index and MT-2A protein expression) and tumor grade (68). Furthermore, an inverse correlation between MT expression and the extent of apoptosis has been identified in Barrett's esophagus. In fact, MT expression increased with the progression of the histologic metaplasia-dysplasia-adenocarcinoma sequence. It has been suggested that MT over-expression may protect Barrett's esophageal cells from apoptosis, ensuring their proliferation and the subsequent development of adenocarcinoma (69).

5.2. MT and survival

The survival rates for patients with MT-negative esophageal squamous cell carcinomas are better than those for patients with MT-positive tumors (70). MT expression was identified in 45% of patients with lung small-cell carcinoma with a statistically significant higher over-expression in a short-term survival group that included patients with advanced disease and the probability for survival not exceeding 24 months (71). MT over-expression in cases of ductal breast carcinoma was related to both lower survival rates and higher recurrence rates (72, 73). A correlation of metallothionein isoform expression profile with the more invasive ER-negative breast cancer cells has also been identified (74). In colorectal cancer, MT over-expression was a prognostic indicator of poor survival rate independent of major clinicopathological parameters (75). In endometrial adenocarcinoma, high MT content has been associated with poor histological grade, reduced patient survival, and local tumor recurrence. Moreover, high MT intensity distribution scores have been identified in aggressive papillary serous adenocarcinoma (76).

5.3. MT and radiation injury

The protective effect of MT in radiation injury has been documented. MT synthesis was induced by ultraviolet irradiation in human skin *in vivo,* suggesting a physiological photo-protective role for MT (77). Repeated doses of radiation exposure were more effective in inducing MT synthesis than one large dose. In normal cells, MT may provide a protective effect against radiation-induced genotoxicity and cytotoxicity, while in tumor cells, this phenomenon may participate in drug and radiation resistance (78,79). The presence of MT may be one of several factors involved in the radio-resistance of tumor tissue and the adaptive response in low-dose ionizing radiation (49). In particular, Cai and Cherian discovered that MT bound to zinc had high-capacity antioxidant properties that protect cells from radiation-induced DNA damage (78,79,21).

5.4. MT and drug protection

Acquisition of apoptotic resistance may be an important step in malignant transformation (80). Sensitivity to drug-induced apoptosis is modulated by a number of factors, including tumor-suppressor proteins (p53, Bax) and the expression of anti-apoptotic proteins (bcl-2). The

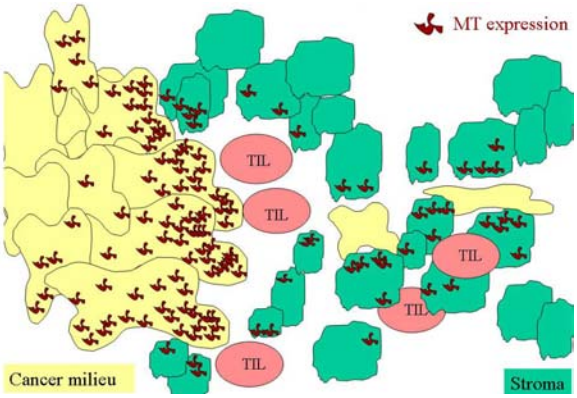

Figure 2. MT tumor and stroma reaction.

conferring of a multi-drug resistant phenotype by cancer cells is associated with the expression of drug efflux pumps (MDRs and MRPs) (35). MT has been correlated with resistance to apoptosis induced by doxorubicin in cardiomyocytes (81). Cells that lack MT due to gene deletion are more sensitive to the cytotoxicity of anti-cancer drugs, mutagens, and oxidants (24). MT has also been found to protect cells from apoptosis induced by etoposide (35). In cases of esophageal squamous cell carcinoma, MT-negative tumors responded better to chemoradiotherapy (82).

MT anti-apoptotic protective effects during chemotherapy and radiotherapy have two major consequences. On the one hand, they lead to resistance to the provided treatment, affecting the patient's chances for survival. On the other hand, they provide normal cell protection from negative chemotherapy and radiotherapy toxicity. From a clinical point of view, as far as the stimulation of MT expression in specific tissue is concerned, normal cells may be useful in preventing the side-effects of chemotherapy and radiotherapy, while the inhibition of MT expression in cancer cells may enhance the therapeutic response.

6. METALLOTHIONEIN IMMUNOMODULATING ACTIVITY

MT expression is induced by exposure to cellular stressors, such as heavy metal cations (83, 84), reactive oxygen species (85), and bacterial endotoxin (86). MT can be released to the extracellular environment in response to these stressors and can be found in physiological fluids, such as blood, bile, and urine (87). Furthermore, extracellular MT has been demonstrated to influence immune functions. Borghesi *et al.* has shown that MT present in the extracellular environment can interact with the plasma membrane of lymphocytes and modulate immune cell functioning (88). MT can be induced by acute-phase cytokines (IL-1, IL-6, TNF-alpha, IFN-gamma) (89,90,91). MT has also been found to confer resistance to the TNF cytotoxic effect in MCF breast carcinoma cells (92). MT suppressed murine cytotoxic lymphocyte function and decreased detectable MHC class I and CD8 molecules on lymphocytes, which are important for the interaction between CTL and target cells (93). When the gradient chemotactic movement of leukocytes was demonstrated under metallothionein, MT appeared to modify the character of the immune response (94). E. Canpolat *et al.* has proposed that MT could serve as a negative regulator of the immune response, suppressing the autoimmune attack on self-tissues. The induction of MT by glucocorticoids may explain the role of such treatment in autoimmune diseases. Nevertheless, a reportedly elevated MT synthesis in neoplasms may suppress the proper immune response in the tumor (95). Finally, MT may play a role in both the suppression of the anti-tumor response and in the increase in tumor cell proliferation.

7. METALLOTHIONEIN IN STROMA

MT expression has been reported in both healthy and cancerous tissues. In healthy tongue epithelium, the expression of MT was typically localized in basal and parabasal cells, which are known to be the dividing cells; in the more superficial areas of epithelium where the cells are differentiated MT expression was lacking (65). Likewise, MT expression in healthy head and neck epithelium was reported to be typically localized in the parabasal layer of the epithelium (96-99). MT has also been reportedly expressed in healthy tissues adjacent to a tumor in lung cancer (100) and in the stroma of head and neck carcinoma (squamous cell carcinomas of oral cavity, pharynx, and maxillary sinus) (101). MT immunoreactivity in head and neck squamous cell carcinomas and breast adenocarcinomas has been reportedly expressed by healthy tumor-adjacent tissue even in cases where the cancer cells did not express MT (96-99). MT has been found in healthy cartilage in the vicinity of head and neck cancer. In breast cancer, MT expression has been identified in a tumor-adjacent healthy vascular wall, while healthy gland cells in the tumor vicinity also expressed MT. It has been suggested that cancer cells migrating from the tumor may induce a response of MT expression in adjacent tissues (96-98). MT expression in tumor cells would seem to be associated with an increased resistance to apoptosis induced by infiltrating immune cells. The interaction between tumor cells and immune cells involves tumor escape from host immunological surveillance. Tumor cells express agents which, on the one hand, are able to inhibit the activity of infiltrating immune cells, but, on the other hand, protect tumor cells from unwanted apoptosis. MT expression by tumor -adjacent tissue may also be associated with increased tumor aggressiveness, local tumor spread, and the response to an increasing accumulation of immune cytotoxic cells in tumor microenvironments. MT expression in healthy tumor-adjacent tissue has been shown to be significantly higher in patients with lymph node metastases (98). Thus the expression of MT in the stroma has been associated with tumor spread. The tumor stroma may respond to the tumor growth and aggressiveness by the expression of MT, thus protecting itself from and developing a resistance to apoptosis (Figure 2).

8. METALLOTHIONEIN IN DECIDUA

Alterations in apoptotic levels in the endometrium corresponding to menstrual cycle changes and to the respective layers of the endometrium have been

observed (102). Endometrial ectopic cells typically have a different apoptotic level than eutopic endometrial cells (103). This phenomenon is probably linked to alterations in the eutopic endometrium profile of immune cell infiltration and to unique feature of endometrial cells (104-112). The mucous membrane of the entire reproductive tract is infiltrated by immune system cells and activated immune cytotoxic cells are predominant in this infiltrate (NK cells in the endometrium and CTLs in the tubal mucosa) (104). The activity of these infiltrating immune cells (113-116) is regulated by mucous membrane cells. The coexistence of immune cells and mucous membrane epithelial cells is related to the development of a resistance to immune-mediated apoptosis in epithelial cells, especially endometrial cells (117). Over the course of the menstrual cycle phases, the level of MT expression fluctuates, reaching its highest level during the mid-secretory cycle phase when the endometrium is infiltrated by abundant dNK cells (117,118). During the implantation of an ectopic pregnancy in the tubal mucosa the increase in MT is accompanied by an increasing infiltration of immune cytotoxic cells (99,119). Similarly, during spontaneous abortion, when the decidua is infiltrated by abundant cytotoxic cells, MT expression increases (99). MT expression is at a statistically significantly higher level at this time than during the mid-secretory cycle phase while the number of infiltrating dNK cells in the endometrium is statistically significantly higher than during the mid-secretory cycle phase (118). In the reproductive tract mucosa, changes in the number of infiltrating immune cells and alterations in their activity in both the endometrium and tubal mucosa accompany changes in MT expression. This indicates the possibility of alterations in the resistance to immune-mediated apoptosis in mucosal cells. Similarly, in ectopic endometrium, changes in the immune cytotoxic cell infiltrate (120) and the increasing activity of these cells accompany alterations in MT expression in both the stroma and the glandular epithelium (112,117). In the case of eutopic endometrium, MT expression is predominant in the glandular epithelium, whereas in ectopic endometrium, MT expression is predominant in the stroma. In scar endometriosis resulting from cesarean section, where the activity of the immune cytotoxic cells is higher in comparison to the levels found in the endometrium during the mid-secretory cycle phase and in ovarian endometriosis, MT expression is also at its highest level (112). The ability of a woman's reproductive system epithelial cells to develop a resistance to immune-mediated apoptosis is a feature that under normal physiological conditions enables the proper interaction between epithelial cells and immune cytotoxic cells as a mechanism to compensate for the increase in the activity of immune cells required for essential reproductive processes.

9. PERSPECTIVES

Multistep carcinogenesis is related to a breakdown in the immune response that enables tumor progression and dissemination. This breakdown is a crucial for tumor survival. MT's participation in geno- and cytotoxicity influences this process. The evaluation of MT expression in cancer and its stroma seems to correlate with the level of immune system inhibition in the cancer-adjacent stroma.

10. ACKNOWLEDGMENTS

I wish to thank Professor Jacek Skladzien for his advice, helpful discussions, and friendly words of support. I would like also to thank Christine Maisto for her assistance. This work was funded by the Polish Ministry of Science, Grant Number: N403 032 31/2079 in 2006/2008.

11. REFERENCES

1. Liu J., Yang G. J.A. Thompson-Lanza, A. Glassman, K. Hayes, A. Patterson , R.T. Marquez, N. Auersperg, Y. Yu, W.C. Hahn, G.B. Mills & R.C. Bast Jr : A genetically defined model for human ovarian cancer. *Cancer Res* 64, 1655-1663 (2004)

2.Slaughter D.P., HW. Southwick & W. Smejkal: "Field cancerization" in oral stratified squamous epithelium. *Cancer (Phila)* 6, 963-968 (1953)

3. Copper M.P., BJ. Braakhuis, N. de Vries, GA. van Dongen, JJ. Nauta & GB. Snow: A panel of biomarkers of carcinogenesis of the upper aerodigestive tract as potential intermediate endpoints in chemoprevention trials. *Cancer* 71, 825-830 (1993)

4. Franklin W.A., AF. Gazdar, J. Haney, II. Wistuba, FG. La Rosa, T. Kennedy, DM. Ritchey & YE. Miller: Widely dispersed p53 mutation in respiratory epithelium. A novel mechanism for field carcinogenesis. *J Clin Inverst* 100, 2133-2137 (1997)

5. Prevo L.J., C.A. Sanchez, P.C. Galipeau & BJ. Reid: p53 mutant clones and field effects in Barrett's esophagus. *Cancer Res* 59, 4784-4787 (1999)

6. Rosenthal A.N., A. Ryan, D. Hopster & I.J. Jacobs: Molecular evidence of a common clonal origin and subsequent divergent clonal evolution in vulval intraepithelial neoplasia, vulval squamous cell carcinoma and lymph node metastases. *Int J Cancer* 99, 549-554 (2002)

7. Chu T., C.Y. Shen, H.S. Lee & H.S. Liu: Monoclonality and surface lesion-specific microsatellite alterations in premalignant and malignant neoplasia of uterine cervix: a local field effect of genomic instability and clonal evolution. *Genes Chromosomes Cancer* 24, 127-134 (1999)

8. Jothy S., B. Slesak, A. Harlozinska, J. Lapinska, J. Adamiak & J. Rabczynski: Field effect of human colon carcinoma on normal mucosa: relevance of carcinoembryonic antigen expression. *Tumour Biol* 17, 58-64 (1996)

9. Forsti A., J. louhelainen, M. Soderberg, H.Wijkstrom & K. Hemminki: Loss of heterozygosity in tumour-adjacent normal tissue of breast and bladder cancer. *Eur J Cancer* 37, 1372-1380 (2001)

10. Stern R.S., S. Bolshakov, A.J. Nataraj & H.N. Ananthaswamy: p53 mutation in nonmelanoma skin cancers occurring in psolaren ultraviolet a-treated patients : evidence for heterogeneity and field cancerization. *J Investig Dermatol* 119, 522-526 (2002)

11. Califano J., P. van der Riet, W. Westra, H. Nawroz, G. Clayman, S. Piantadosi, R. Corio, D. Lee, B. Greenberg, W. Koch & D. Sidransky: Genetic progression model for

head and neck cancer: implications for field cancerization. *Cancer Res* 56, 2488-2492 (1996)
12. Braakhuis B., M.P. Tabor, J.A. Kummer, C.R. Leemans & R.H. Brakenhoff: A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 63, 1727-1730 (2003)
13. Keller SE, H.L. Ioachim, T. Pearse & D.M. Siletti: Decreased T-lymphocytes in patients with mammary cancer. *Am J Clin Pathol* 65, 445-9 (1976)
14. Ioachim H.L: The stroma reaction of tumors: An expression of immune surveillance. *J Cell Biochem* Suppl 1, 57, 465-475 (1979)
15. Miescher S, T.L. Whiteside, L. Moretta & V. Von Fliedner: Clonal and frequency analyses of tumor infiltrating T lymphocytes from human solid tumors. *J Immunol* 138, 4004-4011 (1987)
16. Reichert T.E., H. Rabinowich, J.T. Johnson & T.L. Whiteside: Mechanisms responsible for signaling and functional defects. *J Immunother* 21, 295-306 (1998)
17. Reichert T.E., L. Strauss, E. Wagner, W. Gooding & T. Whiteside: Signaling abnormalities, apoptosis and reduced proliferation of circulating and tumor-infiltrating lymphocytes in patients with oral carcinoma. *Clin Cancer Res* 8, 3137-3145 (2002)
18. Gastman B.R., Y. Atarshi, T.E. Reichert, T. Saito, L. Balkir, H. Rabinowich & T.L. Whiteside: Fas ligand is expressed on human squamous cell carcinoma of the head and neck and it promotes apoptosis of T lymphocytes. *Cancer Res* 59, 5356-5364 (1999)
19. Saito T., I. Kuss, G. Dworacki, W. Gooding, J.T. Johnson & T.L. Whiteside: Spontaneous ex vivo apoptosis of peripheral blood mononuclear cells in patients with head and neck cancer. *Clin Cancer Res* 5, 1263-1273 (1999)
20. Kagi J.H. & A. Scheffer: Biochemistry of metallothionein. *Biochemistry* 27, 8509-8515 (1998)
21. Cherian M.G, A. Jayasurya & B.H. Bay: Metallothioneins in human tumors and potential roles in carcinogenesis. *Mutat Res* 533, 201-209 (2003)
22. Apostolova M.D. & M.G. Cherian: Nuclear localization of metalothionein during cell proliferation and differentiation. *Cell Mol Biol* 46, 347-356 (2000)
23. Fan L.Z & M.G. Cherian: Potential role of p53 on metallothionein induction in human epithelial breast cancer cells. *Br J Cancer* 87, 1019-1026 (2002)
24. Theocharis S.E, A.P. Margeli, J.T. Klijanienko & G.P. Kouraklis: Metallothionein expression in human neoplasia. *Histopathology* 45, 103-118 (2004)
25. Andrews G.K.: Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochem Pharmacol* 59, 95-104 (2000)
26. Lohmann R.D. & D. Beyersmann: Cadmium and zinc mediated changes of the Ca(2+)-dependent endonuclease in apoptosis. *Biochem Biophys Res Commun* 190, 1097-103 (1993)
27. Cohen J.J. & R.C. Duke: Glukocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J Immunol* 132, 38-42 (1984)
28. McCabe M.J., S. Jiang & S. Orrenius: Chelation of intracellular zinc triggers apoptosis in mature thymocytes. *Lab Invest* 69, 101-110 (1993)
29. Perry D.K., M.J. Smyth, H.R. Stennicke, G.S. Salvesen, P. Duriez, G.G. Poirier & Y.A. Hannun: Zinc is a potent inhibitor of the apoptotic protease, caspase-3. A novel target for zinc in the inhibition of apoptosis. *J Biol Chem* 272, 18530-18533 (1997)
30. Chai F., A.Q. Truong-Tran, L.H. Ho & P.D. Zalewski: Regulation of caspase activation and apoptosis by cellular zinc fluxes and zinc deprivation: A review. *Immunol Cell Biol* 77, 272-278 (1999)
31. Chimienti F, M. Seve, S. Richard, J. Mathieu & A. Favier: Role of cellular zinc in programmed cell death: temporal relationship between zinc depletion, activation of caspases, and cleavage of Sp family transcription factors. *Biochem Pharmacol* 62, 51-62 (2001)
32. Watjen W., H. Haase, M. Biagioli & D. Beyersmann: Induction of apoptosis in mammalian cells by cadmium and zinc. Induction of apoptosis in mammalian cells by cadmium and zinc. Environmental health perspectives. *Environ Health Perspect* 110;suppl 5, 865-867 (2002)
33. Ishido M., R. Ohtsubo, T. Adachi & M. Kunimoto: Attenuation of both apoptotic and necrotic actions of cadmium by bcl-2. *Environ Health Perspect* 110, 37-42 (2002)
34. Klaassen C.D, Liu J. & S. Choudhuri: Metallothionein: an intracellular protein to protect against cadmium toxicity. *Annu Rev Pharmacol Toxicol* 39, 267-294 (1999)
35. Shimoda R, W.E. Achanzar,W. Qu, T. Nagamine, H. Takagi, M. Mori & M.P. Waalkes: Metallothionein is a potential negative regulator of apoptosis. *Toxicol Sci* 73, 294-300 (2003)
36. Williams L., & L. Bell: Asynchronous development of the rat colon. *Anat Embryol* 183:573-578 (1991)
37. Smith C.A., G. T. Williams, R. Kingston, E.J. Jenkinson & J.T. Owen: Antibodies to CD3/T cell receptor complex induce death by apotosis in immature T cells in thymic cultures. *Nature* 337(6203), 181-184 (1989)
38.Kerr J.F, A.H. Wyllie & A.R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)
39.Wyllie A.H., R.G. Morris, A.L. Smith & D. Dunlop: Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. *J Pathol* 142, 67-77 (1984)
40. Shimizu T., M. Kubota, A. Tanizawa, H. Sano, Y. Kasai, H. Hashimoto, Y. Akiyama & H. Mikawa: Inhibition of both etoposide-induced DNA fragmentation and activation of poly-ADP-ribose synthesis by zinc ion. *Biochem Biophys Res Commun* 169, 1172-1177 (1990)
41. Martin S.J. & D.R. Green: Protease activation during apoptosis: death by a thousand cuts? *Cell* 82, 349-352 (1995)
Nagata S: Apoptosis by death factor. *Cell* 88, 355-365 (1997)
42.Widlak P: DNA microarrays, a novel approach in studies of chromatin structure. *Acta Biochim Pol* 51, 1-8 (2004)
43.Sharif-Askari E, A. Alam, E. Rheaume, P.J. Beresford, C. Scotto, K. Sharma, D. Lee, W.E. DeWolf, M.E. Nuttall, J. Lieberman & R.P. Sekaly: Direct cleavage of the human DNA fragmentation factor-45 by granzyme B induces caspase-activated DNase release and DNA fragmentation. *EMBO J* 20, 3101-13 (2001)

44. Hockenbery D.M.: Bcl-2, a novel regulator of cell death. *Bioessays* 17, 631-638 (1995)
45. Ko L.J. & C. Prives: p53: Puzzle and paradigm. *Genes Dev* 10,1054-1072 (1996)
46. Meplan C., M.J. Richard & P. Hainaut: Metalloregulation of the tumor suppressor protein p53:zinc mediates the renaturation of p53 after exposure to metal chelators in vitro and in intact cells. *Oncogene* 19, 5227-5236 (2000)
47. Ostrakhovitch E.A., PE. Olsson, S. Jiang & M.G. Cherian: Interaction of metallothionein with tumor suppressor p53 protein. *FEBS Lett* 580, 1235-1238 (2006)
48. Kondo Y, E. Woo, A.E. Michalska, K.H. Choo & J.S. Lazo: Metallothionein null cells have increased sensitivity to anticancer drugs. *Cancer Res* 55, 2021-2023 (1995)
49. Kondo Y, J.M. Rusnak, D.G. Hoyt, C.E. Settineri, B.R. Pitt & J.S. Lazo: Enhanced apoptosis in metallothionein null cells. *Mol Pharmacol* 52, 195-201 (1997)
50. Cui Y, J. Wang, X. Zhang, R. Lang, M. Bi, L. Guo & S.H. Lu: ECRG2, a novel candidate of tumor suppressor gene in the esophageal carcinoma, interacts directly with metallothionein 2A and links to apoptosis. *Biochem Biophys Res Commun* 302, 904-915 (2003)
51.Sakurai A, S. Hara, N. Okano, Y. Kondo, J. Inoue & N. Imura: Regulatory role of metallothionein in NF-kappaB activation. *FEBS Lett* 455, 55-58 (1999)
52. Dong G, Z. Chen, T. Kato & C. Van Waes: The host environment promotes the constitutive activation of nuclear factor-κB and proinflammatory cytokine expression during metastatic tumor progression of murine squamous cell carcinoma. *Cancer Res* 59, 3495-3504 (1999)
53. Arlt A. & H. Schafer: NFκB- dependent chemoresistance in solid tumors. *Int J Clin Pharmacol Ther* 40, 336-347 (2002)
54. Van Antwerp D.J, S.J. Martin, T. Kafri, D.R. Green & I.M. Verma: Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 274(5288), 787-789 (1996)
55. Sciavolino P.J., TH. Lee & J. Vilcek: Overexpression of metallothionein confers resistance to the cytotoxic effect of TNF with cadmium in MCF-7 breast carcinoma cells. *Lymphokine Cytokine Res* 11, 265-270 (1992)
56. Leyshon-Sorland K., L. Morkrid & H.E. Rugstad: Metallothionein: a protein conferring resistance in vitro to tumor necrosis factor. *Cancer Res* 53, 4874-4880 (1993)
57. Veis D.J., C.M. Sorenson, J.R. Shutter & S.J. Korsmeyer: Bcl-2 defficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75, 229-240 (1993)
58. Gross A., J.M. McDonnell & S.J. Korsmeyer: Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev* 13, 1899-1911 (1999)
59. Friedman M, J.W. Lim, E. Manders, A.D. Schaffner, G.L. Kirshenbaum, H.M. Tanyeri, D.D. Caldarelli & J.S. Coon: Prognostic significance of Bcl-2 and p53 expression in advanced laryngeal squamous cell carcinoma. *Head Neck* 23, 280-285 (2001)
60. Friedman M, P. Grey, T.K. Venkatesan, I. Bloch, P. Chawla, D.D. Caldarelli & J.S. Coon: Prognostic significance of bcl-2 expression in localized squamous cell carcinoma of the head and neck. *Ann Otol Rhinol Laryngol* 106, 445-450 (1997)
61. Gallo O, V. Boddi, A. Calzolari, L. Simonetti, M. Trovati & S. Bianchi: *bcl-2* protein expression correlates with recurrence and survival in early stage head and neck cancer treated by radiotherapy. *Clin Cancer Res* 2, 261-2 (1996)
62. Tamai K.T., E.B. Gralla, L.M. Ellerby & D.J. Thiele: Yeast and mammalian metallothioneins functionally substitute for yeast copper-zinc superoxide dismutase. *Proc Natl Acad Sci USA* 90, 8013-8017 (1993)
63. Kane D.J., T.A. Sarafian, R. Anton, H. Hahn, E.B. Gralla, J.S. Valentine, T. Ord & D.E. Bredesen: Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* 262(5137), 1274-1277 (1993)
64. Sundelin K., M. Jadner, L. Norberg-Spaak, A. Davidsson & H.B. Hellquist: Metallothionein and Fas (CD95) are expressed in squamous cell carcinoma of the tongue. *Eur J Cancer* 33, 1860-1864 (1997)
65. Jayasurya A., B.H. Bay, W.M. Yap & N.G. Tan: Correlation of metallothionein expression with apoptosis in nasopharyngeal carcinoma. *Br J Cancer* 82, 1198-1203 (2000)
66. Jayasurya A., B.H. Bay, W.M. Yap, N.G Tan & B.K. Tan: Proliferative potential in nasophryngeal carcinoma: correlation with metallothionein expression and tissue zinc levels. *Carcinogenesis* 21, 1809-1812 (2000)
67. Jin R., V.T. Chow, P.H. Tan, S.T. Dheen, W. Duan & B.H. Bay: Metallothionein 2A expression is associated with cell proliferation in breast cancer. *Carcinogenesis* 23, 81-86 (2002)
68. Li Y., J.M. Wo, L. Cai, Z. Zhou, D. Rosenbaum, C. Mendez, M.B. Ray, W.F. Jones & Y.J. Kang: Association of Metallothionein expression and lack of apoptosis with progression of carcinogenesis in Barrett's esophagus. *Exp Biol Med (Maywood)* 228, 286-292 (2003)
69. Coyle P., G. Mathew, P.A. Game, J.C. Myers, J.C. Philcox, A.M. Rofe & G.G. Jamieson: Metallothionein in human oesophagus, Barrett's epithelium and adenocarcinoma. *Br J Cancer* 87, 533-536 (2002)
70. Joseph M.G., D. Banerjee, W. Kocha, R. Feld, L.W. Stitt & M.G. Cherian: Metallothionein expression in patients with small cell carcinoma of the lung: correlation with other molecular markers and clinical outcome. *Cancer* 92, 836-842 (2001)
71. Goulding H., B. Jasani, H. Pereira, A. Reid, M. Galea, JA Bell, C.W. Elston, J.F. Robertson, R.W. Blamey, R.A. Nicholson & K.W. Schmidt: Metallothionein expression in human breast cancer. *Br J Cancer* 72, 968-972 (1995)
72. Schmidt K.W., I.O. Ellis, J.M. Gee, B.M. Darke, W.E. Lees, J. Kay, A. Cryer, J.M. Stark, A. Hittmair & D.M. Ofner: Presence and possible significance of immunohistochemically demonstrable metallothionein over-expression in primary invasive ductal carcinoma of the breast. *Virc Arch A Pathol Anat Histopatol* 422, 153-159 (1993)
73. Tai S.K., O.J Tan, V. Chow, R. Jin, J.L. Jones, P.H. Tan, A. Jayasurya & B.H. Bay: Differential expression of metallothionein 1 and 2 isoforms in breast cancer lines with different invasive potential: identification of a novel nonsilent metallothionein-1H mutant variant. *Am J Pathol* 163, 2009-2019 (2003)
74. Janssen L., W. van Duijn, F.J. Kubben, G. Griffioen, C.B. Lamers, J.H. van Krieken, C.J. van de Velde & H.W. Verspaget: Prognostic significance of metallothionein in

human gastrointestinal cancer. *Clinical Cancer Res* 8, 1889-1896 (2002)
75. McCluggage W.G., P. Maxwell, P.W. Hamilton & B. Jasani. High metallothionein expression is associated with features predictive aggressive behaviour in endometrial carcinoma. *Histopathology* 34, 51-55 (1999)
76. Anstey A., R. Marks & C. Long: In vivo photoinduction of metallothionein in human skin by ultraviolet irradiation. *J Pathol* 178, 84-88 (1996)
77. Cai L. & M.G. Cherian: Zinc-metallothionein protects from DNA damage induced by radiation better than glutathione and copper- or cadmium-metallothioneins. *Toxicol Lett* 136, 193-198 (2003)
78. Cai L., M. Satoh, C. Tohyama & M.G. Cherian: Metallothionein in radiation exposure: its induction and protective role. *Toxicology* 132, 85-98 (1999)
79. Achanzar W.E., K.B. Achanzar, J.G. Lewis, M.M. Webber & M.P: Waalkes: Cadmium induces c-myc, p53, and c-jun expression in normal human prostate epithelial cells as a prelude to apoptosis. *Toxicol Appl Pharmacol* 164, 291-300 (2000)
80. Wang G.W., J.B. Klein & Y.J. Kang: Metallothionein inhibits doxorubicin-induced mitochondrial cytochrome c release and caspase-3 activation in cardiomyocytes. *J Pharmacol Exp Ther* 298, 461-468 (2001)
81. Sunada F., M. Itabashi, H. Ohkura & T. Okumura: p53 negativity, CDC25B positivity, and metallothionein negativity are predictors of a response of esophageal squamous cell carcinoma to chemoradiotherapy. *World J Gastroenterol* 11, 5696-5700 (2005)
82. Klaassen C.D. & L.D. Lehman-McKeeman: Induction of metallothionein. *J Am Coll Toxicol* 8, 1315-1320 (1989)
83. Palmiter R.D.: Molecular biology of metallothionein gene expression. *Experientia Suppl* 52, 63-80 (1987)
84. Sato M. & I. Bremmer: Oxygen- free radicals and metallothionein. *Free Radic Biol Med* 14, 325-337 (1993)
85. Durnam D.M, J.S. Hoffman, C.J. Quaife, E.P. Benditt, H.Y. Chen, R.L. Brinster & R.D. Palmitier: Induction of mouse metallothionein-I m RNA by bacterial endotoxin is independent of metals and glucocorticoid hormones. *Proc Natl Acad Sci USA* 81, 1053-1056 (1984)
86. Bremner I., RK. Mehra & M. Sato: Metallothionein in blood, bile and urine. *Experientia Suppl* 52, 507-517 (1987)
87. Borghesi L.A., J. Youn, E.A. Olson & M.A. Lynes: Interactions of metallothionein with murine lymphocytes: plasma membrane binding and proliferation. *Toxicology* 108, 129-140 (1996)
88. Karin M., R.J. Imbra, A. Heguy & G. Wong: Interleukin 1 regulates human metallothionein gene expression. *Mol Cell Biol* 5, 2866-2869 (1985)
89. Schroeder J.J & R.J. Cousins: Interleukin-6 regulates metallothionein gene expression and zinc metabolism in hepatocyte monolayer cultures. *Proc Natl Acad Sci USA* 87, 3137-3141 (1990)
90. Sato M, M. Sasaki & H Hojo: Tissue specific induction of metallothionein synthesis by tumor necrosis factor-alpha. *Res Commun Chem Pathol Pharmacol* 75, 159-172 (1992)
91. Sciavolino P.J., T.H. Lee & J. Vilcek: Overexpression of metallothionein confers resistance to the cytotoxic effect of TNF with cadmium in MCF-7 breast carcinoma cells. *Lymphokine Cytokine Res* 11, 265-270 (1992)
92. Youn J & M.A. Lynes: Metallothionein-induced suppression of cytotoxic T lymphocyte function: an important immunoregulatory control. *Toxicol Sci* 52, 199-208 (1999)
93. Yin X., D.A. Knecht & M.A. Lynes: Metallothionein mediates leucocyte chemotaxis. *BMC Immunol* 6, 21 (2005)
94. Canpolat E. & M.A. Lynes: In vivo manipulation of endogenous metallothionein with a monoclonal antibody enhances a T-dependent humoral immune response. *Toxicol Sci* 62, 61-70 (2001)
95. Popiela T.J., L. Rudnicka–Sosin, M. Dutsch-Wicherek, M. Klimek, P. Basta, K. Galazka & L. Wicherek: The metallothionein and RCAS1 expression analysis in breast cancer and adjacent tissue regarding the immune cells presence and their activity. *Neuro Endocrinol Lett* 27, 786-94 (2006)
96. Popiela T.J., M. Klimek, L. Wicherek, M. Dutsch-Wicherek, K. Galazka & L. Rudnicka-Sosin L: The characterization of the exposure to immune mediated apoptosis and the regulation of immune cytotoxic activity in the environment of a neoplasm and in decidua. *Neuro Endocrinol Lett* 27, 779-85 (2006)
97. Dutsch-Wicherek M., T.J. Popiela, M. Klimek, L. Rudnicka-Sosin, L. Wicherek, J.P. Oudinet, J. Skladzien & R. Tomaszewska: Metallothionein stroma reaction in tumor adjacent healthy tissue in head and neck squamous cell carcinoma and breast adenocarcinoma. *Neuro Endocrinol Lett* 26, 567-574 (2005)
98. Wicherek L, K. Galazka, T.J. Popiela, M. Dutsch-Wicherek, A. Czekierdowski, W. Pabian, T. Banas, M. Migdal & M. Klimek: Metallothionein expression and infiltration of cytotoxic lymphocytes in uterine and tubal implantation sites. *J Reprod Immunol* 70,119-131 (2006)
99. Theocharis S, C. Karkantaris, T. Philipides, E. Agapitos, A. Gika, A. Margeli, C. Kittas & A. Koutselinis: Expression of metallothionein in lung carcinoma: correlation with histological type and grade. *Histopathology* 40, 143-151 (2002)
100. Muramatsu Y., Y. Hasegawa, H. Fukano, T. Ogawa, M. Namuba, K. Mouri, Y. Fujimoto, H. Matsuura, Y. Takai & M. Mori: Metallothionein immunoreactivity in head and neck carcinomas; special reference to clinical behaviors and chemotherapy responses. *Anticancer Res* 20, 257-264 (2000)
101. Tabibzadeh S: The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodelling. *Mol Hum Reprod* 2, 77-92 (1996)
102. Harada T., F. Taniguchi, M. Izawa, Y. Ohama, Y. Takenaka, Y. Tagashira, A. Ikeda, A. Watanabe, T. Iwabe, N. Terakawa: Apoptosis and endometriosis. *Front Biosci* 12,3140-3151(2007)
103. King A, T. Burrows & Y.W. Loke: Human Uterine Natural Killer Cells. *Nat Immun* 15, 41-52 (1996)
104. McCoy C.E. & L.A. O'Neil: The role of Toll-like receptors in macrophages. Front Biosci 13,62-70 (2008)
105. Chen W.J: Dendritic Cells and CD4+CD25+ T Regulatory Cells: Crosstalk Between Two Professionals

in Immunity versus Tolerance. *Front Biosc.*11, 1360-1370 (2006)
106. Weiss A., S. Goldman & E. Shalev: The matrix metalloproteinases (MMPS) in the decidua and fetal membranes. *Front Biosci* 12, 649-659 (2007)
107. Eidukaite A., A. Siaurys & V. Tamosiunas V: Aberrant expression of CD95 and CD69 molecules among CD56 cells in women with endometriosis. Am J Reprod Immunol 55,276-281 (2006)
108. Yang J.H., M.Y. Wu, D.Y. Chang, C.H. Chang, Y.S. Yang & H.N. Ho: Increased interleukin-6 messenger RNA expression in macrophage-cocultured endometrial stromal cells in adenomyosis. *Am J Reprod Immunol* 55,181-187 (2006)
109. Givan A.L, H.D. White, J.E. Stern, E. Colby, E.J. Gosselin, P.M. Guyre & C.R. Wira: Flow cytometric analysis of leukocytes in the human female reproductive tract: comparison of fallopian tube, uterus, cervix, and vagina. *Am J Reprod Immunol* 38, 350-359 (1997)
110. Zhang C., N. Maeda, C. Izumiya, Y. Yamamoto, T. Kusume, H. Oguri, C. Yamashita, Y. Nishimori, K. Hayashi, J. Luo & T. Fukaya: Killer immunoglobulin-like receptor and human leukocyte antigen expression as immunodiagnostic parameters for pelvic endometriosis. *Am J Reprod Immunol* 55,106-114 (2006)
111. Wicherek L., M. Dutsch-Wicherek, K. Galazka, T. Banas, T. Popiela, A. Lazar & B. Kleinrok-Podsiadlo: Comparison of RCAS1 and metallothionein expression and the presence and activity of immune cells in human ovarian and abdominal wall endometriomas. *Reprod Biol Endocrinol* 4,41 (2006)
112. Agic A., H. Xu, D. Finas, C. Banz, K. Diedrich & D. Hornung: Is Endometriosis Associated with Systemic Subclinical Inflammation? *Gynecol Obstet Invest* 62, 139-147 (2006)
113. Ugur Y., A.N. Cakar, M.S. Beksac & A. Dagdeviren : Activation Antigens during the Proliferative and Secretory Phases of Endometrium and Early-Pregnancy Decidua. *Gynecol Obstet Invest* 62,66-74 (2006)
114. Winger E.E: CD57+ cells and recurrent spontaneous abortion. *Am J Reprod Immunol* 58,311-314 (2007)
115. Fahey J.V., P.K. Wallace, K. Johnson, P.M. Guyre & C.R. Wira: Antigen presentation by human uterine epithelial cells to autologous T cells. *Am J Reprod Immunol* 55, 1-11 (2006)
116. Wicherek L., T.J. Popiela, K. Galazka, M. Dutsch-Wicherek, M. Oplawski, A. Basta & M. Klimek: Metallothionein and RCAS1 expression in comparison to immunological cells activity in endometriosis, endometrial adenocarcinoma and the endometrium according to menstrual cycle changes. *Gynecol Oncol* 99, 622-630 (2005)
117. Klimek M, L. Wicherek, K. Galazka, T. Tetlak, T.J. Popiela, M. Kulczycka, L. Rudnicka-Sosin & M. Dutsch-Wicherek: Cycle dependent expression of endometrial metallothionein. *Neuro Endocrinol Lett* 26, 663-666 (2005)
118. Wicherek L, K. Galazka & A. Lazar: The analysis of Metallothionein, RCAS1 immunoreactivity regarding immune cells concentration in endometrium and tubal mucosa in ectopic pregnancy during the course of tubal rupture. *Gynecol Obstet Invest* 65, 52-61 (2007)
119. Garai J., V. Molnar, T. Varga, M. Koppan, A. Torok & J. Bodis: Endometriosis: harmful survival of an ectopic tissue. *Front Biosci* 11, 595-619 (2006)

Key Words: Metallothionein, Apoptosis, Cancer, Tumor, Neoplasia, Review

Send correspondence to: Romana Tomaszewska MD, PhD; Pathomorphology Department of the Jagiellonian University, Grzegorzecka Str, 31-531 Krakow, Poland, Tel: 4812424-7900, Fax: 4812424-7923, E-mail: romatom@mp.pl

http://www.bioscience.org/current/vol13.htm