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REVIEW

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THE ROLE OF METALLOTHIONEIN IN BREAST CANCER

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Summary

Metallothioneins (MTs) are a family of small cysteine-rich proteins involved in many physiological and pathological processes. Genes that encode the four isoforms of metallothioneins (MT1-M4) are located on chromosome 16q13. Strucutures of these four isoforms allow metallothioneins their various biological functions. Many studies have shown that MT plays an important role in carcinogenesis, tumour growth, its progression from local to metastatic disease and may contribute to resistence to chemotherapy and radiotherapy. Due to the fact that breast cancer is one of the leading causes of death in women worldwide it is important to better understand the biology of breast cancer. So, findings of MT could evenutally help as a prognostic tool and could lead to a possible new specific anti-cancer treatment.

KEYWORDS: *metallothionein; breast cancer; prognostic factor; treatment*

BACKGROUND

Since the discovery of metallothioneins (MTs) in 1957, many studies have been conducted to understand its complex role in various biological processes, both the physiological and pathophysiological.

Structure of MT

Metallothionein is a low molecular mass (from 6 do 10 kDa) cysteine-rich intracellular metal-binding protein constituting the major fraction of intracellular protein thiols(1). It is included in essential metal homeostasis, heavy metal detoxification and cellular antioxidative defense(2). The primary structure of MT proteins is characterized by Cys-X-Cys or Cys-X-Y-Cys, and Cys-Cys repetitive sequences where X is any other amino acid than cysteine(2,3). There are two metal-binding domains that have been characterized in MT, the α- and β- clusters which consist of 61 amino acids in human MT(3,4,5). Due to the great flexibility, these proteins have a very dynamic 3D structure and the binding of metals is required for its folding and 3D conformation(6,7). Under physiological conditions, MTs mostly contain zinc (Zn) (7,8). They can be subdivided in four main isoforms (MT1-MT4) according to their structure, metal binding properties, encoded genes, amino

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acids environment, etc., with MT1 existing in several isoforms.

Expression of MT

MT1 and MT2 are present as a major isoforms in almost all tissues and MT3, due to its structure containing seven additional amino acids, is somewhat different and was identified as a growth inhibitory factor (GIF). MT4 is specific to squamous epithelium and is expressed in keratinocytes(3). MTs can be found both in prokaryotic and eukaryotic organisms and most organisms possess MT-encoding genes. In humans, 14 MT genes are localized on chromosome 16q13-22, and both essential (e.g. copper, zinc) and nonessential (e.g. cadmium) metals induce the synthesis of MT(1,3,9). A family of genes encode for the two major classes of MTs; MT1 (MT1A, MT1B, MT1E, MT1F, MT1G, MT1H, MT1M, MT1X) and MT2A, which are expressed ubiquitously, and for MT3 and MT4 that are found in specialized cells such as brain and reproductive organs. MT is expressed in many tissues and organs, but its synthesis is mostly active in the liver, kidney, pancreas and intestines where the main inducers of MT synthesis are Zn and Cd.

In physiological conditions, Zn and Cu are metals involved in MT induction(10). Other inductors of MT synthesis are glucocorticoids, anti-inflammatory cytokines (IL-1, IL-6, TNF-α), catecholamines, phorbol esters, hydrogen peroxide, glucagon, free radicals, EGF and many others(4,6,8,11). In some specific states of physical stress such as immobillization, starvation, exposure to extreme temperature the synthesis of MTs is increased as well as in states of oxidative stress, such as response to radiation or response to free radicals, alkylating agents and oxidants(3).

Besides physiological induction of MTs, there are pathological conditions during which the expression is increased. For example, during anemia in erythroblasts, an induction of MT in the bone marrow is increased. In erythrocyte precursors the induction of MT is controlled by erythropoietin, and in mature erythrocytes it is induced by metals(12). As stated earlier, synthesis of MTs is induced and regulated by indicators of biological stress. MTs are involved in metal ion homeostasis, heavy metal detoxication, acute stress phase, defense against oxidative stress, protective role against DNA damage(6-8,10,11,13,14).

THE PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES OF MT

MTs are involved in many physiological and pathophysiological processes: in cell growth and proliferation, apoptosis, homeostasis and metal metabolism, protection against free radicals and oxidative stress, immune defense response, angiogenesis, carcinogenesis, radio and chemoresistance, and many others(2,4,13,15,16).

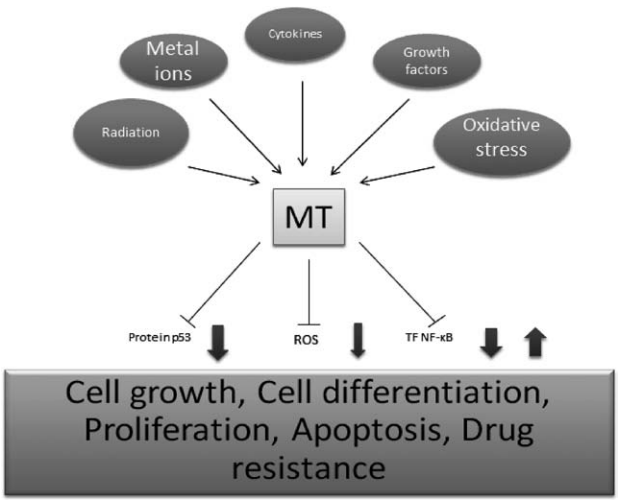

Figure 1. Presentation of the induction of MT by various stimuli and the downstream effects of MT overexpression. Based on Figure 3. from Ruttky-Nedecky B et al. (17).

They are usually expressed in low levels, but their synthesis is shown to be inducible during oxidative stress to protect the cells against cytotoxicity, radiation and DNA damage(4). One of their main characteristics is their participation in protection against the toxic effects of metals, especially cadmium (Cd) and copper (Cu). They participate in the kinetics and metabolism of these metals as their transporters in the cell. Cellular Zn metabolism is regulated by binding and releasing Zn ions through -SH groups(13). The basal level of MT is likely to play a role in Zn resorption by adding Zn to various other transporters that are essential for resorption of Zn, among which ZnT-1 to ZnT-4 and DCT-1 are significant. It was suggested that MT could control the cellular Zn distribution and was shown that the antioxidant properties of MT can be enhanced in the presence of Zn. These functions of MT are important for regulation of physiological processes that are depen-

dent on Zn and the pathological processes in which oxidative stress mobilizes Zn(9).

It has already been mentioned that MT belongs to the group of stress proteins, i.e. proteins of the acute phase of inflammation that are synthesized in the liver. Its synthesis is markedly increased after lipopolysaccharide (LPS) injection mainly due to IL-6 induced induction. Two to four hours after the inflammatory reaction, increased MT expression is visible. Studies have shown that this induction is mediated by the accumulation of Zn in the liver, but is also stimulated by proinflammatory cytokines, especially via IL-6 and glucocorticoids(3).

The main inducers of MT synthesis are Zn and Cu under physiological conditions, however activity of Cd is pronounced in intoxication. The main source of Cd is cigarette smoking but smaller amounts are also ingested through food through the digestive tract. MTs act as its transporters to the cell, otherwise, non-MT-bound Cd is toxic to the cell(2). Increased MT expression is seen in other states of intoxication, e.g. inhalation of gasoline vapors can induce increased expression of MT in organs that play a role in detoxification and excretion of toxic products(lungs and kidneys), as well as in the central nervous system due to the entry of toxic components through the blood-brain barrier or directly through the olfactory tract(18). Thanks to its antioxidant properties, MTs are known to protect the cell from oxidative stress and the harmful effects of free radicals.

Besides its main role in homeostasis of metals and consequently its involvement in protection of free radicals and oxidative stress, it is known to be involved in basic processes of the cell like cell cycle progression, cell proliferation and differentiation and programmed cell death. Many studies have shown MTs play a protective role inhibiting apoptosis due to prevention against p53 (Zn-containing protein) activation and associated apoptotic effects(5,19,20). Any change of the cell that disrupts the balance between proliferation and programmed cell death (apoptosis) can lead to development of cancer.

Considering the mentioned roles of MTs, it has been shown that they play an imoprtant role in carcinogenesis. MTs are expressed in the tissue of many tumors and through years they have been studied as immunohistochemical biomarkers. Its increased expression is shown in human breast, ovarian, gastrointestinal, urinary, lung, nasopharyngeal and thyrois tumors(13). Studies have shown increased MT1 and MT2 in both epithelial and mesenchymal tumors. Their increased expression is associated with greater proliferative potential of tumor cells and is correlated with a more rapid course of the disease and poorer prognosis(4,5,13,21). For better understanding of MT roles in carcinogenesis, a workshop was held by the National Cancer Institute and was focused on three topics: MTs in metal carcinogenesis, role of Zn in tumor cell pathobiology and the role of MTs in tumor cells and potential in chemotherapy(22). Over the years, a great number of studies have been conducted to define the levels of participation and verify the impact of MTs in carcinogenesis, as well as its involvement in therapy resistance.

THE ROLE OF MTs IN BREAST CANCER

Available experimental evidence and data on correlation between expression of MTs and different tumor types suggest that MTs are not expressed equally in all tumor types. Expression of MTs in tumor tissue depends on several factors such as tumor cell type, tumor growth rate, hystological type, tumor differentiation, environmental stimuli or interaction with other cell signaling pathways(20). Among various other types of tumors, breast cancer has been included in the studies and it was shown that MTs have an important role in its biology. Since breast cancer is the most common malignancy that affects women, understanding factors that are included in its origin and course can be significant, as well as investigating new biochemical predictive and prognostic markers.

MT as a prognostic biomarker in breast cancer

MTs act as biomarkers and can be detected in the blood of patients. Increased MTs serum levels can be related to the existence of the tumor, its degree of progression and the stage of the disease (20). Many studies have shown that MTs expression is upregulated in breast cancer(4,19,20,25). Such increased expression is a valuable prognostic marker for disease progression and drug resistance, and is also associated with the worse outcome and lower overall survival in breast cancer patients (23-27).

Immunohistochemical staining has shown MTs cytoplasmatic location in breast cancer cells. Intranuclear localization of MTs indicates enhaced proliferation of tumor cells, since MTs located in the nucleus protects DNA from damage and apoptosis, which supports tumor growth. Cytoplasmatic levels of MTs reach their maximum during the G1/S cell cycle which demonstrates its role in tumor proliferation(19,20). Normally, in lesions without increased cancer risk MTs are localized in myoepithelial cells and the majority of luminal cells are negative(28). In breast cancer, MTs are specifically expressed in myoepithelial cells that contain proteinase inhibitors and can have role in the suppression of angiogenesis, invasion and metastasis of breast cancer. They can produce several growth factors such as transforming growth factor that can cause tumor growth(26). The extracellular MTs has an effect on T-cell function and may contribute to immunosupression of cell-mediated immunity(29). Since it is known that the body develops an immune response against the tumor and its main effectors are T-lymphocytes, there is a connection of MTs involvement in tumor biology and regulation of immune response. The therapeutic potential of the immune system-tumor interaction is used, such as blocking immune inhibitory checkpoints in tumors with an immune infiltrate, especially tumor infiltrating lymphocytes which can be predictive of response to therapy and have a prognostic role, e.g. in triple-negative breast cancer(30).

MTs are expressed differently in different breast cancer types (ductal, lobular, pappilary). While ductal breast cancer is MTs positive in 26% to 100% of cases, lobular breast cancer is less MTs positive, even in advanced stage of disease(26,31). Higher expression of MTs has been noted in tumors with low expression of ER and PR receptors, which are tumors that have worse prognosis(20,26,32,33). MT1E isoform is highly expressed in ER receptor negative tumors. Interestingly, in this type of tumor there is no correlation with tumor size, locoregional nodal metastases, even though high MT expression correlates with poor outcome and lower overall survival(26,34).

Dysregulated expression of MT isoforms was found to correlate with different types of cancer(20). MT1, MT2 and MT3 are overexpressed in breast cancer. MT3 overexpression in breast cancer correlates with poor prognosis due to increased invasiveness. In ductal breast cancer low expression points to epigenetic changes. MT2A isoform overexpression increases invasiveness, modulates cell cycle, enhances cell invasion and migration via the upregulation of matrix metalloproteinase (MMP-9)(20,26). In ductal breast cancer the MT2A isoform is also implicated in chemoresistance to doxorubicin(20). The expression of the MT2A isoform also correlates with higher expression of proliferative biomarker Ki-67, while on the other hand MT1E and MT3 have no correlation with proliferation(26). Tumor suppressor p53 and ER receptors play an important role in the induction of MTs in epithelial breast cancer cells(26,32). MT1E, which is expressed in ER-negative breast cancer, activates effector genes downstream of ER, potentiates myoepithelial differentiaton and tumor invasiveness(20). Higher expression of MTF1 and MT2A isoforms has been shown in higher breast cancer histological grades and the expression of MTF1 influences histological differentiation in ductal breast cancer(20). It is important to mention that there are as well several tumor markers such as laminin, fibronectin, collagen type IV, catepsin D, CD 44 and c-erbB2 that are not connected with MT expression(14,33).

A meta-analysis study evaluated associations between MTs as an immunohistochemical biomarker and tumor type, stage, grade, prognosis and survival. Invasive staining in ductal breast cancer was shown as opposed to small or no staining present in lobulary and papillary cancer(34). There were no associations found between MTs immunopositivity and tumor size and tumor stage, although there are data which associates higher MTs expression with a poor prognosis(26,35,36). It is also unclear whether MTs expression is associated with metastases in breast cancer; some studies have shown no association, whereas others have reported strong association with lymph node metastases(37). A strong positive association was established between MTs staining and histological grade(34). Expression of MT1F and MT2A was found to be higher in histological grade 3 breast cancer than in grade 1 or grade 2(26,36).

Besides its significance in tumor invasiveness, MTs expression may also contribute to the development of resistance to cytostatic drug therapy(19,20,22,34). This can be caused by the transport of Zn from MTs to several transcription factors, such as p53. Thionein can regulate the activation of

transcription of Sp1 which is a Zn finger transcription factor involved in cell growth. During interphase MTs are found in the nucleus where it donates Zn to transcription factors(13). Due to its antioxidative properties, MT could protect malignant cells and induce chemo and radioresistance(25). Since it acts as an oxidative stress buffer, the increase in oxidative stress (radiation, cytostatic drug therapy) will affect the expression of MTs. Higher expression of MTs can cause resistance to cisplatin, which is known as an important cytotoxic agent used as monochemotherapy and polychemotherapy, as well as radiosensitizer during radiotherapy. Resistance to cisplatin is due to the binding of MTs to Pt, which inactivates the mechanism of action of cisplatin. (26). Removal of free radicals and metabolites of anti-tumor drugs can cause inactivation of drug activity and neutralize the drug itself(13). In addition to cisplatin, MTs affects the effect of other anticancer drugs, such as doxorubicin, oxaliplatin, 5-fluorouracil(20).

CONCLUSION

Although much about metallothionein remains undiscovered, we can say with certainty that their role in breast cancer is significant. Many studies have shown a correlation between metallothionein expression and tumor growth, regardless of positive or negative effect. A higher expression of metallothionein in breast cancer correlates with higher tumor grade, higher tumor stage and worse outcome. Metallothionein expression correlates with previously known important prognostic factors like estrogen and progesterone receptors, tumor supressor p53, and non-histone nuclear protein Ki-67. The aforementioned prognostic factors in combination with metallothionein in the future could help to improve the accuracy of prognosis in breast cancer patients. Further studies are needed to define its role in different breast cancer molecular subtypes, and possibly to develop new specific anti-cancer treatment.

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Sažetak

ULOGA METALOTIONINA U KARCINOMU DOJKE

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Metalotioneini su skupina proteina bogatih cisteinima koji su uključeni u mnoge fiziološke i patološke procese. Geni koji kodiraju četiri izoforme metalotioneina locirani su na lokusu 16q13. Strukture ovih četiriju izoformi omogućuju metalotioneinima njihove razne biološke funkcije. Mnoge su studije pokazale da metalnotionein ima važnu ulogu u karcinogenezi, rastu tumora, njegovoj progresiji od lokalne prema metastatskoj bolesti te je povezan sa razvojem rezistencije na kemoterapiju i radioterapiju. Rak dojke jedan je od vodećih uzroka smrti u svijetu i važno je bolje razumijeti ulogu metalotioneina u različitim podtipovima karcinoma dojke. Ovakvi podaci mogli bi pomoći kao prognostički alat i voditi pronalasku novog specifičnog liječenja karcinoma.

KLJUČNE RIJEČI: *metalotionein; karcinom dojke; prognostički čimbenik; liječenje*