ORIGINAL ARTICLE

Results in treatment of early breast cancers and the level of selected metalloproteinases

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Abstract

BACKGROUND: Breast cancer is the most common malignancy in women in the U.S. and Europe. In the early stages of the disease, women are treated surgically, which is supplemented with hormonal therapy, immuno-, chemo- or radiotherapy. Postoperative qualification for further treatment is based on clinical stage, the pathology of the tumor and classic prognostic factors. Despite that, among patients with breast cancer in early stages of clinical advancement, there is a relatively large proportion of observed tumor recurrence. These observations oblige the search for additional prognostic factors that determine the progression of the disease faster, according to which, could emerge a group of women at increased risk of recurrence of the disease.
AIM: The aim of this paper is to determine the meaning of the expression of selected metalloproteinases as prognostic factors in breast cancer.
METHODS: The study group consisted of 108 patients ages 26 to 86 years treated surgically from 1994 to 2000 because of primary breast cancer in the early clinical stage, ie stage I and II according to TNM classification.
RESULTS: Between two of the tested metalloproteinases (MMP-2 and MMP-11) only MMP-2 appears to have prognostic significance in early forms of breast cancer, and its strong expression is associated with shorter survival.

INTRODUCTION

Breast cancer is the most common malignancy in women in the United States and Europe, including Poland. This represents approximately 23% of all cases of cancer in women (Jemal *et al.* 2009) and the risk of cancer of this organ in our country is growing.

According to the American Cancer Society (ACS) in the United States, a country with the highest number of cases, it is estimated that in 2011 there are 207,000 new cases of cancer, and one in eight women born today will develop breast cancer.

The most important risk factor for breast cancer in women is age. Average age of breast cancer diagnosis is 61 years. Cancer occurs more frequently among women of higher socioeconomic classes, residents of developed countries, more often in women living in the city than in rural areas, and often in unmarried women (Jassem 1998; Kreienberg *et al.* 2002)

Exposure to endogenous and exogenous estrogen is one of known risk factors for breast cancer. It

is frequently observed in women who experience early menarche, late menopause, women who for a long time been using hormone replacement therapy, in women with obesity, nulliparous, or those in whom the first pregnancy occurred after 30 years life (Collaborative Group on Hormonal Factors in Breast Cancer 2002). As a risk factor also mentioned is hyperinsulinemia and a diet rich in animal fats and alcohol. Approximately 10% of all cases of breast cancer are in patients with a positive family history, some of them are related genetically and usually with a mutation within the BRCA1 and BRCA2 genes (Cortesi *et al.* 2010). The factors that reduce the risk of developing breast cancer include late occurrence of menarche, unilateral or bilateral oophorectomy, pregnancy before age twenty, and breastfeeding. Also stressed is the importance of regular exercise, proper body mass index and diet low in fat, which is associated with prevention of hyperinsulinemia.

In the detection of cancerous changes in the breasts, in addition to the patient's history and examination by palpation is mammography, ultrasound, and in some cases the magnetic resonance imaging.

Diagnosis shell be confirmed by examination of cytological material obtained by fine-needle biopsy (rarely) or more histological examination of tissue samples obtained by core biopsy and needle biopsy, stereotactic biopsy or open surgery.

Currently in early stages of breast cancer the basic treatment is modified radical mastectomy or the breast conserving therapy (tumorectomy or quadrantectomy) combined with radiation. During the surgical procedure one also removes the axillary lymph nodes on the side of the breast tumor. Surgical treatment is usually supplemented depending on the type of surgery, tumor characteristics and general condition of patient by radiation therapy, hormone therapy, chemotherapy or immunotherapy. This depends mainly on the presence of cancer metastasis to lymph nodes and the presence of steroid receptors.

It is estimated that in clinical stage I of breast cancer 5-year survival is around 90%, in II A-80%, II B-70%, in III-40%, and in IV stage-10% (Jassem 1998). However, the relapse occurs even after 10 or 20 years after diagnosis and primary treatment of the disease.

It is known that breast cancer is a systemic disease from the beginning, and the results of treatment and patient survival is dependent on distant metastases. It is therefore important to assess the risk of their occurrence and coordinate individualized treatment. With this in mind, one routinely takes into account such factors as patient age as well as size, type and degree of histological tumor differentiation, lymph node status, the presence of steroid receptors, HER2 protein expression and the presence of cancer cells in the vessels surrounding the tumor (Untch *et al.* 2004; Goldhirsch *et al.* 2009).

With the introduction of mammography screening, an increasing trend toward early detection of breast cancers has been observed. In these patients, it is important to select treatment that will provide maximum benefit with the least side effects. It is interesting that among patients with operable breast cancer at less advanced stages of disease development a fairly large proportion of observed tumor recurrence is observed. These observations oblige us to seek additional factors that determine the faster progression of disease and its recurrence.

Failures in the treatment of solid tumors depend on tumor cell metastases, especially distant. Traditionally, it is believed that breast cancer metastases are initially formed through the lymph nodes and in the later stages, hematogenously.

However, more recent reports indicate that cancer cells are present in the bloodstream from the beginning of the disease, and therefore should be treated as generalized regardless of its duration (Untch *et al.* 2004).

In breast cancer the most common metastases to regional lymph nodes are created by the invasion of cancer cells to mammary gland lymphatic vessels. These vessels form a plexus located in the pectoralis major fascia from there they direct to the axillary lymph nodes (Jassem 1998). Breast cancer also spreads through the blood and in this way, cancer cells metastasize to bone, lung, liver and brain, but also described is the occurrence of tumor metastasis to the ovaries, to the other breast, adrenal gland, skin and subcutaneous tissue (Kreienberg *et al.* 2002). Regardless of the location of their metastases, formation takes place through the same road and consists of several phases.

Cancer cells must initially overcome the intercellular adhesion and detach from the primary tumor mass, move through the blood vessel endothelium or lymphatic system, and then after reaching the target location must overcome a natural barrier tissue such as the basement membrane and invade the extracellular matrix (ECM). This process is possible by the enzymatic digestion of proteins forming the barrier. At this stage, the key role is played by proteolytic enzymes, including metalloproteinases (Garbett *et al.* 2000). By digestion of basement membranes, and tissue matrix (ECM), metalloproteinases allow angiogenesis, local expansion (Sharma *et al.* 2004), and distant metastasis of cancer (Polette & Brimbaut 1998).

Metalloproteinases are more than twenty enzymes (Table 1) (Nelson *et al.* 2000) of the type of extracellular endopeptidase, with some common amino acid sequences, containing a zinc ion (De Clerck 2000; Egeblad & Werb 2002; Jezierska & Motyl 2009; Matrisian *et al.* 1990; McCawley & Matrisian 2000; McCawley & Matrisian 1998; Minn *et al.* 2005; Nagase *et al.* 1992; Polette *et al.* 2004; Rudoplh-Owen *et al.* 1998). The zinc ion is part of the catalytic center of these enzymes, hence of crucial importance for their function (Figures 1 and 2).

These enzymes play a role in physiological processes such as embryogenesis, angiogenesis, wound healing,

bone growth, menstruation, childbirth and postpartum uterine involution, development and disappearance of Graafian follicles, involution of the breasts after lactation (Benaud *et al.* 1998; Tlsty 2001) and pathological conditions, such as growth and formation of tumor metastases (Bister *et al.* 2004; Cai *et al.* 2007; Decock 2007; Decock 2005; Gaibyan *et al.* 2004; Jäälinojä *et al.* 2000; Koblinski *et al.* 2000; McCarthy *et al.* 1999; Nielsen *et al.* 2001; Settner *et al.* 2009; Väisänen *et al.* 2000) the formation of cirrhosis, hypertensive vascular remodeling and atherosclerotic plaque formation and changes associated with chronic inflammation- for example, in rheumatoid arthritis and periodontitis.

These enzymes can be divided into several groups depending on their function (Jones *et al.* 1999; Pasco *et al.* 2003; Pei & Weiss 1995; Remacle *et al.* 1998; Reed *et al.* 2000; Sato *et al.* 1997). And so they are: collagenases (MMP-1, MMP-8), gelatinases (MMP-2, MMP-9), membrane-type metalloproteinases (MMP-14-17) and stromelysins (MMP-11, MMP-13). A large part of this group of enzymes is involved directly in the process of tumor expansion through proteolytic activity.

Metalloproteinase 2 (MMP-2) belongs to the gelatinases, is released from the cell into the extracellular space as a proenzyme and activated by cutting off the N-terminal propeptide (Morgunova *et al.* 1999). It was shown that the role of the activator MMP-2 is likely to be one of transmembrane metalloproteinase (MT1-MMP) (Ishigaki *et al.* 1999; Jones *et al.* 1999; Morgunova *et al.* 1999; Polette & Brimbaut 1998) and in the process of activation of MMP-2 it becomes part of a complex molecule inhibitor of metalloproteinase-2 (TIMP2) (Lafleur *et al.* 2003; Nakapoulou *et al.* 2002), which when combined with proMMP-2, allows its activation. Gelatinase A exhibits proteolytic activity mainly in relation to type IV collagen and constituent of basal membranes (Cai *et al.* 2007). It has been proven, however, that the substrates of this enzyme are also other types of collagen (I, V, VII, X, XI, XIV) and elastin, fibronectin, aggrecan, osteonectin, laminin, proteoglycans and other metalloproteinases such as MMP-1, MMP-9 and MMP-13.

Its function, which involves the basal membrane protein digestion with subsequent interruption of continuity, is typical for the whole group of zinc ion dependent proteases. This mechanism is one of the cornerstones of the process of expansion and metastasis of cancer. Additionally, demonstrating the enzymatic activity in relation to other metalloproteinases, it activates some of them, thus exacerbating the process.

Increased expression of MMP-2 are found in many types of cancers such as breast cancer, bronchus, colon, stomach, bladder, prostate and ovarian cancers (Chandru *et al.* 2007; Ornstein & Cohn 2002; Perigny *et al.* 2008; Talvensari-Mattila *et al.* 2005; Trudel *et al.* 2003; Väisänen *et al.* 2000). The literature has emphasized its relationship with the tendency to produce distant metastases, a shorter survival and poorer prognosis, especially in breast cancer with metastases to lymph nodes.

Metalloproteinase 11 (MMP-11) is a metalloproteinase first identified in breast cancer. It is produced mainly by fibroblast tissue matrix (Basset *et al.* 1997; Louis *et al.* 2005; Masson *et al.* 1998; Wang & Tetu 2002; Wiseman & Werb 2002; Wolf *et al.* 1993). Its role is not fully explained, because unlike the other enzymes of this group, it shows no strong proteolytic activity against components of the basal membrane. Proteolytic activity was demonstrated, however on protein growth factors and cytokines of intercellular space (Bigg *et al.* 1997). It has been proven that its substrates include, α-1 proteinase inhibitor and α-2 anti-plasmin. Since the proteinase inhibitor α-1 is the main inhibitor of elastase, thus indirectly by its proteolysis, enhances elastase-dependent process of tissue damage. However the distribution of α-2 antiplasmin increases the amount of free plasmin, which may increase the activation of other metalloproteinases (Louis *et al.* 2005).

It is suspected that this enzyme plays a role in control reactions between tumor cells and the environment (ECM). Its effect is more concerned with survival of cancer cells in the tissues of the host, and the process of tumor growth initialization rather than the process of metastasis (Carter *et al.* 1989; Noël *et al.* 1995; Rio & Foidart 2000). It is interesting that elevated expression of this enzyme was found in dysplastic foci such as those found in the airways, which show precancerous changes in contrast to areas of meta-and hyperplasia (Lafleur *et al.* 2003). MMP-11 expression was observed both in fibroblasts adjacent to the tumor (Selvey *et al.* 2004) and in the cytoplasm of epithelial cancer cells, but there are reports of greater prognostic importance of the presence of this enzyme in cancer cells (Engel 1997).

The importance of metalloproteinases in the course of cancer is the subject of research since about 15 years. It is suggested that the increased expression of MMP-11 and MMP-2 is an unfavorable prognostic factor in breast cancer (Abrial *et al.* 2005; Bria *et al.* 2010), and the relationship between the increased expression and lymph node involvement was proven in studies of other cancers (non-small cell bronchial carcinoma, colorectal, head and neck and skin) (Baker at al. 2002; Bister *et al.* 2004; Skoglund *et al.* 2004). Nakopoulou studies highlighted the association of increased expression of these enzymes with a worse survival rate and axillary lymph node involvement in breast cancer (Benaud *et al.* 1998). The increasing ability to detect breast cancer in its early stages of clinical advancement oblige us to explore additional prognostic factors, which will allow individualize further systemic therapy. It may help to reduce the recurrence of cancer. Also of interest would be to determine the level of expression of the described metalloproteinases in patients with early forms of breast cancer without axillary lymph node involvement or the presence of single tumor cell metastasis to lymph nodes in terms of outcomes.

OBJECTIVES OF WORK

The aim of this paper is to determine the meaning of the expression of selected metalloproteinases as prognostic factors in breast cancer. To achieve this goal, the following will be defined:

1. The level of expression of metalloproteinases 2 and metalloproteinase 11 in breast cancer changes
2. The relationship between outcomes and the degree of expression of studied metalloproteinases-MMP-2 and MMP-11

MATERIAL AND METHODS

The study group consisted of 108 patients ages 26 to 86 years treated surgically at the Department of Gynecology and Oncology CMUJ from 1994 to 2000 because of primary breast cancer in the early clinical stage, ie stage I and II according to TNM classification and who remained in outpatient care at the Oncology Clinic of Gynecology and Oncology Clinic CMUJ.

The average age of patients studied was 55.2 years. Most of them, as many as 45 (42%) women were 55 years or more at the time of diagnosis. Groups between 46 and 55 years of age was 38 (35.0%) women, 22 (20.3%) patients were aged 36 to 45 years, while 3 (2.7%) had less than 35 years at the time of diagnosis

Analysis of family history revealed that in 73 (67.6%) cases, no first degree and second degree relatives were ill with breast cancer and / or ovarian cancer. Data from the obstetric history showed that 12 (11.1%) women had never been pregnant, 12 (11.1%) women had been pregnant once, 30 (27.8%) women were pregnant twice, and 54 (50.0%), three or more times. 15 (13.5%) women were not pregnant, 21 (19.4%) gave birth once, 40 (37.0%) gave birth twice, and 32 (30.1%) women gave birth three or more times; because 60 (55.6%) women breast-fed for longer than six months, 17 (15.7%) women breast-fed, but less than six months, and 31 (28.7%) women did not breast-feed.

Menstruation history indicate that 18 (16.8%) women got their first menstruation before the age of 10, 48 (44.4%) women got their first menstruation between the ages of 11–12, 36 (33.3%) between 13–14 years of age and 6 (5.5%) women got their first menstruation after 14 years of age.

In the study group, 65 (60.2%) women were postmenopausal. Within this group, the last menstruation being between 45–47 years of age included 9 (13.8%) of the women, between the ages of 48–50 included 33 (50.7%) women, between the ages of 51–52 included 17 (26.2%) women and above 53 years of age, 6 (9.2%) women.

Only 9 (8.3%) women used a hormone replacement therapy for 5 years or longer, 6 (5.5%) used it less than 5 years, and the remaining 93 (86.2%) women were not taking hormone replacement therapy.

The first stage of treatment was surgery. In 101 (93.5%) cases, modified radical mastectomy was performed involving the total excision with skin and lymph nodes on the operated side, sparing the pectoral muscles. In 7 (6.5%) cases, breast conserving surgery was performed, with removal of part of axillary lymph nodes on the side of the operated breast followed by radiotherapy.

Chemotherapy was given to 40 (37%) patients. It was used primarily in patients with metastasis to axillary lymph nodes, or in the presence of adverse prognostic factors such as low histological grade of tumor differentiation, the young age of the patient, overexpression of the HER2 protein, as well as in the absence of expression of steroid receptors. In 36 (33.3%) cases, the chemotherapy regimen was based on 5 – fluorouracil, cyclophosphamide and methotrexate (CMF), while in 4 (3.7%) cases, patients received anthracyclines (AC).

In 49 (45%) patients in whom expression of steroid receptors was confirmed hormone therapy was prescribed, of which 41 (38%) cases used Tamoxifen, in 5 (4.2%) cases Anastrozole, and in 3 (2,8%) cases Anastrozole, and Tamoxifen.

Aromatase inhibitors were used in postmenopausal patients with coexistence of risk factors for thromboembolism or those who poorly tolerate Tamoxifen which would rule out its use.

After the surgery the patient remained in control of the outpatient Oncology Clinic of Gynecology and Oncology Department CMUJ. Over the first five years, visits took place every 3–6 months, and by next year, every 6–12 months.

This work has gained a positive opinion of the Commission of Bioethics at the Jagiellonian University No. KBET/16/B/2006.

Immunohistochemical recognition of tested metalloproteinases

The level of expression of MMP-2 and MMP-11 was determined by immunohistochemistry in tumor biopsies fixed in 15% formalin solution. For this purpose, 3 micrometer sections were prepared which, after immersion in 20% rabbit serum (DAKO A / S) were subjected to incubation with mouse monoclonal antibodies against MMP-2 and MMP-11 (Lab Vision). The resulting material was subjected to incubation at room temperature for about 12 hours. In a further step rabbit, biotinylated secondary antibodies against mouse antibodies (DAKO A / S) suspended in 3% human plasma at 1:400 ratio were added. Samples were incubated at room temperature for 30 min. with peroxidase-complexes of Biotin-Streptavidin. Visualization was performed using diaminobenzidine, and nuclei were stained with hematoxylin.

These reactions are made in line with the correct application of immunohistochemical methods, conducting the positive controls.

Statistical methods

The collected research material was presented in narrative form based on the number and frequency of tested

characteristics. Quantitative variables are presented by analyzing the distribution parameters of the arithmetic mean and standard deviation.

The analysis was performed for the treatment outcomes as end points of death. Survival time was counted from the date of surgery. The results of treatment are presented using the Kaplan-Meier survival at 10 years of observation. For the complete observation are those who have failed treatment or completed a 10-year observation.

The effect of the level of expression of metalloproteinases in the course of these curves was analyzed using the logrank test.

Statistical significance for these tests was adopted for which the level of significance was less than or equal to 0.05 ($p \leq 0.05$).

Calculations were performed using the statistical package STATISTICA 8 PL licensed for the Jagiellonian University.

RESULTS

The evaluation of the expression levels of metalloproteinases (MMP-2 and MMP-11); was based on the intensity and character of the staining of slides, recognizing diffuse or granular forms. Identified were four levels of intensity of expression: 0 – negative, ie no observable reaction to the presence of metalloproteinases, 1 – weak, ie when 20% of cells examined showed expression for metalloproteinases, 2 – medium, where 20–50% of cells showed expression of metalloproteinases (in this case also marked the grainy nature of the staining) and 3 – strong, where over 50% of the cells examined showed expression of metalloproteinases (Talvensaari-Mattila *et al.* 1999). Different degrees of expression of the enzymes are present photographs in Figures 1 and 2.

In immunohistochemical assays performed, metalloproteinase 2 (MMP-2**)** in 61 (56.5%) cases showed no expression of MMP-2, in 23 (21.3%) cases MMP-2 expression was weak, in 11 (10.2%) average, and in 13 (12.0%) cases, strong expression

In the immunohistochemical assays performed, metalloproteinase 11 in 32 (29.62%) cases showed no expression of MMP-11, in 34 (31.5%) MMP-11 expression was weak, in 16 (14.8%) cases average expression and 26 (24%) cases of strong expression

In the group of 99 women who survived the 10-year observation period were 61 (61.6%) cases where no expression of MMP-2 was confirmed, in 21 (21.2%) cases weak expression, in 9 (9.1%) cases average, and in 8 (8.1%) cases, strong expression of MMP-2.

In the group of women whose observation ended with the death no cases with lack of expression of MMP-2 were observed; in 2 (22.2%) expression of MMP-2 was weak, in 2 (22.2%) average and 4 (55.6 %) cases of strong expression.

In the comparison of survival curves, Kaplan-Meir for patients with different levels of expression of MMP-2, indicates the presence of statistically significant differences in (p=0.05) survival time after surgery. The survival curve of patients in whom the level of expression of MMP-2 = 3 deviates significantly from the survival curves of patients with MMP-2 = 0, MMP-2 = 1 and MMP-2 = 2. The 10-year survival of patients in the group with a strong expression of MMP-2 is significantly lower than the 10-year survival of patients with absence, poor or average level of expression of MMP-2 (Figure 3).

In the group of 99 women who survived the 10-year observation period, 31 (31.3%) cases showed no expression of MMP-11, in the next 31 (31.2%) cases weak expression, in 14 (14.1%) cases average, and in 23 (23.2%) cases, strong expression of MMP-11.

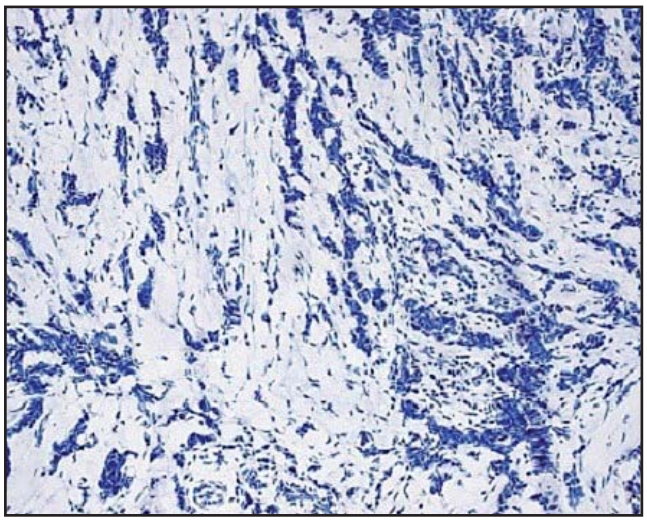

Fig. 1. Immunohistochemical reaction of the tested metalloproteinases. Lack of expression (0).

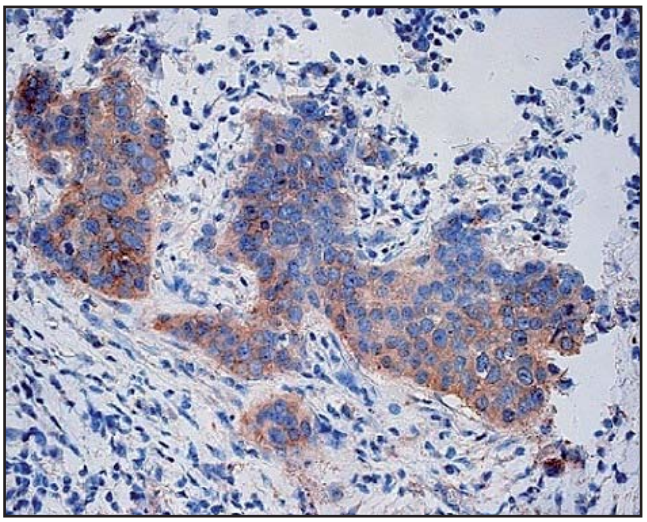

Fig. 2. Immunohistochemical reaction of the tested metalloproteinases. Strong expression (3 +), intense granular reaction.

The group, which resulted in fatal outcome, observation included: in 1 (11.1) case showed no expression of MMP-11, in 3 (33.3%) expression of MMP-11 was weak, in 2 (22.2%) average expression, and in 3 (33.3%) cases strong expression.

There was no significant statistical difference in the level of MMP-11 expression between the groups in which the therapeutic procedure was effective compared to that in which death were observed. Statistically 10-year survival factor (p=0.487) was not significantly dependent on the level of expression of MMP-11, although in cases of women who survived the 10-year observation period no expression or low expression of MMP-11 was almost about 50% higher compared to the group where the tumor relapsed or patient died (Figure 4).

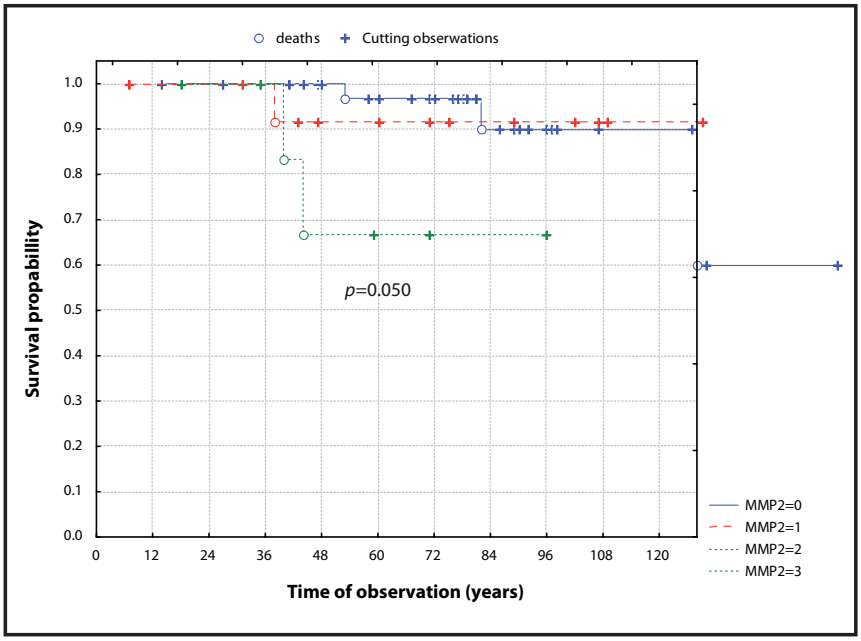

Fig. 3. 10-year probability of survival, depending on the level of expression of MMP-2.

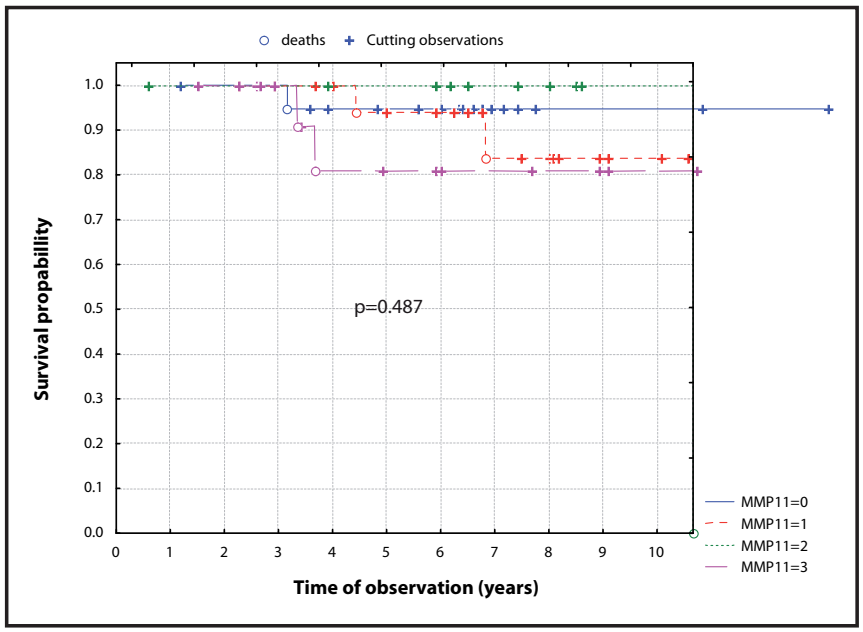

Fig. 4. 10-year probability of survival, depending on the level of expression of MMP-11.

Despite the lack of statistical significance in the group where treatment was completed successfully, dominating is the lack of expression of MMP-11.

DISCUSSION

Breast cancer is the most common malignancy in women. In developed countries, changing lifestyles, having fewer offspring, late first pregnancy, high-energy diet and long-term exposure to estrogen makes the incidence of this disease continue to grow.

Breast cancer is a disease of diversity, and often inspite of similar clinical stage and histological differentiation of tumor, patients are burdened with different risk of relapse rate (Cianfrocca & Goldstein 2004). Still are sought some new, additional prognostic factors. Including these prognostic factors in the qualification for further treatment could help in the emergence of a high-risk group of patients who benefit from additional treatment.

Intensively examined prognostic factors are associated with tumor cell biology. Cancer cells, including breast cancer cells, use proteolysis in the local processes of expansion and development of distant metastases (Weigel & Dowsett 2010). They produce proteolytic enzymes, including metalloproteinases, as well as stimulating the fibroblasts, digesting components of the extracellular matrix (ECM); among others, proteoglycans and glycosaminoglycans, thereby gain the space needed for tumor growth. Furthermore, the tumor cells digesting collagen type IV, the main component of basement membranes, overcome this natural barrier, gaining the opportunity to invade the blood and lymph vessels, and thus, the creation of distant metastases (Chandru *et al.* 2007; Kim 2006; Mendes 2007; Talvensaari-Mattila *et al.* 1999; Trangas *et al.* 2001). Destruction of extracellular matrix components also reduces the strength of intercellular adhesion, facilitating detachment of cells from the tumor mass, which also facilitates

the process of metastasis. Metalloproteinases and pro-angiogenic factors also indirectly participate in the next stage of expansion which is the process of tumor neo-angiogenesis (Settner *et al.* 2009).

Physiologically, the activity of MMPs is strictly controlled, while in the process of neoplastic changes its control is disordered, resulting in an increased expression of proteolytic enzymes (Trudel *et al.* 2003; Ornstein & Cohn 2002). Thus, increased expression of metalloproteinases is associated with increased tumor invasiveness (Benaud *et al.* 1998; Decock 2007; Nelson *et al.* 2000; Remackle *et al.* 1998).

The best known enzyme of this group is MMP-2. The prognostic value of this metalloproteinase in cancer is confirmed by many authors. Most work relates to its prognostic significance in solid tumors such as stomach cancer, prostate, bladder, small cell lung, ovarian and endometrial cancer (Micke & Őstman 2004; Perigny *et al.* 2008; pories *et al.* 2008; Talvensaari-Mattila *et al.* 2005; Trudel *et al.* 2003). Cai *et al.* (2007), observed increased expression of MMP-2 and MMP-9 in the early stages of ovarian cancer and was closely associated with damage to the basement membrane. In studies, Trudel *et al.* (2003) also confirmed the prognostic value of MMP-2 in prostate cancer. In Talvensaari-Mattila *et al.* studies the value of MMP-2 as a prognostic factor in breast cancer patients with the presence of steroid receptors in tumor tissues has been confirmed. Its increased expression was associated with a shorter relapse-free period and with a shorter survival (Nakopoulou *et al.* 2005; Talvensaari-Mattila *et al.* 1998; 1999; 2001; 2003).

The objective of the study was to analyze the level of MMP-2 in the serum of patients with breast cancer too. Leppa (2004) confirmed the association of elevated serum levels with worse prognosis in the group of patients with metastases to axillary lymph nodes. The relationship of MMP-2 expression to breast cancer was also confirmed by Moses and colleagues (1998), and Pories *et al.* (2008), observing elevated levels in the urine of patients with breast cancer (Moses 1998).

In the analyzed material in the group of women who survived the 10-year observation period, in 61%, MMP-2 expression was negative, in 82% negative or weak, and in only 18% of cases, average or strong. However, in cases where the observation resulted in fatal outcome, in 77.7%, the expression of MMP-2 was average or strong, and in only 22.2%, weak. In this group there were no cases with lack of expression of MMP-2. In the Hirvonen studies (Hirvonen *et al.* 2003), the ratio of 10-year survival in patients without the presence of metastases to regional lymph nodes occurred 100% in the group with the lack of expression of MMP-2, and 87% in the group with positive expression of MMP-2.

After extracting the group with a strong expression of MMP-2, it was lower by another 3%.

The results of treatment are better than obtained in our material; however, as mentioned, the study group consisted only of patients with locally advanced disease, so with no lymph node involvement. The studies Talvensaari showed 5-year survival of women with similar severity of disease, were 100% for the lack of expression of MMP-2 and 73% in the presence of MMP-2 (Talvensaari-Mattila *et al.* 2003)

Of the more than 20 metalloproteinases usually analyzed as potential prognostic factors in breast cancer are MMP-2, MMP 9 and MMP 11. Expression of MMP-2 is often analyzed together with the expression of MMP-9, which is its direct activator (Braun *et al.* 2005). Due to the very different results for the MMP-9 as a prognostic factor in breast cancer-even in the literature are reports of its importance as a positive prognostic factor in patients without metastasis to axillary lymph nodes (Pellikanen *et al.* 2002) – as the second test it was decided to choose metalloproteinase MMP-11, an enzyme from a completely different group of protease-dependent zinc ion, which are the stromelysins. MMP 11 is different from other metalloproteinases. In contrast to the other, it is secreted from cells after its activation in the Golgi apparatus. Therefore it requires no other cofactors for its enzymatic activation. Its increased expression is observed in breast cancer, but also in pancreatic cancer (Łukaszewicz *et al.* 2008), stomach (Hua *et al.* 2005), esophagus (Sharma *et al.* 2004) and small cell lung cancer (Janecki-Delebecq *et al.* 2000). In a study by Sharma et al (2004), increased MMP-11 expression in esophageal cancer was associated with worse outcomes. What is also interesting, there was a correlation between increased expression of MMP-11 and p53 protein, which might suggest its role in regulating the expression of MMP-11. In studies by Janecki-Delebecq et al, elevated levels of MMP-11 expression was associated with the occurrence and metastasis of non-small cell cancer to local lymph nodes.

Most work is devoted to studying the role of MMP-11 in breast cancer; in the assessment of the prognostic value of metalloproteinases, in this type of cancer test results are more ambiguous than in the case of MMP-2. Initially it was thought that MMP-11 occurs only in tumor stromal cells, especially in fibroblasts (Pierzchała *et al.* 2004). But in their work, Tetu Ahmad confirmed their expression also in the cytoplasm of tumor cells. Described in the literature is the relationship of increased expression of MMP-11 with worse prognosis in cancer. (Ahmad *et al.* 1998; Chenard *et al.* 1996; Eiseler *et al.* 2009; Hanby *et al.* 1998; Nakopoulou ey al. 2002; Pei & Weis 1995; Pellikainem *et al.* 2002; Sato *et al.* 1997). It is known that in the figures of ductal carcinoma *in situ* – comedo type, which involves a high risk of developing invasive forms MMP-11 elevated expression was observed.

Many authors have not observed relation of MMP-11 expression with survival and relapse-free time in breast cancer (Carter *et al.* 2003; Pacheco *et al.* 1998; Rudoph-Owen & Matrisian 1998). In the analysis of our material is also not a statistically significant relationship between

increased expression of MMP-11 and shorter survival. It should be noted that in the group of women who have survived a 10-year observation period, the percentage of cases with no or weak expression of MMP-11 was 62.6%.

Perhaps the role of this enzyme is to initiate tumor growth through paracrine effects on the cells surrounding the tumor. Perhaps the evaluation of prognostic significance of MMP-11 requires observation of a larger group of women with breast cancer.

It should be noted that the predictive value of Stromelysin 3 (MMP-11) in breast cancer was recently used in clinical practice. It enters into the composition of Oncotype DX diagnostic test (Paik *et al.* 2004; Van't Veer *et al.* 2002). This is a panel of 21 genes for proteins with prognostic significance in breast cancer, whose genes are determined by RT-PCR (real time PCR). The value of the test has been confirmed in clinical studies on thousands of patients with breast cancer in N0 stage, characterized by the presence of steroid receptors. This test is helpful in extracting the group requiring treatment beyond the use of additional chemotherapy and hormonal therapy. The St. Gallen expert panel in 2009 recommended its use, but still with caution in terms of recognized prognostic factors.

CONCLUSION

Between two of the tested metalloproteinases (MMP-2 and MMP-11) only MMP-2 appears to have prognostic significance in early forms of breast cancer, and its strong expression is associated with shorter survival.

SUMMARY

Breast cancer is the most common malignancy in women in the U.S. and Europe. The ratio of cases of this cancer is constantly increasing. In the early stages of the disease, women are treated surgically, which is supplemented with hormonal therapy, immuno-, chemo-or radiotherapy. Postoperative qualification for further treatment is based on clinical stage, the pathology of the tumor and classic prognostic factors.

Despite that, among patients with breast cancer in early stages of clinical advancement, there is a relatively large proportion of observed tumor recurrence. These observations oblige the search for additional prognostic factors that determine the progression of the disease faster, according to which, could emerge a group of women at increased risk of recurrence of the disease. The immediate cause of death from breast cancer is metastasis of cancer cells to distant organs. The process of their formation is associated with the action of proteolytic enzymes, including matrix metalloproteinases (MMPs).

Metalloproteinases are a group of zinc-dependent ion proteases involved in the proteolysis of extracellular matrix components (ECM), including adhesive proteins and collagen. Their role has been confirmed in physiological processes such as embryogenesis, repair and reconstruction processes of tissue, angiogenesis, mammary gland involution and cyclic changes in endometrial and pathological diseases such as cardiovascular diseases, chronic inflammatory diseases and cancer. They participate in the process of migration and metastasis formation of cancer cells.

The aim of this study was to determine the meaning of the expression of selected MMPs as prognostic factors in breast cancer. To achieve this goal, expression levels of MMP-2 and MMP-11 in the breast cancer tumors and the correlation between those expression and 10-years survival were determined. Our material consisted of 108 patients from ages 26 to 86 years treated surgically because of early primary breast cancer, ie in stage I and IIA according to the TNM classification in 1994–2000

In 101 cases, modified radical mastectomy and in 7 cases breast conserving therapy with axillary lymphadenectomy was performed After the operation, the level of expression of selected MMPs, ie MMP-2 and MMP-11, was established. The effect of expression of selected MMPs on the results of treatment by means of the Kaplan-Maier curves were analyzed with the logrank test. The statistically significant level of significance was adopted to be less than or equal to 0.05. The analysis showed no statistically significant dependence of the results of treatment; the level of expression of MMP-11 demonstrated a statistically significant correlation between the ratio of 10-year experience, and the level of expression of MMP-2 (p=0.05). Based on the results of research and clinical observations, we concluded, that of the two metalloproteinases (MMP-2 and MMP-11) only MMP-2 appears to have prognostic value in early forms of breast cancer, and its strong expression is associated with shorter survival.

REFERENCES

1 Abrial SC, Penault-Llorca F, Delva R (2005). High prognostic significance of residual disease after neoadjuvant chemotherapy: a retrospective study in 710 patients with operable breast cancer. Breast Cancer Res Treat. **94**(3): 255–63.
2 Ahmad A, Hanby A, Dublin E, Poulsom R, Smith P, Barnes D, et al (1998). Hart I: Stromelysin 3: an independent prognostic factor of relapse- free survival in node-positive breast cancer and demonstration of novel breast carcinoma cell expression. Amer J Pathol. **152**: 721–728.
3 Baker EA, Stephenson TJ, Reed MW, Brown N (2002). Expression of proteinases and their inhibitors in humane breast cancer progression and survival. Mol Path. **55**: 300–304.
4 Basset P, Bellocq JP, Lefebvre, Noel A, Chenard MP, Wolf C, et al (1997). Stromelysin-3: a paradigm for stroma-derived factors implicated in carcinoma progression. Crit Rev Oncol. **26**: 43–53.
5 Benaud C, Dickson RB, Thompson EW (1998). Roles of the matrix metalloproteinases in mammary gland development and cancer. Breast Cancer Res Treat. **50**: 97–116.

6 Bigg H.F, Shi Y.E, Steffensen B, Overall CM (1997). Specific high affinity binding of tissue inhibitor of metalloproteinases (TIMP-4) to the COOH- terminal hemopexin-like domain of human gelatinase A. TIMP-4 binds progelatinase A and the COOH- terminal domain in a similar manner to TIMP-2. J Biol Chem. **272**: 496–500.
7 Bister V, Salmela M, Karjalainen-Lindsberg M, Urja J, Lohi J, PuolakkainenP, et al, (2004). Differential expression of three matrix metalloproteinases, MMP-19, MMP-26 and MMP-28 in normal and inflamed intestine and colon cancer. Dig Dis and Science. **49**: 653–661.
8 Braun S, Vogl FD, Naume B (2005). A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med. **353**(8): 793–802.
9 Bria E, Carlini P, Cuppone F, Vaccaro V, Milella M, Cognetti F (2010). Early recurrence risk: aromatase inhibitors versus tamoxifen. Expert Rev Anticancer Ther. **10**(8): 1239–53.
10 Cai K, Yang W, Capo-Chichi C (2007). Prominent expression of metalloproteinases in early stages ovarian tumorgenesis. Mol Carcinog. **46**: 130–143.
11 Carter CL, Allen C, Henson DE (1989). Relation of tumor size, lymph node status, and survival in 740 breast cancer cases. Cancer. **63**(1): 181–187.
12 Chandru H, Sharada AC, Manjunath S (2007). Expression of matrix metalloproteinase (MMP-2) extracellular matrix metalloproteinase inducer (EMMPRIN) in benign and advanced breast cancer tissue samples. Biomed Chem. **1**: 348–352.
13 Chenard MP, O'Siorain L, Shering S (1996). High levels of stromelysin-3 correlate with poor prognosis in patients with breast carcinoma. Int J Cancer. **69**: 448–451
14 Cianfrocca M, Goldstein LJ (2004). Prognostic and predictive factors in early-stage breast cancer. Oncologist. **9**(6): 606–16.
15 Collaborative Group on Hormonal Factors in Breast Cancer (2002). Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. The Lancet. **360**: 187–195.
16 Cortesi L, Masini C, Cirilli C, Medici V, Marchi I, Cavazzini G, Pasini G, et al (2010). 10-year overall survival in a Caucasian population with high probability of hereditary breast cancer. BMC Cancer. **10**: 1186–1206.
17 Decock J (2007). Matrix metalloproteinase expression patterns in luminal A type breast carcinomas. Dis Markers. **23**(3): 189–196.
18 Decock J (2005). Plasma gelatinase levels in patients with primary breast cancer in relation to axillary lymph node status, Her2/neu expression and other clinicopathological variables. Clin Exp Metastasis. **22**(6): 495–502.
19 Egeblad M, Werb Z (2002). New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. **2**: 161–174.
20 Eiseler T, Döppler H, Yan IK, Goodison S, Storz P (2009). Protein kinase D1 regulates matrix metalloproteinase expression and inhibits breast cancer cell invasion. Breast Cancer Res. **11**(1): 246–254.
21 Engel GL (1997). From biomedical to biopsychosocial. Being scientific in the human domain. Psychosomatics. **38**(6): 521–8.
22 Gaibyan L, Lenahan C, Pories S (2004). Metalloproteinases: Promising tumor markers for breast cancer management. Cur Surg. **61**: 255–259.
23 Garbett EA, Reed MWR, StephensonTJ, Brown NJ (2000). Proteolysis in human breast cancer. Mol Pathol. **53**: 99–106.
24 Goldhirsch A, Ingle JN, Gelber AS, Coates AS, Thürlimann B, Senn HJ et al (2009). Meeting highlights: International Expert consensus on the Primary Therapy of early Breast cancer 2009. Annals Oncol. **20**: 1319–1329.
25 Hanby A, Dublin E, Poulsom R, Smith P, Barnes D, Rubens R, et al (1998). Stromelysin 3: an independent prognostic factor for relapse-free survival in node-positive breast carcinoma cell expression. Am J Path. **152**: 721–728.
26 Hirvonen R, Talvensaari-Matilla A, Paakko P, Turpeenniemi-Hujanen T (2003). Matrix metalloproteinase 2 (MMP-2) in T(1-2) N(0) breast carcinoma. Breast Cancer Res Treat. **77**: 85–91.
27 Hua D, Rui-Fang G, Wen-Mei L, Min Z, You-Yong L (2005). Matrix metalloproteinase 11 depletion inhibits cell proliferation in gastric cancer cells. Bioch Bioph Res Com. **326**: 274–281.
28 Ishigaki S, Toi M, Ueno T, Matsumoto H, Muta M, Koike M, et al (1999). Significance of membrane type 1 matrix metalloproteinase expression in breast cancer. Jap J Cancer Res. **90**: 516–522.
29 Jäälinojä J, Herva R, Korpela M, Höyhtyä M, Turpeenniemi-Hujanen T (2000). Matrix metalloproteinase 2 (MMP-2) immunoreactive protein is associated with poor grade and survival in brain neoplasms. J Neurooncol. **46**(1): 81–90.
30 Janecki-Delebecq T, Porte H, Zerimech F, Copin MC, Gouyer V, Daquenbronne E, et al (2000). Overexpression level of stromelysin-3 is related to the lymph node involvement in non-small cell lung cancer. Clin Cancer Res. **6**: 1086–1092.
31 Jassem J (1998). Rak sutka" Springer PWN Warszawa.
32 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ (2009). Cancer Statistics. Ca Cancer J Clin. **59**: 225–249.
33 Jezierska A, Motyl T (2009). Matrix Metalloproteinase-2 involvment in breast cancer progression: a mini review. Med Sci Monit. **15**(2): 32–40.
34 Jones JL, Glynn P, Walker RA (1999). Expression of MMP-2 and MMP-9, their inhibitors and the activator MT1-MMP in primary breast carcinomas. J Pathol. **189**: 161–168.
35 Kim HJ (2006). Expression of MT-1 MMP, MMP-2, MMP-9 and TIMP2 mRNAs in ductal carcinoma in situ and invasive ductal carcinoma of the breast. Yonsei Med J. **47**(3): 333–42.
36 Koblinski JE, Mamoun A, Bonnie FS (2000). Unraveling the role of proteases in cancer. Clin Chim Act. **291**: 113–135.
37 Kreienberg R, Volm T, Mobus V, Alt D (2002). Menagment des mammacarcinoms. Springer Verlag, Berlin.
38 Lafleur MA, Tester A, Thompson EW (2003). Selective involvement of TIMP-2 in the second activational cleavage of pro-MMP-2: refinement of the pro-MMP-2 activation mechanism. FEBS Lett. **553**(3): 457–63.
39 Leppa S (2004). A high serum matrix metalloproteinase-2 level is associated with an adverse prognosis in node-positive breast carcinoma. Clin Cancer Res. **10**: 1057–63.
40 Louis K, Guerineau N, Fromigue O, Defamie V, Collazos A, Anglard P, et al (2005). Tumor cell- mediated induction of the stromal factor Stromelysin-3 requires heterotypic cell contact-dependent activation of specific protein kinase C isoforms. J Biol Chem. **14**: 1272–1283.
41 Łukaszewicz M, Mroczko B, Szmitkowski M (2008). Rola metaloproteinaz i ich inhibitorów w raku trzustki. Postępy Hig Med Dośw. **62**: 141–147.
42 Masson R, Lefebvre O, Noël A, Fahime ME, Chenard MP, Wendling C, (1998). In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. J Cell Biol. **140**(6): 1535–41.
43 Matrisian LM (1990). Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet. **6**: 121–125.
44 McCarthy K, Maguire T, McGreal G, McDermott E, O'Higgins N, Duffy MJ (1999). High levels of tissue inhibitore of metalloproteinase-1 predict poor outcome in patients with breast cancer. Int J Cancer. **84**: 44–48.
45 McCawley L, Matrisian LM (2000). Matrix metalloproteinases: multifunctional contributors to tumor progression. Mol Med Today. **6**: 149–156.
46 McCawley L, Matrisian LM (1998). Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol. **10**: 640–646.
47 Mendes O (2007). MMP-2 role in breast cancer brain metastasis development and its regulation by TIMP2 and ERK1/2. Clin Exp Metastasis. **4**: 158–169.
48 Micke P, Östman A (2004). Tumour-stroma interaction: cancer associated fibroblasts as novel targets in anti-cancer therapy? Lung Canc. **45**: 163–175.
49 Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, et al (2005). Genes that mediate breast cancer metastasis to lung. Nature. **436**: 518–524.
50 Morgunova E, Tuutila A, Bergmann U, Isupov M, Lindqvist Y, Schneider G, et al (1999). Structure of human pro-matrix metalloproteinase-2: activation mechanism revealed. Science. **284**: 1667–1670.
51 Moses MA (1998). Increased incidence of matrix metalloproteinases in urine of cancer patients. Cancer Res. **58**(7): 1395–99.

52 Nagase H, Barett A. J, Woessner JF (1992). Nomenclature and glossary of the matrix metalloproteinases. Matrix suppl. 421–424.
53 Nakapoulou L, Katsarou S, Giannopoulou I, Alexandrou P, Tsirmpa I, Panayotopoulou E, et al (2002). Correlation of tissue inhibitor of metalloproteinase-2 with proliferative activity and patient's survival in breast cancer. Mod Pathol. **15**: 26–34.
54 Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM (2000). Matrix metalloproteinases: biologic activity and clinical implications. J Clin Onc. **18**: 1135–1149.
55 Nielsen BS, Rank F, Lopez JM, Vizoso F, Lund LR, Dan, K, et al (2001). Collagenase-3 expression in breast myofibroblasts as a molecular marker of transition of ductal carcinoma in situ lesions to invasive ductal carcinomas. Cancer Res. **61**: 7091–7100.
56 Noël A, Santavicca M, Stoll I, L'Hoir C, Staub A, Murphy G, et al (1995). Identification of structural determinants controlling human and mouse stromelysin-3 proteolytic activities. J Biol Chem. **29**: 2866–72.
57 Ornstein DL, Cohn K (2002). Balance between activation and inhibition of matrix metalloproteinase-2 (MMP-2) is altered in colorectal tumors compared to normal colonic epithelium. Digest Dis Sci. **47**: 1821–1830.
58 Pacheco MM, Mourao M, Mantovani EB, Nishimoto IN, Brentani MM (1998). Expression of gelatinases A and B, Stromelysin 3 and matrylysin genes in breast carcinomas: clinico-pathological correlations. Clin Exper Meta. **16**: 577–585.
59 Paik S, Shak S, Tang G (2004). A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. **351**(27): 2817–26.
60 Pasco S, Ramont L, Maquart FX, Monboisse JC (2003). Control of melanoma progression by various matrikines from basement membrane macromolecules. Crit Rev Onc Hem. **49**: 229–233.
61 Pei D, Weiss SJ (1995). Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature. **375**: 244–247.
62 Pellikanen JM, Kataja V, Ropponen K, Kellokoski J, Pietilainen T, Bohm J (2002). Reduced nuclear expression of transcription factor AP-2 associates with aggresive breast cancer. Clin Cancer Res. **8**: 3487–3499.
63 Perigny M, Bairati I, Harley I, Beauchemin M, Harel F, Plante M, et al (2008). Role of Immunohistochemical Overexpression of MMP-2 and MMP-11 in the prognosis of death by ovarian cancer. Am J Clin Pathol. **129**: 226–231.
64 Pierzchała RP, Pasz-Walczak G, Jeziorski A (2004). Nowe czynniki rokownicze w raku piersi- przegląd piśmiennictwa. Wsp Onkol. **8**: 429–434.
65 Polette M, Brimbaut P (1998). Membrane-type metalloproteinases in tumor invasion. Int J of Biochem Cell Biol. **30**: 1195–1202.
66 Polette M, Nawrocki-Raby B, Gilles Ch, Clavel Ch, Birembaut P (2004). Tumor invasion and matrix metalloproteinases. Crit Rev Oncol. **49**: 179–186.
67 Pories SE, Zurakowski D, Roy R, Lamb CC, Raza S, Exarhopoulos A, *et al.* (2008). Urinary metalloproteinases: noninvasive biomarkers for breast cancer risk assessment. Cancer Epidemiol Biomarkers Prev. **17**(5): 1034–42.
68 Remacle AG, Noel A, Duggan C, Mc Dermott E, O'Hoggins N, Foidart JM, et al (1998). Assay of matrix metalloproteinases types 1, 2, 3 and 9 in breast cancer. Brit J Cancer. **77**: 926–931.
69 Reed W, Hannisdal E, Boehler PJ, Gundersen S, Host H, Marthin J (2000). The prognostic value of p53 and c-erb B-2 immunostaining is overrated for patients with lymph node negative breast carcinoma: a multivariate analysis of prognostic factors in 613 patients with a follow-up of 14–30 years. Cancer. **88**(4): 804–13.
70 Rio MC, Foidart JM (2000). Demonstration in vivo that stromelysin-3 functions through its proteolytic activity. Oncogene. **19**: 1605–1612.
71 Sato H, Okada Y, Seiki M (1997). Membrane type matrix metalloproteinases (MT-MMPs) in cell invasion. Thromb Haemost. **78**: 497–500.
72 Selvey S, Haupt LM, Thompson EW, Matthaei KI, Irving MG, Griffiths LR (2004). Stimulation of MMP-11(Stromelysin 3) expression in mouse fibroblasts by cytokines, collagen and co-culture with human breast cancer cell lines. Cancer. **4**: 1186–1194.
73 Settner R, Bogusiewicz M, Rechberger T (2009). Matrix metalloproteinases and theirs inhibitors in ovarian cancer progression-diagnostic and therapeutic implications. Ginekol Pol. **80**: 47–53.
74 Sharma R, Chattopadhyay TK, Mathur M, Ralhan R (2004). Prognostic Significance of Stromelysin-3 and tissue inhibitor of matrix metalloproteinase-2 in esophageal cancer. Oncology. **67**: 300–309.
75 Skoglund J, Emterling A, Gunnar A, Anglart P, Sun X (2004). Clinicopathological significance of stromelysin-3 expression in colorectal cancer. Oncology. **67**: 67–72.
76 Talvensaari-Mattila A, Blanco-Sequeiros G, Turpenniemi-Hujanen T (2001). Matrix metalloproteinase 2 (MMP-2) is associated with the risk for a relapse in postmenopausal patients with node-positive breast carcinoma treated with antiestrogen adjuvant therapy. Breast Cancer Res Treat. **65**: 55–61.
77 Talvensaari-Mattila A, Paakko P, Hoyhtya M, Blanco-Sequeiros G, Turpeeniemi T (1998). Matrix metalloproteinase-2 immunoreactive protein: a marker of aggressiveness in breast carcinoma. Cancer. **83**: 1153–1162.
78 Talvensaari-Mattila A, Pääkkö P, Turpeenniemi-Hujanen T (1999). MMP-2 positivity and age less than 40 years increases the risk for recurrence in premenopausal patients with node-positive breast carcinoma. Breast Cancer Res Treat. **58**(3): 287–93.
79 Talvensaari-Mattila A, Pääkkö P, Turpeenniemi-Hujanen T (2003). Matrix metalloproteinase-2 (MMP-2) is associated with survival in breast carcinoma. T Br J Cancer. **689**(7): 1270–5.
80 Talvensaari-Mattila A, Markku S, Soini Y, Turpeenniemi-Hujanen T (2005). Prognostic Value of Matrix Metalloproteinase-2 (MMP-2) Expression in Endometrial. Cancer Anticancer Res. **25**: 4101–4105.
81 Tlsty TD (2001). Stromal cells can contribute oncogenic signals. Canc Biol. **11**: 97–104.
82 Trangas T, Scorilas A, Karameris A, Arnogiannaki N, Ardavanis A, Bassilopoulos P, et al (2001). Overexpression of matrix-metalloproteinase-9 in human breast cancer: a potential favourable indicator in node– negative patients. Br J Cancer. **84**: 1488–96.
83 Trudel D, Fradet Y, Meyer F, Harel F, Tetu B (2003). Significanceof MMP-2 expression in prostate cancer: an immunohistochemical study. Cancer Res. **63**: 8511–8515.
84 Untch M, Sittek H, Bauerfeind I, Reiser M, Hepp H (2004). Diagnostik und Therapie des Mammacarcinoms-State of the Art 2004. Zuckschwerdt Verlag München
85 Väisänen A, Matti Kallioinen T, Turpeenniemi-Hujanen T, Taskinen P (2000). Prognostic value of MMP-2 immunoreactive protein (72 kD type IV collagenase) in primary skin melanoma. Jour of Path. **186**: 51–58.
86 Van 't Veer LJ, Dai H, van de Vijver MJ (2002). Gene expression profiling predicts clinical outcome of breast cancer. Nature. **415**(6871): 530–6.
87 Wang S, Tetu B (2002). Stromelysin-3 expression by mammary tumor-associated fibroblast under in vitro breast cancer cell induction. Int J Cancer. **99**: 792–799.
88 Weigel MT, Dowsett M (2010). Current and emerging biomarkers in breast cancer: prognosis and prediction. Endocr. Relat. Cancer. **17**: 245–60.
89 Wiseman BS, Werb Z (2002). Stromal effects on mammary gland development and breast cancer. Science. **296**: 1046–1049.
90 Wolf C, Rouyer N, Luts Y, Adida C, Loriot M, Bellocq JP (1993). Stromelysin-3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastioc cells and possibly implicated in tumour progression. Proc Natl Acad Sci USA. **90**: 1843–47.