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Review

The role of matrix metalloproteinases in pathogenesis of human bladder cancer

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Matrix metalloproteinases (MMPs) play an important role in many physiological and pathological processes, including neoplastic processes. They belong to a group of enzymes called endopeptidases and have the ability to hydrolyze all proteins in the extracellular matrix (ECM). They are produced in most connective tissue cells, macrophages, leukocytes, endothelial cells, microglial cells and in cancer cells. Neoplastic diseases are one of the main causes of death in Poland and in the world, therefore learning about the process of carcinogenesis seems to be particularly important. The process of carcinogenesis is currently widely studied and MMPs play one of the key roles in the development of cancer. They do this by regulating local tumor growth, stromal invasion, stimulating angiogenesis and metastasis formation. Bladder cancer is the 7th most common cancer in the male population and the 11th most common cancer in the world. In bladder cancer, most studies have been devoted to MMP-2 and MMP-9, that are enzymes responsible for the degradation of type IV collagen in the first place, which through the destruction of basement membranes and ECM, play an essential role in the tumor invasion process. Since bladder cancer is characterized by the ability to relapse, from the point of view of clinical practice it seems particularly important to develop a marker of early bladder tumor recurrence. MMPs detected in the urine and serum of patients with bladder cancer are potential factors that could play such a role.

Keywords: matrix metalloproteinases, tissue inhibitors of metalloproteinases, bladder cancer, carcinogenesis, angiogenesis

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Abbreviations: AIDS, acquired immune deficiency syndrome; CAF, cancer-associated fibroblasts; CIS, carcinoma in situ (ang. intraepithelial neoplasia); ECM, extracellular matrix; EGF, epidermal growth factor; EMMPRIN, extracellular matrix metalloproteinase inducer; EMT, epithelial-mesenchymal transition; EPO, erythropoietin; FDA, Food and Drug Administration; FGF-β2, fibroblast growth factors type β2; GPI, glycophosphatidylinositol; HIF-1, hypoxia-inducible factor 1; IL-1, interleukin 1; IL-10, interleukin 10; IL-12, interleukin 12; IL-1ß, interleukin-1ß; IL-6, interleukin 6; IL-8; CXCL-8, interleukin 8; INF-γ, interferon γ; MMPs, matrix metalloproteinases; MT-MMPs, membrane-type of MMPs; PDGF, platelet-derived growth factor; RA, rheumatoid arthritis; RX(R/K)R, furin recognition motif; SPARC, secreted protein acidic and rich in cysteine; TAM, tumor-associated macrophage; TAM-M1, tumor-associated macrophage type M1; TAM-M2, tumor-associated macrophage type M2; TGF-β1, transforming growth factor β1; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; VEGF-R2, vascular endothelial growth factor – type 2 receptor

INTRODUCTION

Biochemically, extracellular matrix metalloproteinases (MMPs) belong to a group of enzymes called endopeptidases and have the ability to hydrolyze all extracellular matrix proteins (ECM). They are produced in most connective tissue cells, macrophages, leukocytes, endothelial cells, microglial cells and, importantly, also in neoplastic cells (Deryugina & Quigley, 2006; Page-McCaw *et al.*, 2007; Fic *et al.*, 2011). MMPs play an important role in physiological and pathological processes, taking part in initiating and regulating inflammatory and carcinogenic processes, and also play a significant role in embryogenesis and organ maturation (Deryugina & Quigley, 2006; Fic *et al.*, 2011; Wątroba *et al.*, 2019). The role of MMPs in the pathological processes of fibrosis in liver cirrhosis, the development of bronchopulmonary dysplasia in preterm neonates, as well as in the degeneration of articular cartilage in rheumatoid arthritis (Mądro *et al.*, 2012; Bryda & Wątroba, 2018; Wątroba & Bryda, 2019).

CHARACTERISTICS OF MATRIX METALLOPROTEINASES

MMPs contain a zinc ion in their structure and their typical components are propeptide that inhibits catalysis by blocking the active centre and the catalytic domain, which is responsible for the hydrolysis of peptide bonds of a given substrate (Kurzepa *et al.*, 2014). The so-called hinge region or hemopexin-like domain are non-permanent components of MMPs (Lipka *et al.*, 2008). The regulation of the activity of MMPs is under the constant control of tissue inhibitors of metalloproteinases (TIMPs), which form non-covalent complexes with MMPs in the ratio 1:1 (Brew *et al.*, 2000; Fic *et al.*, 2011).

TIMP-1 is structured as an alkaline glycoprotein of 184 amino acids with a molecular weight of 28 kDa (Murphy *et al.*, 1991). TIMP-1 has an inducible expression mechanism, and its synthesis is stimulated by erythropoietin (EPO), tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) and platelet (PDGF) and epithelial (EGF) growth factors (Arza *et al.*, 2001; Brew & Nagase, 2010). The primary function of TIMP-1 is to inhibit the activity of MMPs by forming non-covalent bonds with both the active and inactive forms (Brew *et al.*, 2000; Brew & Nagase, 2010).

TIMP-2 is a protein composed of 194 amino acids with a molecular weight of 21 kDa, which is characterized by a constitutive expression mechanism and has the ability to block the activity of both MMP-2 and MMP-9 and has an influence on growth factor-dependent induction of mitogenesis (Ozenci *et al.*, 1999; Lambert *et al.*, 2004; Kim *et al.*, 2015).

TIMP-3 is an insoluble polypeptide with a molecular weight of 30 kDa and has the lowest inhibitory activity for MMP-2, -3, -7 and -9 and also has anti-angiogenic activity (Palosaari *et al*., 2003; Chetty *et al*., 2008). TIMP-3 has an inducible expression mechanism and inhibits the activity of most matrilisins, gelatinases and collagenases (Gill *et al*., 2003; Trojek, 2012; English *et al*., 2018).

Regulation of biological activity of MMPs depends on gene transcription, proenzyme activity and TIMPs activity. The ability to initiate MMPs expression have IL-1β, TNF-α, interleukin 6 (IL-6), interleukin 8 (IL-8, CXCL-8), lectin and extracellular MMP inducers (EMMPRIN) (Opdenakker *et al*., 2001; Schmidt *et al*., 2006).

MMPs are secreted into the ECM in the form of inactive proenzymes and their activation takes place in two stages, including a reversible process based on the principle of a "cysteine switch" and not related to the separation of the propeptide, and the irreversible process of permanent separation of the propeptide catalyzed by proteolytic enzymes (Fig. 1) (Mott & Werb, 2004; Okamoto *et al*., 2004; Kim & Hwang, 2011; Kapoor *et al*. 2016; Franco *et al*., 2017).

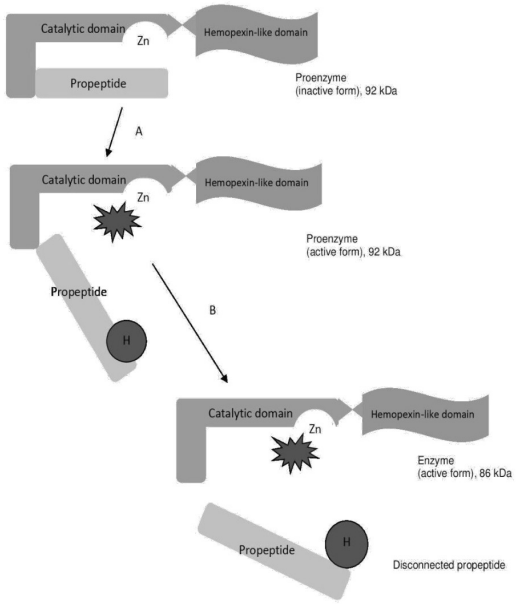

Figure 1. Diagram of the structure and activation of the MMP proenzyme (on the example of MMP-9).
The domains present in the gelatinases are: propeptide, a catalytic domain with a zinc atom (Zn), a hinge region and a hemopexin-like domain. A – non-proteolytic activation using mercury (Hg) compounds or some denaturing compounds. During this process, there is no disconnection of the propeptide (no change in mass in relation to the proenzyme). If the propeptide activating agent is removed, the propeptide rejoins the active site preventing catalysis („cysteine switch"). B – proteolytic activation involves the detachment of the propeptide. It is an irreversible process associated with a reduction in the mass of the enzyme by the mass of the propeptide (Wątroba *et al*., 2021 modified).

GELATINASES (MMP-2, -9)

They belong to the group of MMPs with the ability to degrade gelatine (Bauvois, 2012). They have a fibronectin-like hydrophobic fragment and a domain that is structurally similar to type V collagen (Kurzepa *et al*., 2014). The molecular weight of the latent form of MMP-2 (pro-MMP-2) is 72 kDa and that of pro-MMP-9 is 92 kDa (Opdenakker *et al*., 2001; Verslegers *et al*., 2013). The molecular weights of the proteolytically activated MMP-2 and MMP-9 are 66 kDa and 86 kDa respectively, and their primary substrate is type IV collagen, which is part of the basement membrane of the endothelium (Opdenakker *et al*., 2001; Kurzepa *et al*., 2014). Additionally, gelatinases have the ability to bind laminin, aggrecan, elastin and fibronectin, and activate pro-inflammatory cytokines such as IL-8, IL-1β, and TNF-α (Opdenakker *et al*., 2001; Wątroba *et al*., 2019).

COLLAGENASES (MMP-1; -8; -13, -18)

In their structure, collagenases contain the hemopexin domain combined with the catalytic domain and show the ability to degrade entactin, fibronectin, aggrecan and almost all collagen subtypes (Loftus *et al*., 2002).

MATRILYSINS (MMP-7, -26)

Matrilysins are the smallest group among all MMPs and are classified as endometalloproteinases. They do not have a hemopexin domain in their structure and their catalytic activity includes the degradation of fibronectin, fibrinogen, and type IV collagen. In humans, they are a marker of the degree of malignancy of breast and lung cancer, while MMP-7 plays a role in the destabilization of atherosclerotic plaque and its activity positively correlates with the degree of heart failure (Bolon *et al*., 1997; Galis & Khatri, 2002; Lipka *et al*., 2008; Wysocka *et al*., 2014).

STROMELYSINS (MMP-3, -10, -11)

Stromelysins are structurally similar to gelatinases. In addition to the structural constants typical of MMPs, it has a hemopexin-like domain along with a hinge region (van Hove *et al*., 2012). MMP-3 plays the role of apoptotic processes, regulation of gene expression and modelling of the cytoskeleton, and also has the ability to activate other pro-forms of MMPs, such as pro-MMP-1, -3, -7, -8, -9, -13, which accelerate the degradation of extracellular proteins not only by MMP-3 but also by activation of pro-MMP-9 (Cauwe & Opdenakker, 2010). MMP-10 is 85% similar to MMP-3 in terms of the amino acid composition of the catalytic domain and has a similar substrate spectrum. It plays a role in the processes of angiogenesis and regulation of the maturation of the skeletal system (Batra *et al*., 2012). MMP-11 can be activated intracellularly through the furin recognition motif of RX(R/K)R present on its C-terminal fragment and its biological role is not fully understood (Nagase *et al*., 2006; Yang *et al*., 2016).

MEMBRANE TYPE OF MMPS – MT-MMPS (MMP-14, -15, -16, -17, -24, -25)

MT-MMPs include a group of enzymes functionally and structurally related to elements of plasmalemma, some of them belong to type I membrane proteins (MMP-14, MMP-15, MMP-16, MMP-24), while others are glycophosphatidylinositol (GPI) related proteins. Apart from MMP-17, enzymes from this group are necessary in the MMP-2 activation process, while MMP-14 plays an important role in the angiogenesis process (Wysocka *et al*., 2014).

OTHER MMPS (MMP-12, -19, -20, -21, -28)

This group includes enzymes which, due to their structures and functions, cannot be classified into other groups. Enamelysin (MMP-20) plays an important role in the process of tooth enamel embryogenesis and inhibition of its activity with congenital enamel hypoplasia (Lu *et al*., 2008). MMP-19 activity has been found in the synovial vessels in the rheumatoid arthritis (RA), while epilysin (MMP-28), present in keratinocytes, plays a role in the process of hemostasis (Manicone *et al*., 2011; Chang *et al*., 2018).

THE ROLE OF MATRIX METALLOPROTEINASES IN CARCINOGENESIS

MMPs play an important role in many physiological and pathological processes, including neoplastic processes (Kwiatkowski *et al*., 2009). Neoplastic diseases are one of the main causes of death in Poland and in the world, therefore learning about the carcinogenesis process seems to be particularly important. Despite numerous attempts to implement targeted therapy in neoplastic diseases, mortality in about 90% of cancer patients is a result of metastatic disease development and cell resistance to available therapeutic agents (Dofara *et al*., 2020).

The first work dealing with the problem of carcinogenesis and cancer formation is considered to be the one written by Percivall Pott in 1775, assessing the influence of carbon black on the risk of scrotal cancer in chimney sweeps (Peters & Gonzalez, 2018).

The carcinogenesis process is currently widely studied and MMPs play one of the key roles in cancer development. They do this by regulating local tumor growth, stromal invasion, promoting angiogenesis and metastasis formation. There are usually 4 stages in the carcinogenesis process: initiation, promotion, progression, and metastasis. MMPs take part in each of them (Pittayapruek *et al*., 2016; Chojnacki *et al*., 2017). The pathway for cancer cells to metastasize is very complex. In the primary tumor microenvironment, under the influence of numerous factors such as hypoxia, acidosis and with the participation of numerous inflammatory cytokines, well-differentiated epithelial cells transform into undifferentiated mesenchymal cells capable of migration. This process is called the epithelial-mesenchymal transition (EMT) (Valastyan & Weinberg, 2011).

In normal tissues, there is an ongoing interaction between cells and ECM, which takes place through direct cell-cell contact and through numerous chemokines, growth factors, and cytokines that regulate tissue homeostasis. In the neoplastic process, this homeostasis is disturbed at an early stage. Neoplastic cells secrete enzymes, including MMP-1, MMP-2, MMP-9, which have a destructive effect on the ECM and synthesize transforming growth factor type β1 (TGF-β1), fibroblast growth factor type β2 (FGF-β2), interleukin 1 (IL-1), IL-6 that recruit and are involved in the transformation of other stromal cells (Taddey *et al*., 2013).

Cancer-associated fibroblasts (CAFs) play a special role among the cells involved in local tumor progression. When CAFs are active, they can release signalling molecules that stimulate tumor growth. CAFs emit, among others collagen, fibronectin, and MMP-1, MMP-3, MMP-7, MMP-9, MMP-13, which in turn can release other growth factors contained in the ECM, such as vascular endothelial growth factor (VEGF). In addition, CAFs form the pathways by which cancer cells can migrate with CAFs (Bremnes *et al*., 2011).

Tumor-associated macrophages (TAMs) are another important extracellular matrix cells involved in tumor development. In the microenvironment of tumors, they appear in two forms with different functions. TAMs type M1 (TAMs-M1) play an anti-tumor and immunostimulatory role by secreting interferon γ (INF-γ) and interleukin 12 (IL-12), while TAMs type M2 (TAMs-M2) act immunosuppressive and through the secretion of interleukin 10 (IL- 10) enhance angiogenesis and the secretion of MMP-1, MMP-3, and MMP-14, promoting tumor development (Räsänen & Vaheri, 2010).

Cancer cell invasion is a complex process by which MMPs are involved in the degradation of ECM elements and the basal membranes surrounding the primary tumor. Impairment of the adhesive properties between cells and the reorganization of the cytoskeleton promote the migration of cancer cells and infiltration of the stroma. The reorganization of the ECM matrix by MMPs causes the release of their cytokines and chemokines, which then affect cancer cells (Pittayapruek *et al*., 2016).

Numerous immunohistochemical studies have shown that in brain tumors of particularly high malignancy, such as gliomas, a much higher expression of MMPs is found compared to normal tissue. Similar conclusions can be found in the study of laryngeal cancer, which also confirms the relationship between the degree of MMPs expression and the growth rate and invasiveness of the tumor (Grzelczyk *et al*., 2016; Chojnacki *et al*., 2017; Zhou *et al*., 2019).

In breast, kidney, prostate, and colorectal cancers, the correlation between the degree of expression of genes encoding MMPs proteins and the appropriate TIMPs, and some unfavourable prognostic factors, such as the clinical advancement of the tumor, grading, metastasis, relapse-free time, and overall survival, was confirmed (Grzelczyk *et al*. al., 2016).

Gelatinases, including MMP-9, which are involved in the remodelling of the ECM, play a particularly important role in the invasion of neoplastic cells and the development of the primary tumor. Many types of cells are capable of synthesizing and secreting MMP-9, including macrophages, neutrophils, fibroblasts, and endothelial cells. MMP-9 allows the degradation of collagen of basement membranes, including type IV collagen. This destruction is a key element in the invasion and formation of metastasis by cancer cells. Increased expression of MMP-9 generally promotes the development of the neoplastic process, although it may also play a suppressive role, as in the case of colorectal cancer developing in the course of colitis ulcerosa (Huang, 2018).

MMP-2 is secreted mainly by fibroblasts and other fibroblast-like stromal cells. MMP-2, through its proteolytic activity, takes part in the creation of a microenvironment that promotes the proliferation of cancer cells. This includes the elimination of adhesion molecules such as adherins and integrins, and the remodelling of the cytoskeleton, allowing the separation of neoplastic cells from the primary tissue. Of all the adhesion molecules, it is the loss of e-cadherin expression on the surface of cancer cells that appears to be a key component of EMT. E-cadherin as a transmembrane glycoprotein protects intercellular contacts, playing a key role in the adhesion of epithelial cells. It has been observed that the decrease in e-cadherin expression in breast, endometrial, bladder, colon and oesophageal cancer is associated with a more severe course of the disease and a worse prognosis (Sun *et al*., 2018; Gonzalez *et al*., 2019; Song *et al*.,

2019). MMP-3 and MMP-7 directly cleave the e-cadherin domain, releasing an 80 kDa fragment involved in inhibiting cell adhesion and stimulating cell invasion by a paracrine mechanism (Singh *et al*., 2017).

THE ROLE OF MATRIX METALLOPROTEINASES IN CANCER ANGIOGENESIS

Angiogenesis allows the primary tumor to grow. The process involves creating a network of new blood vessels, thanks to which the tumor gains constant access to nutrients and can release harmful waste products. In addition, the emerging new, pathological blood vessels favour the migration of cells into the lumen of the vessels and the formation of distant metastases (Deryugina & Quigley, 2015; Chojnacki *et al*., 2017).

According to the commonly prevailing view on angiogenesis within the primary tumor, new vessels that are formed there are structurally and functionally immature. The tumor vascular network is chaotic, tortuous, abnormal in diameter, abundant with pericyte defects, and is lined by highly permeable and abnormal endothelial cells (Deryugina & Quigley, 2015; Zhou *et al*., 2019).

Changes occurring in the microenvironment of the primary tumor, such as hypoxia, acidosis, anaerobic metabolism, nutrient deficiency, stimulate the secretion of angiogenic factors. The described processes also increase the expression of MMPs, including MMP-9 secreted by tumor cells, TAMs, and neutrophils. MMP-9 in turn causes the release of VEGF found in the ECM and activates FGF-β2 with a strong pro-angiogenic effect (Noë *et al*., 2001).

Many molecular factors directly or indirectly influence the process of angiogenesis induced by neoplastic cells. VEGF plays a key role in this aspect (De Bock- *et al*. 2011). There is a functional link between VEGF and MMPs in the angiogenesis process. Expression of VEGF and MMP-9 can be induced simultaneously at the level of transcription by type 1 hypoxia induction factor (HIF-1) (Pittayapruek *et al*., 2016).

During tumor growth, its demand for oxygen increases, which leads to local tissue hypoxia, and in order to adapt to local conditions neoplastic cells increase the synthesis and secretion of HIF-1 (Pittayapruek *et al*., 2016). Moreover, hypoxia induces the expression of numerous MMPs as found with MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, and MMP-13 (Ardi *et al*., 2009). The activity opposite to tissue hypoxia is performed by secreted protein acidic and rich in cysteine (SPARC), which simultaneously inhibit the regulation of VEGF and MMP-9, which in the medulloblastoma model leads to the development of smaller tumors and a sparse vascular network (Bhoopathi *et al*., 2010).

Similar conclusions were observed in gastric cancer, where it was found that SPARC inhibit the expression of VEGF and MMP-7, while increased VEGF expression is associated with the overexpression of MMP-2 and MMP-9, which at the same time translates into the course of angiogenesis and the formation of gastric cancer metastases (Zhang *et al*., 2012).

While VEGF is involved in regulating the secretion of MMPs, especially MMP-9, some of the MMPs secreted by stromal cells and tumor cells can regulate the secretion of VEGF. This is confirmed by the finding by Kudo *et al*. (2012) that the relationship between MMP-13 of neoplastic origin and VEGF produced by fibroblasts and endothelial cells, which in turn leads to the stimulation of angiogenesis *in vivo*.

Many MMPs are involved in the angiogenesis process. As mentioned above, MMP-9 releases VEGF and FGF-β2 from the ECM. MMP-1 increases the expression of both VEGF and the VEGF receptor (VEGF-R2). MMP-2 and MMP-9 degrade basement membranes and allow migration of endothelial cells. MMP-1 and MMP-8 are involved in the proliferation of endothelial cells during angiogenesis. MMP-2 and MMP-14 shape the lumen of new vessels, and MMP-14 and MMP-9 contribute to the recruitment of pericytes that stabilize the newly formed vessels (Kudo *et al*., 2012; Fields, 2019; Quintero-Fabián *et al*., 2019).

MATRIX METALLOPROTEINASES IN BLADDER CANCER

Despite the dynamic development of diagnostic and therapeutic methods, prognosis of a 5-year survival in patients with bladder cancer is still unsatisfactory. Bladder cancer is the 7th most common cancer in the male population and the 11th most common cancer in the world. The incidence rate (per 100,000 people per year) is 9.0 for men and 2.2 for women. In Europe, the highest incidence rate is in Belgium (31 in men and 6.2 in women) and the lowest in Finland (18.1 in men and 4.3 in women) (Burger *et al*., 2013; Jablonowski 2013; van Osch *et al*., 2016; Cumberbatch *et al*. 2018; Babjuk *et al*., 2019). In Poland, bladder cancer is the 4th most common cancer in men and accounts for 7% of all malignant neoplasms, and in women it accounts for 2% of malignant tumors. The vast majority of cancers are diagnosed in patients over 50, and over 60% are in the elderly population (over 65). The risk of developing bladder cancer increases with age and is almost 3-4 higher in men than in women. According to the National Cancer Registry, the peak incidence in the group of men is at the 9th decade of life, and in women at the 8th decade of life. Bladder cancer causes 5% of deaths in men and 2% in women from neoplastic causes (Wojciechowska & Dzidkowska, accessed on January 18, 2021).

It has been proven that the incidence of bladder cancer depends on many factors. Among them, cigarette smoking occupies a key position, which affects approximately 50% of patients with bladder cancer. There are no conclusive data on e-cigarettes, however, carcinogens are also detectable in urine in people who use this form of cigarettes. The second most frequent risk factor is occupational exposure to aromatic amines and polycyclic hydrocarbons, which mainly affect people working in the oil industry related to paints, varnishes, metal, and fuel processing. Exposure to ionizing radiation also promotes the development of bladder cancer (including patients receiving pelvic radiation therapy). Endemic infections caused by schistosomal flukes causing chronic cystitis are also considered to be the cause of the development of bladder cancer (Burger *et al*., 2013; van Osch *et al*., 2016; Cumberbatch *et al*., 2018; Babjuk *et al*., 2019).

At diagnosis, approximately 75% of patients have disease confined to the mucosa (pTa, CIS) or submucosa (pT1). The percentage of patients with bladder muscle non-infiltrative disease (pTa, CIS, pT1) is even higher in people under 40 (Jablonowski, 2013). Currently, there are several forms of bladder cancer treatment: surgery, chemotherapy, radiotherapy, and immunotherapy. A special part is played by radical cystectomy, which is the standard treatment for cancer infiltrating the bladder muscle. Unfortunately, almost 50% of patients undergoing cystectomy, will develop metastases within 2 years of

Table 1. Matrix metalloproteinases in bladder cancer

MMP	Tested material	Laborathory method	Results	References
MMP-2	tumor tissue	zymography	Higher expression of the active form of MMP-2 in low differentiated and high clinically advanced tumors.	Kanda *et al*., 2000
MMP-9	tumor tissue	ELISA	Higher expression of MMP-9 in low differentiated tumors.	Wu *et al*., 2018
MMP-28	tumor tissue	immunohistochemical methods	Higher expression in high clinically advanced tumors. High expression correlates with shorter survival times.	Wang *et al*., 2020
MMP-2, MMP-9, TIMP-1 TIMP-2	urine serum	ELISA	Higher expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 in the study group.	Ricci *et al*., 2015
MMP-7	urine	Western-blot	High expression of MMP-7 in the group of patients with metastases.	Szarvas *et al*., 2011
MMP-3 MMP-9	urine	ELISA	High expression in the test group. MMP-3 is highly expressed early in the disease.	El-Sharkawi *et al*., 2014
MMP-14 MMP-15	tumor tissue	ELISA Western-blot	Higher expression of MMP-14 and MMP-15 in the study group.	Kudelski *et al*., 2020

surgery and will die of the underlying disease (Anghel *et al*. 2016).

There are many studies on MMPs in bladder cancer (Table 1). Most studies have been devoted to MMP-2 and MMP-9, i.e. enzymes responsible for the degradation of type IV collagen in the first place, which, through the destruction of basement membranes and ECM, play an essential role in the process of tumor invasion (Kanda *et al*., 2000; Opdenakker *et al*., 2001; Kurzepa *et al*., 2014; Wu *et al*., 2018).

In a study by Kanda and others (Kanda *et al*., 2000) the activity of MMP-2 using zymography in bladder tumor tissue collected from 61 patients was assessed. The correlations between the amount of active and total MMP-2 form, and the degree of histological differentiation (G1, G2, G3), and the clinical advancement of the tumor according to TNM were examined (Table 2). Expression of the active form of MMP-2 was higher in the group of G3 tumors than in G2 and G1 tumors, while no significant difference was found between G2 and G1 tumors (Table 3). Mean expression of the active form of MMP-2 also increased with clinical advancement. Significantly higher expression of the active form of MMP-2 was found in the bladder muscle invasive disease (> pT1) than in non-invasive tumors (pTa, pT1, CIS). Similar conclusions were observed by Wu *et al*. (2018) in relation to MMP-9 in tumour tissue. MMP-9 activity was tested by immunohistochemistry and ELISA. Tumour tissue was used in the research group, while in the control group the normal bladder mucosa was at least 3 cm away from the tumour. MMP-9 expression was found in 57.6% of patients in the research group, compared to 6.3% in the control group. It was confirmed that the expression of MMP-9 was statistically higher in G3 tumors (poorly differentiated) than in G1 and G2. There were no differences between G1 and G2 tumors. Age and gender had no effect on MMP-9 expression. The positive correlation between the degree of MMP-9 expression and the degree of histological differentiation of the tumor was emphasized, therefore MMP-9 can be used as a potential marker of malignancy of bladder tumors.

Table 2. The TNM classification of bladder cancer (Colombel *et al*., 2008 modified)

T – primary tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Noninvasive papillary carcinoma
Tis	Carcinoma in situ
T1	Tumor invades subepithelial connective tissue (lamina propria)
T2	Tumor invades muscle (muscularis propria) T2a tumor invades superficial muscle (inner half) T2b tumor invades deep muscle (outer half)
T3	Tumor invades perivesical tissue T3a microscopic T3b macroscopic (extravesical mass)
T4	Invasion of adjacent structures T4a tumor invades prostate, uterus, vagina T4b tumor invades pelvic or abdominal wall
N – lymph nodes	
Nx	Regional nodes cannot be assessed
N0	No regional lymph node disease
N1	Metastasis in single node 2 cm or less
N2	Metastasis in single or multiple nodes between 2 – 5 cm
N3	Metastasis in lymph node greater than 5 cm
M – metastasis	
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

In a study by Wang *et al*. (2020) they assessed the activity of MMP-28 in biopsy samples from bladder cancer patients and compared it to normal bladder mucosa.

Table 3. The WHO histological grading of bladder cancer (Pasin *et al*., 2008 modified)

Grade	Microscopic characteristics
G1	the cancer cells look very similar to normal bladder cells, they are usually slow-growing and are less likely to spread
G2	the cancer cells look less like normal cells and are slightly faster grow
G3	the cancer cells look very different to normal cells and usually grow more quickly

MMP activity was assessed in tumour tissue by immunohistochemical methods. Significantly increased expression of MMP-28 was found in patients with multiple tumors, deep infiltration (pT2-pT4), large tumour diameter, lymph node metastases and distant metastases. Moreover, patients with elevated MMP-28 expression had a significantly shorter (35.65±1.47 months) survival time than patients with low MMP-28 expression (5636±125 months). MMP-28, called epilysin, belongs to the subfamily MMP-19. MMP-28 plays an important role in the development of some neoplasms, and its expression has been confirmed in colon, pancreatic, ovarian, prostate and lung cancer cells (Marchenko & Strongin, 2001).

In addition to examining the expression of MMPs directly in the tumour tissue, the activity of MMPs in urine and serum was assessed in patients with bladder cancer. In a study by Ricci and others (Ricci *et al*., 2015), MMP-2, MMP-9, TIMP-1, TIMP-2 in urine and blood serum were determined. The study used a morning urine sample and blood serum from patients before treatment. Commercial ELISA tests were used to measure MMPs and TIMPs. There was no presence of MMP-2, MMP-9, TIMP -1, TIMP-2 in urine and blood samples in patients from the control group. In the group of bladder cancer patients, increased MMP-2 activity was observed in 63% (26 out of 41 patients) and MMP-9 in 61% (25 out of 41 patients). The mean concentration in urine was 1.27 ng/ml and 1.30 ng/ml for MMP-2 and MMP-9, respectively. Compared to MMPs, the presence of TIMP-1 and TIMP-2 was found in a higher percentage of patients (95% and 83%, respectively). The concentration of TIMP-1 in urine was significantly higher in the group of G3 tumors than in G1 tumors. Moreover, a higher concentration of TIMP-1 was observed in the group of T1 tumors than in Ta tumors. In contrast to TIMP-1, the concentration of TIMP-2 in urine did not show a significant difference between low- and well-differentiated tumors and did not depend on the clinical stage.

Durkan and others (Durkan *et al*., 2001) found higher TIMPs expression in urine in the group of bladder cancer patients than in the control group, with higher concentrations in the group of patients with infiltrating bladder cancer than in the group of non-invasive tumors (Ta, T1, CIS). There were no differences depending on the degree of histological differentiation of the tumour. Monier *et al*. (2002) came to different conclusions, finding lower concentrations of TIMP-1 in the group of patients with T1-T4 bladder cancer than in patients with Ta grade bladder cancer.

In the analysis of MMP-3 and MMP-9 concentrations in urine by El-Sharkawi and others (El-Sharkawi *et al*., 2014) found significantly higher concentrations of MMPs in the group of bladder cancer patients compared to the control group of healthy patients. However, MMP-3 showed high concentrations in the earlier stages of tumour development (T1 and T2), which remained high in the more advanced forms (T3 and T4), while MMP-9 was highly expressed in advanced bladder tumors (T3 and T4). It was emphasized that MMP-3 may be used in the future as a marker of early forms of bladder cancer.

In the analysis of MMP-7 concentration in urine in patients with bladder cancer by Szarvas and others (Szarvas *et al*., 2011), no difference in the concentrations of the tested MMP was found between the group of patients with cancer confined to the bladder and the control group of healthy patients, while in the group of patients with distant metastases, the concentration of MMP-7 was 4 times higher than in the control group.

Kudelski and others (Kudelski *et al*., 2020) analyzed the activity of membrane metalloproteinases (MMP-14 and MMP-15) and the concentration of TIMP-1 in the tissue of bladder tumors and in healthy bladder tissue. For this purpose, ELISA tests and the Western-blot method were used. The MMP-14 activity in the control group was 7.35 mg/kg of protein. Both low-grade and high-grade tumors showed increased activity of MMP-14, while in low-grade tumors the activity of the tested MMP was about 35% higher, and in high-grade tumors almost 10 times higher than in the control group. The activity of MMP-15 was approximately 3 times higher than that of MMP-14 in the control group. Low-grade tumors were characterized by increased MMP-15 activity, while in the high-grade neoplasms, MMP-15 activity was 4 mg/kg lower than in the control tissue. The lowest concentration of TIMP-1 was found in healthy bladder tissue. The TIMP-1 concentration was the highest in the low-grade neoplasm group (almost 75% higher than in the control group), while in the high-grade group the TIMP-1 concentration decreased significantly but was still higher than in the control group. The authors emphasize the role of MMP-14 as a risk factor for the development of metastatic disease.

DISCUSSION

In 28 countries of the European Union, more than 120,000 people are diagnosed with bladder cancer every year, and over 40,000 people die from this cancer every year. In the EU, EUR 4.9 billion was spent in 2012 on the treatment of bladder cancer, which accounted for 5% of all cancer-related health care expenditure (Leal *et al*., 2016).

Numerous studies confirm the relationship between the expression of MMPs and the development of malignant tumors. MMPs take part in the angiogenesis process, participate in tumour invasion, local infiltration, and the formation of distant metastases. Hence, they become a potential target for drug action. Blocking the expression or activity of MMPs may be a future treatment strategy for cancer patients. An example would be an anti-MMP-14 monoclonal antibody that prevents pro-MMP-2 activation or an antibody that binds to the hemopexin domain of MMP-2 (Yang *et al*., 2016).

Chemically modified tetracyclines that have lost their antimicrobial activity are inhibitors of MMPs by binding calcium and zinc. Chemically modified doxycycline is now approved by the Food and Drug Administration

(FDA) as an inhibitor of MMP-7 and MMP-8, which are involved in the pathogenesis of periodontal disease (Li *et al*., 2013). Metastat blocks the activity of MMP-1, MMP-2, MMP3, MMP-7, MMP-9 and MMP-12 and is used to treat Kaposi's sarcoma developing in patients with acquired immune deficiency syndrome (AIDS) (Dezube *et al*., 2006).

Some substances have the additional function of reducing the enzymatic activity of MMPs. Among them, mention may be made of bisphosphonates, which are used to prevent bone resorption. Zolendronic acid, used in the treatment of patients with prostate cancer with bone metastases, has the ability to block the activity of MMP-2, MMP-9, MMP-12, MMP-14, and MMP-15 (Li *et al*., 2012).

Since bladder cancer is characterized by the ability to recur, from the point of view of clinical practice, it seems particularly important to develop a marker of early bladder tumor recurrence. MMPs detected in the urine of bladder cancer patients are potential factors that could perform this role. In the works of numerous authors, elevated concentrations of MMPs in the urine in the course of neoplastic disease are found (Szarvas *et al*., 2011; El-Sharkawi *et al*. 2014; Ricci *et al*. 2015; Fouad *et al*., 2019).

In summary, MMPs play an important role in the development of neoplastic disease, including bladder cancer. Increased expression of MMPs is associated with invasion and metastasis. MMPs can be used as prognostic factors in bladder cancer. The potential treatment of tumors by targeting TIMPs and MMPs, and the use of MMPs as markers of early relapse, requires further research.

Conflict of interests

The authors have no potential conflicts of interest to declare

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