REVIEW ARTICLE

Extracellular matrix metalloproteinases in pathophysiology, diagnostics and treatment of renal cell carcinoma – current state of knowledge and future perspectives

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▮ Abstract
Introduction and Objective. Despite a significant improvement in the diagnosis and treatment of renal cell carcinoma over the past two decades, this cancer still remains one of the most lethal urological neoplasms.
Review Methods. Databases were searched using the keywords: 'RCC', 'MMP', 'TIMP', 'signaling pathway', and 'pathophysiological'. The titles of these entries were analyzed to assess complementarity, obtaining 721 entries. Relevance was then assessed by analyzing the abstracts, and 387 entries were selected. Based on criteria such as number of citations, research methods, sample size and representativeness, 248 references were finally selected. Finally, 181 items were included in the literature.
Brief description of the state of knowledge. Extracellular matrix metalloproteinases plays an important role in the remodeling of the extracellular matrix. They participate in the initiation and regulation of inflammatory and carcinogenic processes.
Summary. The analysis of available literature shows that, from a biochemical point of view, the most important influence on the development of renal cell carcinoma and the formation of metastases is the imbalance between the activity of metalloproteinases and the concentration of their tissue inhibitors. Hope for the future lies in monoclonal antibodies used to selectively block individual metalloproteinases, which may be of particula importance in highly vascularized tumours, which undoubtedly include renal cell carcinoma. Additionally, assessment of metalloproteinases activity could assist in selecting patients for surgical treatment or active surveillance.

▮ Key words
RCC, MMP, TIMP, signal pathway, pathophysiology

INTRODUCTION AND OBJECTIVE

Despite a significant improvement in the diagnosis and treatment of renal cell carcinoma (RCC) over the past two decades, this cancer still remains one of the most lethal urological neoplasms [1]. Currently, >50% of renal tumours are detected incidentally, usually during imaging diagnostics performed for non-specific symptoms or for other abdominal diseases. Many renal tumours remain asymptomatic in the early stages of the disease [2]. Although many advances in diagnosis have been made, still about 20% – 30% of patients are diagnosed at the metastatic stage of the disease. Less than 10% of patients have the classic triad of symptoms, i.e.,

low back pain, palpable tumour upon physical examination, and haematuria, which indicate an advanced disease and a poor prognosis [3]. About 20% of patients with renal tumours develop paraneoplastic syndromes, which include erythropoietin-induced polycythaemia, Cushing's syndrome, hypercalcaemia and hypertension, due to increased renin secretion [4].

REVIEW METHODS

The aim of this review is to summarize the role of matrix metalloproteinases in the pathophysiology, diagnosis and treatment of renal cell carcinoma. Google Scholar was searched using keywords such as 'RCC', 'MMP', 'TIMP', 'signal pathway', and 'pathophysiology' since 2004, obtaining 3,420 entries. The titles of these entries were then analyzed to assess complementarity, obtaining 721 entries. Next,

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relevance was assessed by analyzing the abstracts of these entries and 387 entries were selected, paying attention to the variety of methodological and topical approaches, which included both review and original articles. Based on criteria such as the number of citations, research methods, size and representativeness of samples, 248 references were finally selected. Of these papers, finally 181 items were included in the literature, following the editorial guidelines. The literature selection flowchart is shown in Figure 1.
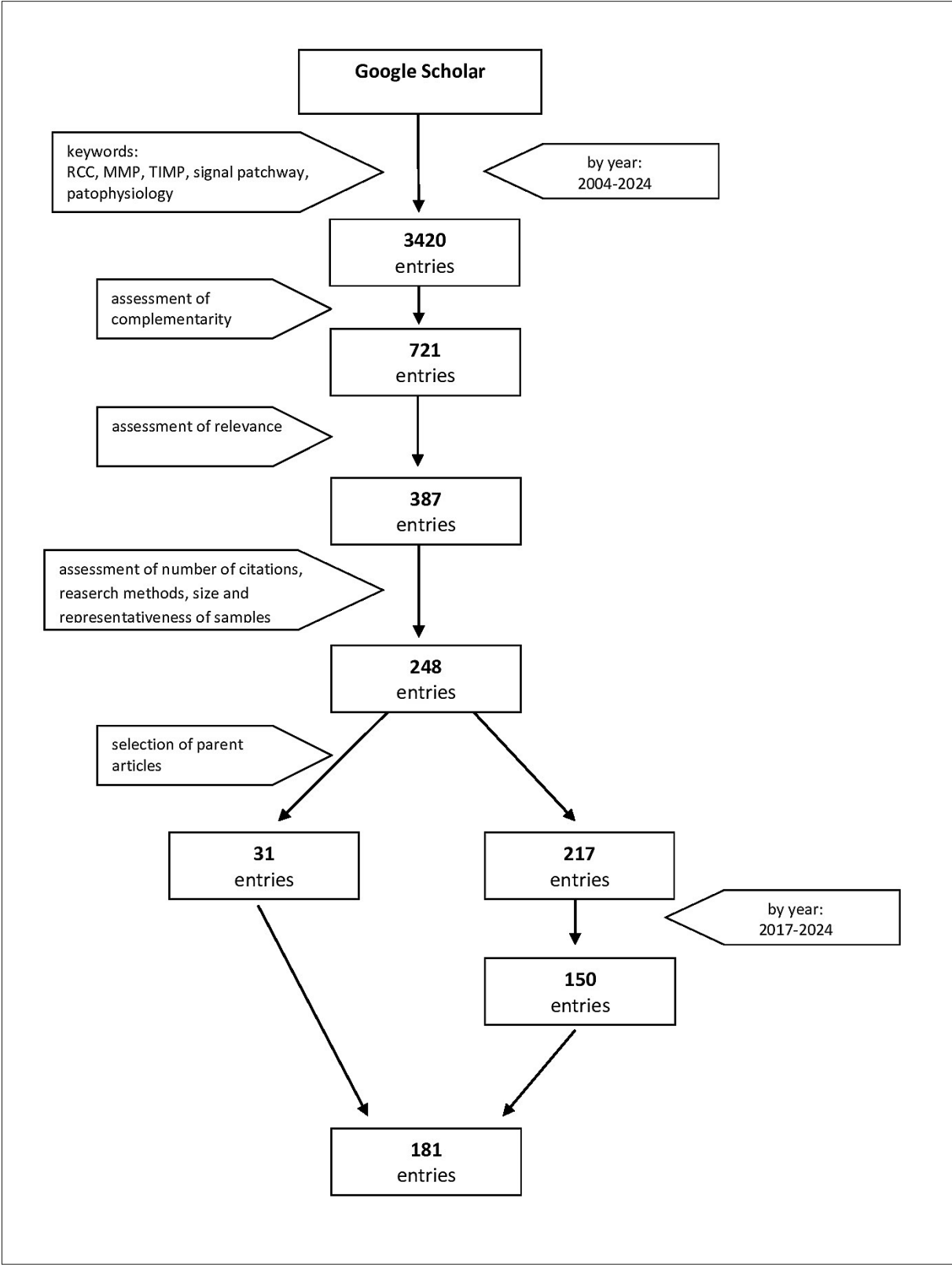

Figure 1. Literature selection flowchart

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DESCRIPTION OF THE STATE OF KNOWLEDGE

RCC is the most common solid lesion detected in the kidneys, comprising 90% of all renal cancers. Other subtypes of renal cell carcinoma, such as medullary carcinoma, Bellini duct carcinoma, and mucinous-epithelial carcinoma, are rare tumours, collectively accounting for less than 5% of all renal tumours, and will not be discussed in this study [5]. RCC includes several subtypes with specific genetic and histopathological characteristics [6]. The most common subtype of RCC is clear cell carcinoma (ccRCC), followed by papillary carcinoma (pRCC), whilst the least commonly diagnosed is the chromophobe subtype (chRCC) [1]. Signalling and inflammatory pathways, such as the rapamycin (mTOR), Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, have a special role in the molecular patomechanism of RCC carcinogenesis and progression, mutations in the von Hippel-Lindau gene, and cytokines, as well as extracellular matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [7]. MMPs are regarded as markers of many diseases, therefore, the assessment of their activity plays an important role not only in diagnosis but also in monitoring treatment, because many of these proteinases are over-expressed in many conditions [8]. MMP-9 is a potential biomarker for such neoplasms as breast, cervical, colorectal, ovarian, and pancreatic cancers, as well as osteosarcoma, non-small cell lung cancer (NSCLC) and giant cell tumour of bone (GCTB), which makes MMP-9 a good candidate for the diagnosis of these conditions, even at an early stage [9, 10, 11, 12, 13]. MMP-7 and MMP-12, in turn, are over-expressed in type 2 diabetes with microangiopathy and macroangiopathy [14, 15, 16].

MATRIX METALLOPROTEINASES – GENERAL CHARACTERISTICS

Structure. MMPs belong to the multi-domain zinc-dependent endopeptidases which function in the neutral pH environment and have a diverse substrate spectrum but similar structural features [17, 18]. The zinc-dependent active site of MMPs is highly conserved and contains three histidine residues bound to catalytic zinc; this site includes two regions, containing a cavity on the protein surface where the zinc ion is located, and also a specific S1′ hinge region [19, 20, 21]. All the MMPs have the same structure of the catalytic domain, which contains three α-helices and five β-harmonics, including four parallel (β2-β1-β3-β5) and one antiparallel (β4), connected by eight loops [22, 23, 24]. The catalytic domain of the enzyme is highly conserved and contains, apart from the catalytic zinc ion, another zinc ion with structural function, three calcium ions and histidine residues, the first of which is adjacent to a glutamic acid (GA) molecule, which is essential for the catalytic process [22, 25].

Between helix α2 and helix α3 there is the Ω-loop, the length of which and amino acid composition vary among individual MMPs, accounting for their diverse selectivity and determining their substrate spectrum [26, 27]. The S1′ hinge region is located in the terminal region of the catalytic domain and forms the outer wall of the "met-turn" [26, 28]. Another structural element of MMPs is the haemopexin residue, which, however, is not present in all MMPs [29]. It has four β-propeller structural elements and is essential for
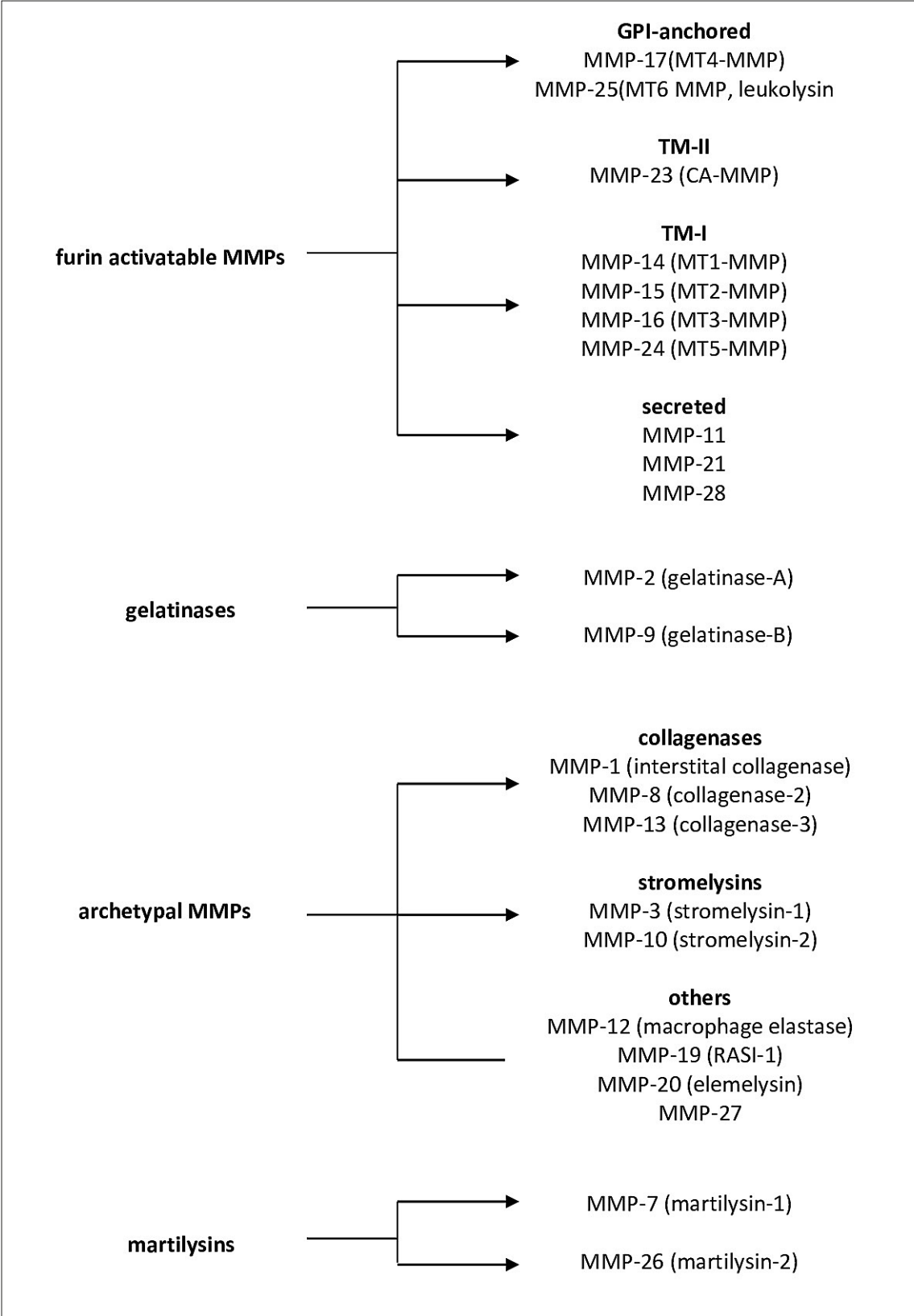
the degradation of the collagen triple helix; it also determines substrate specificity [29, 30]. In some MMPs, such as MMP-23, the haemopexin domain is replaced by an immunoglobulin-like domain, and a cysteine residue-rich domain located behind the C-terminus of the catalytic domain [31].

The catalytic and haemopexin-like domains are linked by a linker containing proline residues, which has diverse lengths and accounts for the flexibility of the domains, stabilizes the enzyme, and hydrolyses some structurally complex substrates [21, 32]. The substrate, as well as structural diversity, allowed to distinguish different groups of MMPs (Fig. 2), comprising such MMPs collagenases as (MMP-1, MMP-8, MMP-13, MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11), matrilysins (MMP-7, MMP-26), membrane-type-MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, MMP-25) and unclassified MMPs (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23), which have been discussed in detail by the authors in previous reviews [33, 34, 35, 36, 37].

Regulation of biological activity. The regulation of biological activity of MMPs is analogous to the regulation of activity of other proteolytic enzymes. This activity depends on gene transcription, proenzyme activation and the action of natural TIMPs, which are endogenous protein regulators and have already been extensively discussed in previous publications by the authors of this article [33, 34, 35, 36]. TIMPs are peptides composed of 184–194 amino acids with a molecular weight of approx 21 kDa, and have a similar, but slightly different affinity for MMPs [37]. In the extracellular matrix (ECM), TIMP-1, TIMP-2 and TIMP-4 are found in a soluble form, whereas TIMP-3 is insoluble and correlative with the ECM [38, 39]. The mechanism of MMPs activity inhibition by TIMPs consists in an reversible blockade of the enzyme by producing stoichiometric complexes with it in a 1:1 ratio [40].

In their structure, all TIMPs have an N-terminal amine domain and a C-terminal carboxyl domain [39, 41]. The ability of TIMP to inhibit the activity of MMPs is the effect of the interactions within their N-terminal domain. The C-terminal domain makes it possible for TIMPs to interact with a haemopexin-like domain present in the structure of some MMPs [42]. TIMPs selectively inhibit MMPs and endopeptidases, such as disintegrins and metalloproteinases, with thrombospondin motifs (ADAMTs) [43, 44]. Apart from TIMPs, a number of biological factors also have the ability to regulate the expression of MMPs; these are interleukin 1β (IL-1β), tumour necrosis factor (TNF), interleukin 6 (IL-6), interleukin 8 (IL-8, CXCL-8), as well as lectins and the extracellular inducer of MMPs (EMMPRIN) [45, 46, 47, 48]. MMP-9 expression is reduced by interleukin-4 (IL-4), interleukin-10 (IL-10), interferon-β (IFN-β), glucocorticoids (GCs) and retinoids [49, 50, 51, 52].

The synthesis of MMP-3 increases in the presence of bacterial lipopolysaccharide (LPS), and decreases as a result of the activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) type α (IκBα), thus showing an expression type similar to MMP-1 [53, 54]. MMP-9 expression occurs in leukocytes, fibroblasts, keratinocytes, vascular endothelial cells, microglia cells and dendritic cells, and also in tumour cells, which is indicative of their inducible expression character [55, 56]. MMP-2 expression is present in smooth muscle and microglia cells, adipocytes, astrocytes, macrophages and vascular endothelial cells, and

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Figure 2. Structural division of MMPs. MT-MMPs – membrane type of matrix metalloproteinases. GPI – glycosylphosphatidylinositol (37, modified)

manifests the nature of constitutive expression [56, 57]. MMP-3 is synthesized in neutrophils, vascular endothelial cells, astrocytes, and neurons, undergoing constitutive or inducible expression depending on the cell type [53].

Activation process. MMPs are secreted into the extracellular space in the form of inactive proenzymes containing a propeptide [37, 58]. The activation of gelatinases can take place in two stages (Fig. 3). The first stage occurs as a 'cysteine switch' and is reversible, and does not involve permanent

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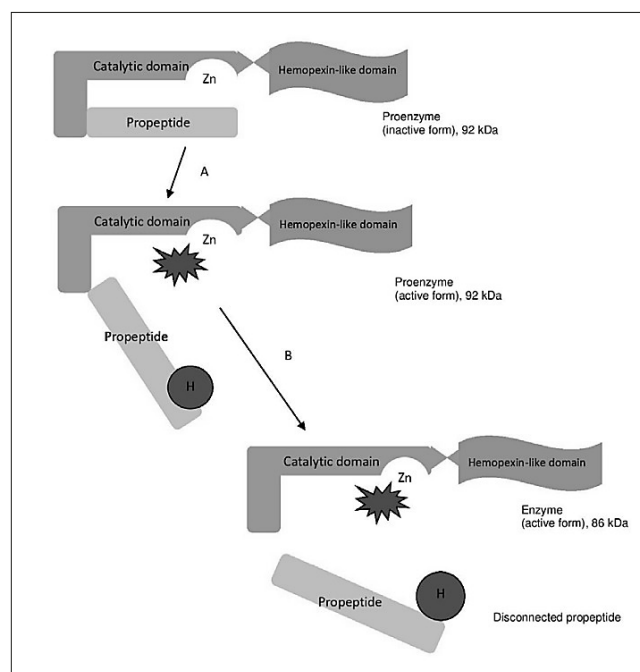
Figure 3. Structure and activation of the MMP proenzyme (using MMP-9 as an example).

The domains present in gelatinases are: propeptide, catalytic domain with a zinc atom (Zn), hinge region and domain with a structure similar to hemopexin. A – non-proteolytic activation, using e.g. mercury compounds or some denaturing compounds. During this process, the propeptide is not detached (no change in mass in relation to the proenzyme). In the case of removal of the activating factor, the propeptide reconnects to the active center, preventing catalysis (so-called 'cysteine switch'). B – proteolytic activation, associated with the detachment of the propeptide. An irreversible process associated with a decrease in the mass of the enzyme by the mass of the propeptide (34).

disconnection of the propeptide [37, 59]. This process also occurs *in vivo,* causing proenzyme-equivalent forms of MMPs to exhibit their catalytic activity [60]. The second step of the activation of MMPs is catalysed by proteolytic enzymes; this is an irreversible process of propeptide disconnection resulting in the reduction of the enzyme's molecular weight by the mass of the propeptide [61]. The activation of pro-MMP-9 occurs *in vitro* in the presence of MMP-2, MMP-3, MMP-10, MMP-13, as well as cathepsin G (CG), α-chymotrypsin (CT-α) and trypsin (TR) [62, 63].

Biological functions. MMPs play an important role in ECM remodelling and have the ability to digest all the proteins and glycoproteins that make up the ECM; they are also involved in both the physiological and pathological processes of its modelling [18, 64, 65]. MMPs take part in the initiation and regulation of inflammatory and carcinogenic processes; they also play a significant role in embryogenesis and organ maturation by regulating cell differentiation, proliferation and apoptosis, and regulating angiogenesis and morphogenesis, which leads to tissue remodelling [34, 65]. In the pathway of ECM degradation, MMPs also play a biological role in signal transduction between cells, transcriptional regulation and control, and immune processes [66, 67]. Apart from their ability to digest ECM proteins and glycoproteins, MMPs also take part in the proteolytic degradation of growth factors, cytokines and membrane receptors [68, 69, 70, 71]. Moreover, there are studies that indicate the intracellular localisation of some MMPs, such as MMP-2. The intracellular activity of MMPs may affect DNA replication processes by means

poly-ADP-ribose (PAR) degradation [30, 72]. MMPs are recognized as markers of many diseases while assessment of their activity plays an important role not only in diagnosis, but also in the monitoring of treatment, because many MMPs are over-expressed in numerous diseases [8].

RENAL CELL CARCINOMA – GENERAL CHARACTERISTICS

Epidemiology. RCC is the most common type of renal cell carcinoma, accounting for about 90% of all cases [73]. Each year, more than 400,000 new cases of RCC and more than 170,000 deaths from RCC are reported worldwide [74]. The incidence of RCC continues to increase, with an estimated 79,000 new cases and 13,920 deaths from RCC in 2022 in the United States alone [75]. The highest incidence of RCC is found in North America (10.9 cases/100,000 population), Western Europe (9.7/100,000) and Australia/New Zealand (9.6/100,000) [76]. In Europe and worldwide, the Czech Republic and Lithuania remain the countries with the highest incidence. Some European countries, such as Croatia, Estonia, Greece and Ireland, continue to note an increasing mortality from RCC [1]. In Poland, RCC accounts for 3% of all cancers diagnosed among adult patients. Epidemiological data indicate that the disease most often develops between the ages of 50–70, and contributes to about 3% and 2% of deaths in male and female subpopulations, respectively [77].

Risk factors. Risk factors for RCC include mainly lifestyle factors, such as obesity, smoking, and hypertension. Diabetes also appears to have an adverse effect on the development of RCC [78]. An extensive retrospective analysis has confirmed that current and former smokers have a 1.6-fold and 1.5-fold increased relative risk of developing RCC, respectively. This risk increases with the growth of the number of pack-years [79]. There is also a distinctive link between obesity and the risk of developing RCC. A study by the European Prospective Investigation into Cancer and Nutrition (EPIC) found that high body mass index (BMI) is associated with a 2.25-fold increased risk of developing RCC, particularly ccRCC and chRCC, while pRCC does not seem to be associated with obesity [80]. Hypertension is associated with a 2-fold higher risk of developing RCC among white Americans and 2.8-fold in African- Americans. It has also been confirmed that systolic blood pressure (SBP) >160 mmHg and diastolic blood pressure (DBP) >100 mmHg are associated with a more than 2-fold higher risk of developing RCC [80]. Primary interventions for RCC prevention include regular physical activity, weight reduction and smoking cessation [81].

Histological differentiation, biology and clinical advancement assessment. RCC comprises several subtypes with specific genetic and histopathological characteristics [82]. As early as 1982, Fhurman proposed an assessment system for histological malignancy of the tumour, based on the size, shape and prominence of cell nuclei (Tab. 1). Fhurman's classification is related to the ability to form metastases and to patient survival, but is not applicable to ccRCC, which accounts for about 75% of the diagnosed cases of RCC.

RCC is a neoplasm that develops from the epithelium of the proximal tubule of the nephron and usually metastasizes

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Table 1. Characteristics of types of renal cell carcinoma (82, 91, 92)

Histological type	Type of epithelium	Clinical features	Most common mutations	General prognosis
Clear cell (75%)	Epithelium of the proximal tubule of the nephron	Fast growth, golden-yellow, well-defined, usually without a capsule	Mutation in the VHL gene (approx. 70% of cases), hypermethylation	Poor prognosis, rapid growth, high metastatic potential
Papillary (10%)	Epithelium of the proximal tubule of the nephron	Slow tumour growth, yellow-brown, well demarcated with a pseudocapsule present	Type I – mutation in the MET gene, amplification or activating mutations. Type II – mutations in the SETD2, CDKN2A or TFE genes	Type I – low malignant potential, is more common and has a better prognosis than type II
Chromophobic (5%)	Epithelium of the distal tubule of the nephron	Well defined, usually without a capsule, light brown	Loss of the entire chromosome, most often: 1, 2, 6, 10, 13, 17, 21.	Prognosis is better than in ccRCC and pRCC

via the vascular route, most commonly to the lungs, liver and bone [83]. Nearly one-half of the ccRCC cases involve detecting an inactivating mutation or deletion in the von Hippen Lindau (VHL) protein suppressor gene, located on the short arm of the 3p25 chromosome. The loss of the VHL protein function contributes to the initiation, progression and acquisition of the ability to form tumour metastases. In a germline mutation in the VHL protein gene, tumours are more often diagnosed at a young age, and bilaterally [84]. The pRCC accounts for about 10% of diagnosed RCC cases and has a better prognosis than ccRCC. Traditionally, pRCC is divided into two subtypes, which differ in biological and clinical terms. In pRCC subtype I, a mutation is found in the gene encoding the Met-receptor tyrosine kinase protein [6]. The Met-receptor and its ligand, the hepatocyte growth factor (HGF), are over-expressed in subtype I of pRCC. The Met-receptor is activated by autophosphorylation through binding to HGF, leading to the activation of downstream signalling pathways, such as PI3K/AKT/mTOR, RAS/MAPK/RAF/ERK and the phospholipase C (PLC) pathway, which are involved in tumour growth and invasion [85]. In pRCC subtype II, there are alterations which are associated with a dysfunction in the activation mechanism of the nuclear factor erythroid-2-related factor 2/antioxidant response element (NRF2-ARE) pathway, whose function involves protecting cells from oxidative stress, defined as an imbalance between pro-oxidant and anti-oxidant systems [6, 86]. The anti-oxidant system plays a fundamental role in cellular defence by eliminating oxidants and producing diverse antioxidant enzymes, such as heme oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase-1 (NQO1) [86].

The expression of these antioxidant enzymes is strongly regulated by the NRF2-ARE signalling pathway, activated via the G120 protein-coupled receptor (GPR120) [87]. Anti-inflammatory factors, released by macrophages under the influence of reactive oxygen species (ROS), through GPR120, induce phosphorylation of extracellular signal-regulated kinase (ERK), leading to NRF2-ARE signalling [88] activation. The main transcription factor NRF2 translocates to the nucleus and binds to ARE-containing promoter regions [86]. Subtype II pRCC comprises a strongly heterogeneous group of tumours that may further sub-stratify in future. Subtype I is more common than subtype II and is regarded to have a better prognosis [89], whereas chRCC is the least frequently diagnosed type of RCC, accounting for about 5% of cases [90].
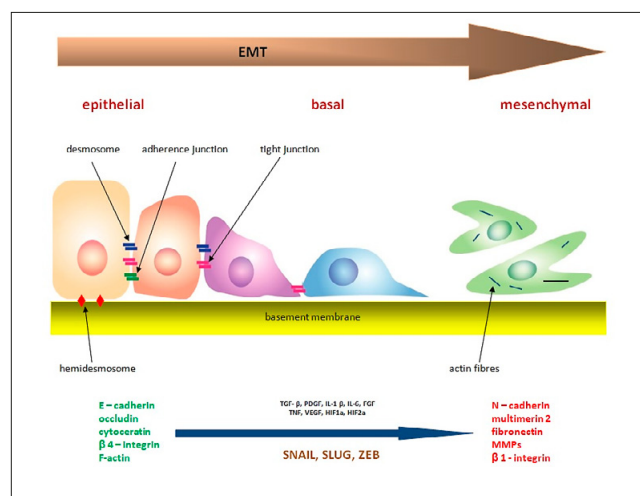
Typical genetic alterations found in chRCC comprise the loss of one copy in chromosomes 1, 2, 6, 10, 13, 17, 21 and the sex chromosome, which is found in about 71% of chRCC cases. Additional genetic alterations involve mutations in the TP53, PTEN and HNF1B genes [91]. chRCC develops from collecting tubule insert cells which play a role in the acid-base regulation. Among all types of RCC, chRCC has the best prognosis [6]. It should be emphasized here that RCC can also undergo transformation to the sarcomatoid form (sRCC), which is associated with a particularly poor prognosis and a 5-year survival rate of 15 – 22% [92].

The tumour, lymph node, metastasis, i.e. TNM classification system is currently recommended for both clinical and scientific application, and its prognostic value has been confirmed in clinical trials. Tumour size, vascular infiltration, infiltration of the renal capsule and adrenal glands, lymph node metastasis and distant organ metastasis, are essential elements of the TNM classification [93]. As for clinical practice, the TNM classification alone cannot be taken as the primary decision-making criterion. The factors of fundamental importance for the choice of the most optimal onco-urological treatment comprise the patient's general condition and preferences, comorbidities, and the experience of a given treatment centre [94].

RENAL CELL CARCINOMA – ROLE OF MATRIX METALLOPROTEINASES

Pathophysiological significance. ECM degradation depends on the cooperation of many enzymes, with MMPs playing a key role in this aspect. MMPs, acting by means of the degradation of ECM elements, damage tissue barriers and enable the invasion of tumour cells, and this process is closely related to local tumour growth, angiogenesis, lymphogenesis, and the acquisition of the ability to form distant metastases [95]. The process of cancer metastasis formation is a complex cascade which starts with the cancer cell acquiring the ability to separate from the primary tumour [96]. Primary tumours are usually solid tumours histologically composed of tumour cells, but also containing in their structure non-malignant cells, such as fibroblasts and macrophages. Primary tumours are surrounded by a highly vascularized cellular stroma, in which inflammation-like reactions occur, promoting ECM remodelling, tumour cell migration and, as a result the formation of metastases [97]. In the micro-environment of the primary tumour, under the influence of factors such as pro-inflammatory cytokines, hypoxia and acidosis, a process known as mesenchymal-epithelial transition (EMT) occurs, involving the transformation of well-differentiated epithelial cells into undifferentiated mesenchymal cells which possess the ability to invade tissues and lymphatic and blood vessels (Figure 4). While the pathogenesis of individual tumours is specific and varies between cancers, the pathway which tumour cells take to form metastasis is similar in all cancers and is strongly dependent on MMPs [33, 97, 98].

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Figure 4. Epithelial-mesenchymal transition (EMT). The epithelial cell loses polarity and intercellular connections, and gains the ability to migrate and be invasive, becoming a mesenchymal cell.
FGF – fibroblast growth factor; HIF1a – hypoxia-inducible factor type 1a; HIF2a – hypoxia-inducible factor type 2a; Il-1β – interleukin 1β; IL-6 – interleukin 6; PDGF – platelet-derived growth factor; TGF-β – transforming growth factor β; VEGF – vascular endothelial growth factor (97, modified).

Not all tumour cells have the same ability to form metastases, due to the heterogeneity of tumour cells. Within the tumour, there are cells with different capacities for self-regeneration, differentiation, resistance to apoptosis, promotion of lymphogenesis and angiogenesis, and sensitivity to chemotherapy and radiotherapy. The ability to form metastases and tumour aggressiveness [99] are also associated with this biological diversity. Tumour cells with high metastatic potential have the ability to form cytoplasmic protrusions, which are called invadiopodia and are composed of F-actin and surrounded by proteins and adhesion molecules [100]. The invadiopodia found in cancer cells are the counterparts of the podosomes located in normal cells. While ivanopodia are involved in tumour invasion, podosomes take part in physiological processes, such as embryonic development, wound healing, inflammatory responses and skeletal remodelling [101]. Ivanopodia are formed in three consecutive stages, i.e. initiation, stabilization and maturation, whilst Src family kinases, activated by epithelial growth factor (EGF), transforming growth factor β (TGF-β) or platelet-derived growth factor (PDGF), play a key role in this process, resulting in the formation of plasma membrane protrusions [102]. Ivanopodia are highly active structures, have a longer half-life than podosomes, and are larger in size, which is a feature that allows them to penetrate deeper into the ECM and degrade it to a greater extent [103]. MMP-14, located in the invadopodia, takes part in ECM degradation and makes it possible for cancer cells to migrate into the blood vessel lumen. It has been confirmed that MMP-14 is involved in the formation of invadopodia, which translates into cell movement and their ability to migrate and invade [104]. In addition to MMP-14, MMP-2 and MMP-9 are also found in mature invadopodia. Ivanopodia survival time, secretion and distribution of MMPs are mainly controlled by Rho GTPases (Rho-GTPa) [105].

The interaction between the cell and ECM is disrupted at an early stage of the tumour process. Tumour cells produce, among others, MMP-1, MMP-2, MMP-9, which degrade ECM elements and basement membranes. In addition, the

tumour cells release a number of growth factors, such as fibroblast growth factor (FGF), TGF-β, PDGF, IL-1β and IL-6, which promote the transformation of cells such as fibroblasts and macrophages [106]. Cancer-associated fibroblasts (CAFs) play an important role because they participate in the process of ECM remodelling when they are activated [107]. They also release enzymes, including MMP-1, MMP-3, MMP-7, MMP-9 and MMP-13, as well as vascular endothelial growth factor (VEGF), which is why they form pathways along which they migrate with tumour cells. In addition, by forming fibronectin fibres, they are responsible for targeted the migration of tumour cells [108]. Increased fibronectin production by primary tumour cells facilitates the migration of cells within the tumour micro-environment [109].

Cancer-associated macrophages (TAMs) exist in the tumour micro-environment in two distinct forms: TAM-M1, which secrete interferon-γ (IFN-γ), and interleukin-12 (IL-12), which has anti-tumour and immunostimulatory effects. TAM-M2 release IL-10, which has immunosuppressive effects, and stimulate the release of MMP-1, MMP-3 and MMP-14, promoting angiogenesis and tumour growth [33]. Due to local hypoxia, tumour cells have to undergo a series of adaptive changes, such as switching from aerobic to anaerobic glycolysis, which, in turn, leads to lactic acid production and local acidosis. Low pH promotes the selection of cells resistant to these local conditions. In response, the cells produce angiogenic factors, including MMPs, thus promoting neo-agniogenesis [110].

Such a relationship has been confirmed in many cancers with regards to MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10 and MMP-13 [111]. The effect of hypoxia is compounded by a hypoxia-inducible factor (HIF). The mutation in the VHL protein gene leads to the accumulation of HIF-2a and HIF-1a, while HIF-1a stimulates the synthesis of MMP-1, MMP-2, MMP-3 in the tumour micro-environment. The VHL-HIF pathway is associated with the over-expression of pro-angiogenic factors, in particular VEGF, which stimulates the development of new blood vessels. The activation of the JAK2 – STAT3 – VEGF signalling pathway plays a significant role in the pro-angiogenic effects of VEGF [112]. The JAK-2 – STAT-3 pathway is an intracellular signal transduction network through which many growth factors and cytokines transmit signals from the cell membrane to the cell nucleus, inducing gene transcription [113]. JAK-2 protein has seven domains, including two domains located in the C-terminal region, comprising a kinase domain (JH1), which plays a role in STAT phosphorylation, and also a pseudokinase domain (JH2), which has no catalytic activity, but modulates the JH1 domain and the domains homologous to Src-2 kinase (JH3–4), which interact with phosphorylated protein tyrosine residues, and FERM domains (JH5–7) located in the N-terminal region and responsible for binding to receptors and regulating catalytic activity [114, 115]. The STAT3 protein was originally described as a factor of acute phase response (APRF). The gene encoding STAT3 contains 24 exons and is located on chromosome 17 (17q21.2) [116]. STAT3 consists of six domains, which include an amino end, a helical domain, a DNA-binding domain, a linker domain, a domain homologous to Src-2 kinase (SH2), and a trans-activation domain at the carboxyl end [117, 118].

STAT proteins have different functional domains, in which the N-terminal domain mediates binding to multiple DNA sites, with DNA binding specificity conferred by

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400–500 residues of the SH2 domain, which are involved in dimerization; while the C-terminal domain plays a role in activation [119]. Four isoforms of STAT3 have been identified: designated as α, β, γ and δ. STAT3γ, with a molecular weight of 72 kDa, and STAT3δ, with a molecular weight of 64 kDa, are produced by proteolytic processing and are believed to play an important role in the regulation of granulocyte development [118, 120]. STAT3α and STAT3β are produced by alternative folding of exon 23 and have different biological functions, and additionally contain a tyrosine phosphorylation activation site, Y705 [121, 122].

The activation of STAT3 by tyrosine phosphorylation leads to the formation of homodimers or heterodimers through the SH2 domain, and induces nuclear translocation to regulate gene transcription [122]. The process of STAT activation is physiologically regulated by the mechanisms which include negative feedback via cytokine-induced SH2 proteins and suppressor cytokine signalling proteins (SOCS), blocking STAT binding to DNA in the nucleus, which is mediated by protein-activated STAT inhibitor (PIAS), and STAT inactivation by tyrosine phosphatases, which include T-cell protein tyrosine phosphatase TC45 (TC-PTP) [123, 124].

Signal transduction mediated by STAT3 is negatively regulated by a type II low molecular weight phosphatase with dual specificity (LMW-DSP2), resulting in a reduction in STAT3 nuclear translocation [119, 125]. Both constitutive and abnormal STAT3 activation promote the process of carcinogenesis [119]. The JAK2 – STAT-3 pathway is activated upon the binding of activating factors to the extracellular catalytic domain of FERM of JAK-2, responsible for binding to receptors [126]. This interaction results in the autophosphorylation of JAK-2 within tyrosine residues, which activates the kinase domain. The kinase domain, in turn, enables the phosphorylation of tyrosine residues on intracellular domains of the receptor to form docking sites for SH2 domain-containing proteins [126, 127]. This enables cytoplasmic STAT3 proteins to bind to phosphorylated tyrosine residues on the receptor using their SH2 domains. In the next step, the N-terminal tyrosine residues of STAT-3 are phosphorylated, resulting in the formation of STAT-3 dimers, dissociation from the receptor and translocation to the cell nucleus, where integration into the corresponding DNA sequences and induction of VEGF gene transcription takes place [113, 128].

Because of the transcription of the VEGF genes, STAT-3 is considered an important oncogenic transcription factor, firstly, because it induces VEGF transcription and plays a key role in controlling cellular activity and angiogenesis, and secondly, because by inhibiting gene expression through DNA methylation of the tumour suppressor gene promoter and its silencing, STAT-3 promotes tumour growth [129, 130, 131]. Thanks to a broader understanding of the role of VEGF, in recent years there has been a shift in treatment strategies of metastatic ccRCC. Checkpoint inhibitors (ICIs) are now in use together with anti-angiogenic drugs, because VEGF inactivation and VEGF receptor (VEGFR) blockade affect tumour vascularization and immune cell and cytokine activity [132].

In the above-described EMT process, epithelial cells transform into less differentiated mesenchymal cells, which weaken, and consequently, lose their apical-basal polarity and cell-cell junctions, thus gaining the ability to move and invade [133]. A key component of EMT is the loss of E-cadherin expression on the surface of the tumour cells. E-cadherin is a trans-membrane glycoprotein made up of a large extracellular domain which forms intercellular junctions, and of an intracellular domain taking part in transmitting information to the cell nucleus. Given the above, E-cadherin is a component of signalling pathways, involved in maintaining normal tissue architecture and intercellular adhesion [134]. During EMT, the 'cadherin switch' takes place; this is a phenomenon involving the loss of E-cadherin and increased expression of mesenchymal N-cadherin, leading, in turn, to the loss of intercellular connections and enabling cell spreading. Moreover, the loss of E-cadherin leads to the mis-localization of catenin β (β-ct) and catenin p120 (p120-ct), enable activation of mitogen-activated protein kinase (MAPK) pathways [135]. Moreover, signalling pathways, such as Wnt and TGF-β activate SNAIL and SLUG-dependent pathways, which inhibit the expression of E-cadherin and stimulate the expression of N-cadherin, which stimulates cell proliferation [84, 136]. SLUG is a transcription factor belonging to the zinc finger family, containing in its structure, a C-terminal domain and an N-terminal domain (SNAG), and is over-expressed in cancer cells, thereby enhancing migration, invasion, metastasis, cell cycle progression and resistance to apoptosis, by inducing repression of CDH1 transcription through binding to E-box sequences present in its promoter region [137, 138].

In addition, SLUG has an inhibitory effect on the synthesis of claudin-1 (CLDN1), which is a membrane protein regulating the barrier properties of intercellular tight junctions and consisting of four transmembrane domains, two extracellular loops and cytoplasmic tails, which is significant for the loss of cell-cell connections and thus promotes cancer cell invasion [137, 139]. Increased expression of miRNA clusters involved in EMT induction increases SLUG expression [137]. The miR-96/183 cluster promotes the expression of key EMT inducers, such as ZEB1, ZEB2, SNAIL2, MMP-2 and MMP-9, whereas the miR-183/182 cluster promotes EMT by means of inducing the expression of mesenchymal genes and also of SNAIL and SLUG [35, 138]. SLUG uses the SNAG domain to repress target gene expression by recruiting histone deacetylases, mSin3A, Suv39H1, LSD1, Ring1A/B and Ajuba/Prmt5/14–3–3, which are involved in histone modifications through acetylation, methylation and ubiquitination, which is associated with downregulation of CDH1 expression, and thus induction of EMT and progression of carcinogenesis [140].

SNAIL is a transcription factor that promotes cell migration and is strongly expressed in many cancers, especially those with metastasis. Like SLUG, through its N-terminal SNAG domain, it increases its affinity for the CDH1 promoter region through interactions with co-repressors and repressors, the process which plays a role in EMT [137]. Also in SNAIL, the expression level of pro-tumour miRNA clusters affects EMT. The miR-106b/25 cluster, through interactions with the F-box and β-TRCP2 domains, increases tumour cell invasion by stimulating SNAIL expression [141]. The stabilization of SNAIL through interaction of the miR- 81b-3p cluster with the γ-3-monooxygenase tyrosine/5-monooxygenase tryptophan activating protein (Tr-3-M/Tp-5-M) acts as an EMT promoter in breast cancer [142].

Smad-2, Smad-3, Smad-4 pathways, through induction of miRNA-181b expression with subsequent induction of SNAIL expression through interaction with TIMP-3 molecules, promote EMT and metastasis [143, 144]. Apart from the

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expression levels of pro-tumor miRNA clusters, the induction of SNAIL expression also occurs through the activation of RTK, TGF-β, Notch, Wnt, bone morphogenetic protein 2 (BMP-2), and the afore-mentioned Smad-2, Smad-3, Smad-4 signalling pathways [137, 145]. Additionally, cells undergoing EMT acquire the capacity for increased synthesis of MMPs. The main MMPs associated with EMT are MMP-1, MMP-2, MMP-3, MMP-7, MMP-14 and MMP-28. MMP-3 and MMP-7 which are involved in the demarcation of the extracellular domain of E-cadherin, as a result of which the cells lose their adhesion capacity and gain the ability to invade. [136]. A study of oral squamous cell carcinoma cells revealed an increased expression of mesenchymal markers, such as fibronectin and vimentin, and a decreased expression of the epithelial markers E-cadherin and cytokeratin [146].

Anoikis is a type of apoptosis, i.e. programmed cell death which takes place when cells lose intercellular connections or contact with the ECM. The goal of anoikis is to prevent cells from re-attaching to other tissues and to inhibit their dysplastic growth. Tumour cells after EMT must develop an adaptive mechanism involving their resistance to anoikis, with MMPs playing a role in this process [147]. One of the components of anoikis resistance is the above-described 'cadherin switch' which occurs during ETM. The loss of E-cadherin stimulates the Twist pathway, resulting in the synthesis of B-cell lymphoma protein-2 (Bcl-2), which exhibits anti-apoptotic activity [148]. Cells also acquire resistance to anoikis by means of other mechanisms, which include the activation of intrinsic anti-apoptotic pathways, changes in integrin molecules or increased expression of receptors for growth factors [149]. The role of MMPs in the process of anoikis resistance is not well understood. MMPs most likely have both pro-apoptotic and anti-apoptotic roles. MMP-7 has the ability to cut Fas ligand (Fas-L) from the cell surface, thus preventing the activation of the extrinsic apoptotic pathway. MMP-11 may play a similar role in breast cancer cells [110].

The anti-apoptotic activity of MMPs also consists of their ability to disengage tumour-associated major histocompatibility complex (MHC-1) proteins. In addition, TIMP-1 also plays an anti-apoptotic role. TIMP-1 binds to the CD63 receptor (CD63-R) and reacts with the β-1 subunit of integrins. As a result, the TIMP-1/CD63/β-1 complex activates signals leading to cell survival through activation of adhesion kinases, such as focal adhesion kinase (FAK), phosphoinositide 3-kinase (PI3-K) and ERK [150]. Angiogenesis is the ability to form new blood vessels from the existing vascular system. It is a well-established feature of the tumorigenesis process that is essential for primary tumour growth and metastasis formation [151]. Changes in the micro-environment of the primary tumour, such as hypoxia, local acidosis and low nutrient availability, lead to stimulation of the synthesis of proangiogenic molecules. An imbalance between pro-angiogenic and anti-angiogenic factors is crucial for the process of angiogenesis. MMPs are involved in the regulation of angiogenesis as well as in the process of lymphogenesis. Angiogenesis results in nutrient delivery and consequent tumour growth [152]. In addition, MMPs, by degrading ECM, release numerous angiogenesis-stimulating factors, such as VEGF, FGF and TNF [153].

MMPs begin the process of angiogenesis with degrading type IV collagen found in basement membranes. The main role in this process is played by MMP-9, which releases angiogenic factors such as VEGF and FGF contained in the ECM [154]. VEGF is considered to be the most potent factor promoting angiogenesis. MMP-9, released from tumour-associated neutrophils, activates basic fibroblast growth factor 2 (bFGF-2), and participates in the degradation of multimerin 2 (MNRN2) found on the surface of endothelial cells; consequently, this process, promotes cell migration and angiogenesis [155]. In addition, the expression of MMP-1, MMP-2 and MMP-14 is increased in tumour vascular endothelial cells. Through activation of the NF-κB-related signalling pathway, MMP-1 promotes the expression of VEGFR-2, which translates into increased VEGFA binding and stimulation of vascular endothelial cell proliferation [156]. Cyclooxygenase type 2 (COX-2) and MMP-2 are associated with the development of abnormal, dilated, and tortuous blood vessels in the tissues of highly aggressive tumours, with their synthesis occurring in tumour cells in a VEGF-independent manner [157]. MMP-7 stimulates angiogenesis through proteolytic degradation of soluble VEGFR-1, which translates into an increase in endothelial cell proliferation. In addition to their pro-angiogenic effects, MMPs can also have an anti-angiogenic effect by degrading type VIII collagen, producing elastin and releasing urokinase-type plasminogen activator receptors (uPARs). The mechanism of action of key MMPs in EMT and carcinogenesis is summarized in Table 2. The points of MMPs action in carcinogenesis and formation of metastasis are shown in Figure 5.
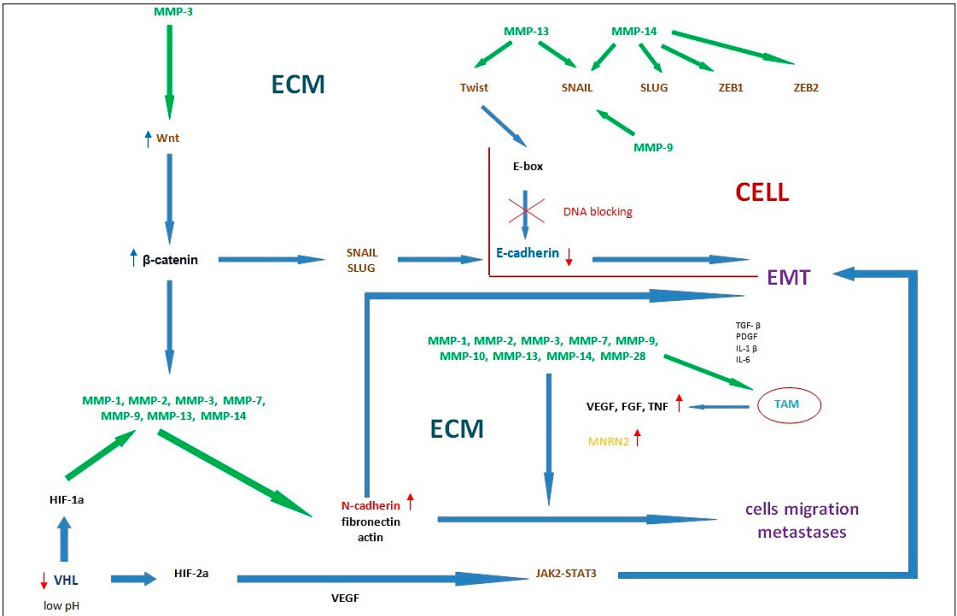
Studies show that all TIMPs exhibit anti-angiogenic activity. TIMP-1 is most likely responsible for regulating angiogenesis. TIMP-2 exhibits anti-angiogenic activity through downregulation of MAPK activity involved in endothelial cell migration and proliferation, which, as a result, leads to an inhibition of angiogenesis and tumour growth [158]. A summary of studies on the role of MMPs are presented in Table 3.

Clinical significance. Although progress in diagnosis and surgical treatment have improved the survival of patients with RCC, this cancer still accounts for many deaths. Therefore, numerous attempts are being made to identify new biomarkers which can help detect patients at high risk for local recurrence and progression to metastatic disease [159]. N. E. Kushlinskii et al. in their study (2020) assessed the activity of MMP-2, MMP-7, MMP-8 and MMP-9, as well as TIMP-1, in patients with RCC. Ninety-four patients (55 men and 39 women) aged 29 -81 years were included in the study. The control group consisted of healthy patients aged 18 – 77 years (60 women and 37 men). The activity of MMPs was assessed with the ELISA method in the patients' blood serum collected before specific treatment [160]. The authors of the study emphasize that an increased expression of MMPs was observed in various malignant processes, which makes these enzymes an important diagnostic and prognostic element in cancers like ovarian cancer, colorectal cancer, and gastric cancer [161].

The authors of the present study confirmed an increased activity of MMP-7, MMP-8 and increased levels of TIMP-1 in the serum of patients with RCC, while the activity of MMP-2 and MMP-9 was lower than in the control group. These differences were not statistically significant. Patients were followed up from 1 – 45 months (mean follow-up period was 26 months), and high MMP-7 and MMP-8 activity was shown to be associated with an unfavourable prognosis in all the

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Table 2. Mechanism of action of key MMPs in EMT and carcinogenesis - abbreviations are explained in the text (104, 105, 108, 110, 111, 134, 135, 136, 153, 154, 155, 156)

MMP	Mechanism of action
MMP-1	• participation in the formation of fibronectin fibers • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-2	• participation in the formation of ivanopodia • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-3	• participation in the formation of fibronectin fibers • demarcation of the extracellular domain of E-cadherin, as a result of which cells lose their ability to adhere and gain the ability to invade • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-7	• participation in the formation of fibronectin fibers • demarcation of the extracellular domain of E-cadherin, as a result of which cells lose their ability to adhere and gain the ability to invade, leads to the mislocalization of β-ct and p120-ct and enables the activation of MAPK pathways • demarcation of Fas ligand from the cell surface, which prevents the activation of the extrinsic apoptosis pathway • proteolytic degradation of soluble VEGFR-1, which translates into increased endothelial cell proliferation • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-9	• participation in the formation of ivanopodia • participation in the formation of fibronectin fibers • release of angiogenic factors contained in the ECM, such as VEGF and FGF activation of bFGF-2 and participation in the degradation of MNRN2, which in turn promotes cell migration and angiogenesis • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-10	• release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-11	• demarcation of Fas ligand from the cell surface, which prevents activation of the extrinsic apoptosis pathway • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • degradation of ECM, which creates pathways along which cancer cells migrate
MMP-13	• participation in the formation of fibronectin fibers • degradation of ECM, which creates pathways along which cancer cells migrate • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF
MMP-14	• participation in the formation of ivanopodia • decomposition of ECM, which creates pathways along which cancer cells migrate • activation of the signaling pathway associated with NF-κB, MMP-1, promotion of VEGFR-2 expression, which translates into increased binding of VEGFA and stimulation of vascular endothelial cell proliferation • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF • intensification of cancer cell migration into the lumen of blood vessels
MMP-28	• decomposition of ECM, which creates pathways along which cancer cells migrate • release of numerous factors stimulating angiogenesis, such as VEGF, FGF and TNF

Figure 5. Points of MMPs action in carcinogenesis and formation of metastasis.
ECM – extracellular matrix; FGF – fibroblast growth factor; HIF1a – hypoxia-inducible factor 1a; HIF2a – HIF1a – hypoxia-inducible factor 2a; Il-1β – interleukin 1β; IL-6 – interleukin 6; MNRN2 – multimerin 2; PDGF – platelet-derived growth factor; TAM – cancer-associated macrophag; TGF-β – transforming growth factor β; TNF – tumour necrosis factor; VEGF – vascular endothelial growth factor; VHL – von Hippel-Landau protein (102, 105, 106, 108, 110, 112, 134, 136, 138)

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Table 3. The most important studies on the role of MMPs in carcinogenesis and clinic of RCC (154, 155, 157, 160, 161, 162, 164, 166, 167, 168, 169, 170)

Author	Tested compounds	Type of tested material and testing technique	Conclusions
Ahn et al. 2008	MMP-9	Bone marrow-derived myelomonocytic cells. Assessment methods – immunostaining, immunofluorescent staining.	In a model system of transplanting tumours into irradiated normal tissue, it was shown that the tumours were unable to grow in the presence of MMP-9 gene knockout, but tumour growth could be restored by transplantation of wild-type bone marrow. MMP-9 may be an important target for adjuvant therapy to enhance tumour response to radiotherapy.
Cho et al.2003	MMP-1, MMP-2, MMP-9, MMP-11, MMP-14, MMP-16	Renal tumour tissue (ccRCC, chRCC, pRCC). Assessment of MMPs expression using cDNA arrays and zymography.	MMP-2, MMP-9, MMP-11, MMP-14, and MMP-16 were upregulated in ccRCC compared with chRCC, whereas MMP-1, MMP-11, and MMP-16 were prominent in pRCC. MMP-9 transcript levels were strongly associated with MMP-9 enzymatic activity, and thus with disease-free and metastasis-free survival. MMP-9 gelatinolytic activity in zymography was strongly associated with MMP-9 mRNA expression, which was more intense in ccRCC than in chRCC), regardless of disease stage. MMP-9 is a significant prognostic predictor in multivariate survival analysis. MMP2 enzymatic activity disappeared, despite consistent transcript expression in RCC.
Chrabańska et al. 2023	MMP-2, MMP-9, CD-44, Ki-67	Renal tumour tissue (non-cc-RCC). Assessment methods – immuno-histochemistry.	Increased CD-44 expression was associated with a significantly higher risk of death from RCC. Increased MMP-2 expression slightly reduced the risk of death. Increased MMP-9 expression worsened overall survival.
Eiro et al. 2018	MMP-2, MMP-9, MMP-11	Human breast cancer cell lines MCF-7 and MDA-MB-231 before and after co-culture with CAFs. Assessment methods - qRT-PCR.	The invasive and angiogenic capacities of MDA-MB-231 cells, respectively, were increased after co-culture with CAFs, especially those from MMP-11 (+) tumours
Gupta et al. 2007	MMP-1, MMP-2	Human breast cancer cell MDA-MB-231 and its lung metastatic derivative LM2-4175 Assessment methods – pRetroSuper technology, bioluminescence. immunohistochemical analysis	MMP-1 and MMP-2 expression stimulates angiogenesis, release of tumour cells into the circulation and creating metastases
Kudelski et al. 2022	MMP-3, MMP-10	Renal tumour tissue. Assessment of MMP activity by ELISA.	High activity of MMP-3 and -10 in tumour tissues was confirmed. MMP-3 activity significantly higher in tumours of higher malignancy.
Kushlinskii et al. 2020	MMP-2, -7, -8, 9 and TIMP-1	Serum. Assessment of MMPs activity by ELISA	Increased activity of MMP-7, -8 and TIMP-1 in serum of patients. High MMP-7 and MMP-8 activity associated with unfavourable prognosis in all stages of renal cancer.
Mehdi et al. 2016	MMP-2, MMP-9, CD-44 , Ki-67	Renal tumour tissue (chRCC, p RCC). Assessment methods - immunohistochemistry.	In the pRCC group, Ki-67 and CD-44 were not associated with overall survival, while patients with low MMP-2 expression had shorter overall survival. Patients with low MMP-9 expression showed longer overall survival than patients with high MMP-9 expression.
Miyata et al. 2007	MMP-10	Renal tumour tissue. Assessment methods - immunohistochemistry.	The relationship between MMP-10 activity and tumour size was confirmed. MMP-10 is an independent risk factor for higher grade pT.
Młynarczyk et al. 2019	MMP-1, MMP-13	Renaltumour tissue. Assessment methods – ELISA and Western blot.	Both collagenases are present in normal and cancerous tissue. A decrease in the amount of MMP-1 and MMP-13 is observed as the tumour progresses, but at the same time their activity increases.

stages of RCC. MMP-7 appears to be an important marker, as in all stages of RCC at values lower than the threshold (<6.3 ng/ml), three-year survival was 93% and dropped to 51% at MMP-7 activities exceeding the above threshold value. A similar correlation was observed for MMP-8, where the threshold value was 51 ng/ml. Then, the three-year survival rate was 78% at values lower than 51 ng/ml and dropped to 58% when the threshold value was exceeded. Also, worse overall survival was found in the group of patients with high TIMP-1 levels and low MMP-2 activity, while MMP-9 activity almost did not correlate with the overall survival of RCC patients. MMP 7 thus has a prognostic role in the group of patients with RCC at clinical stage I (cT1N0M0). Overall survival at three-year follow-up was 100% at levels below the threshold (<6.3 ng/ml), and dropped to 72% once these values were exceeded.

In summary, the authors emphasize that although MMP-7 and MMP-8 activities are elevated in many tumour groups and cannot be used as RCC-specific markers, their increased activity can be used to predict survival, monitor response to treatment and diagnose RCC recurrence [160]. MMP-2 and MMP-9 appear to be the most studied of all MMPs in patients with RCC. Their association with the degree of malignancy, overall survival, propensity for invasion and worse prognosis, has been confirmed [162].

Similar results to those for MMP-2 and MMP-9 were also observed for MMP-1, MMP-3, MMP-7, MMP-11, MMP-12 and MMP-14 in RCC cells. MMP-10, or stromelysin-2, which contributes to the degradation of ECM elements, such as proteoglycans, laminin, fibronectin, and type III and type IV collagen, has also been studied in RCC patients [163]. The study by Yasuyoshi Miyata et al. 2007 on the analysis of MMP-10 activity in patients with RCC, used tumour samples from 103 patients diagnosed with RCC (80 men and 23 women, age range – 39–82 years) who underwent surgical treatment. MMP-10 activity was assessed with immunohistochemistry methods and the results were correlated with clinicopathological features of the tumour, such as size, proliferation index, blood vessel density and overall survival. Patient follow-up time ranged from 20 – 66 months (mean duration – 43 months). MMP-10 activity was found mainly in the cytoplasm of RCC cells in 45 patients (43.7%), and thus defined as MMP-10-positive. The relationship between MMP-10 activity and tumour size

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was confirmed, and MMP-10 expression was considered an independent high-level risk factor (pT).

A similar relationship was observed with respect to histological malignancy grade. The five-year survival rate was statistically lower in MMP-10-positive patients. The authors highlight the role of MMP-10 in RCC cell aggressiveness and suggest that MMP-10 is a potential therapeutic target [164]. MMP-3 and MMP-10 are involved in the activation of other MMPs, and play an important role in normal renal function, and in the process of tumorigenesis; they also perform a significant function in angiogenesis and metastasis. ECM homeostasis plays an important role in the physiological functioning of the kidney, and the enzymes responsible for this are mainly MMPs. Kudelski et al. 2022 evaluated MMP-3 levels and MMP-10 activity in patients with RCC. As mentioned earlier, MMP-3 degrades many proteins, acting also as an activator of MMP-7, MMP-8 and MMP-13. MMP-3 has a higher proteolytic potential than MMP-10 [165].

In order to evaluate MMP-3 levels and MMP-10 activity, renal tumour tissue taken from 20 patients (14 men and 6 women) who had undergone radical nephrectomy was examined. A control group was created from healthy tissue of the contralateral kidney. None of the patients was diagnosed with acute or chronic kidney disease before treatment. MMP-3 DNA content and MMP-10 activity were measured with ELISA and Western-Blot methods. An increased MMP-3 concentration and increased MMP-10 activity were confirmed in tumour tissues in comparison with the control tissue. MMP-3 concentration was statistically higher than MMP-10 activity in the studied material. In addition, the concentration of MMP-3 was significantly higher in tumours with a higher malignancy grade of G3 relative to G2. The data presented here indicate increased ECM degradation in more advanced renal tumours.

The authors of the paper point out that knowledge of the role of MMP-3 and MMP-10 is crucial in understanding the pathophysiology of RCC microenvironment, and can be used in the diagnosis and prognosis of the course of the disease [166]. The activity of collagenases, more specifically MMP-1 and MMP-13 in RCC was studied by Mlynarczyk et al. (2019). The aim of the study was to assess the collagen content and activity of MMP-1 and MMP-13) in RCC tissue. A hydroxyproline assay was used to assess the amount of collagen, while the activity of MMPs was evaluated with the Western-Blot and ELISA methods. It was shown that the collagen content of the tumour tissue decreased with the increasing tumour stage. Both MMP-1 and MMP-13 were expressed in both normal and tumour tissue. In contrast, a systematic decrease in MMP-1 and MMP-13 expression was observed, together with the progressing tumour stage, but at the same time, an increase in the biological of these enzymes activity was also observed. This could be explained by the fact that in healthy tissue they remain in an inactive form and become activated as the tumour progresses [167].

The lack of precise prognostic parameters in RCC results in the fact that the effects of treatment of this cancer vary considerably. The classic prognostic factors can be distinguished into: anatomical (tumour size, infiltration of adjacent structures, lymph node involvement and distant metastasis) and histological (RCC subtype, grading, presence of sarcomatoid transformation, vascular invasion, presence of necrosis) [84]. However, these markers are not sufficient

to predict the clinical course of renal cell carcinoma, hence, there is a need for research into new factors.

MMPs and cancer stem cells play a key role in the process of carcinogenesis, hence Chrabanska et al. (2023), attempted to evaluate the role of CD-44, MMP-2 and MMP-9 as prognostic markers in a study in which 302 patients with RCC – 243 with ccRCC and 59 non-ccRCC (41 pRCC and 18 chRCC) were included. All the patients underwent either radical or partial nephrectomy, and tissue specimens were evaluated according to current ISUP and WHO guidelines [168]. All specimens were evaluated by immunohistochemistry for CD-44, MMP-2 and MMP-9 expression. During a mean follow-up of 48.1 months, there were 79 deaths of patients with ccRCC and 6 pRCC or chRCC. Patients with increased CD-44 expression in RCC had a significantly higher mortality risk than RCC patients with low CD-44 expression. An increased MMP-2 expression slightly reduced the risk of death, whereas the degree of MMP-9 expression has no effect on overall survival of RCC patients. A strong factor affecting overall survival is the histologic subtype of RCC-patients with ccRCC have a higher risk of death than patients with pRCC and chRCC [169].

The vast majority of research concerns the role of MMPs in the pathogenesis, treatment and prognosis of RCC concentrates on ccRCC, i.e. its most common subtype. The literature on pRCC and chRCC is very limited. The objective of one article published in 2022 was to evaluate the expression of MMP-2, MMP-9, CD-44 and Ki-67 specifically in pRCC and chRCC. Ki-67 is a non-histone protein, absent in the cells which do not undergo cell division, making it an excellent marker of tumour cell proliferation [170]. In the above study, the samples from 41 patients with pRCC and 18 patients with chRCC were examined. All the samples were collected from patients during partial or radical nephrectomy, and then evaluated according to current ISUP and WHO guidelines [168]. Immunohistochemical methods were then used to evaluate the expression of MMP-2, MMP-9 and CD-44, Ki-67, and the staining results were evaluated by two independent pathologists. In the patients with pRCC, Ki-67 and CD-44 were not associated with overall survival, but the patients with low MMP-2 expression had shorter overall survival. The patients with low MMP-9 expression manifested longer overall survival than the patients with high MMP-9 expression. No deaths were observed in the pRCC group during the two-year follow-up period, therefore statistical analysis could not be performed [143]. Summary of studies on the role of MMPs are presented in Table 3.

Potential importance in diagnostics and treatment and future perspectives. At the beginning of the 20th century, Eugen von Hippel and Arvid Lindau described a syndrome consisting of retinal haemangioma, cerebellar embryonal haemangioma and spinal cord haemangioma. In 1936, the abbreviation 'VHL', from the names of the discoverers, was first used to describe the disease of hereditary hypervascular tumours [112]. The VHL-HIF-VEGF-VEGFR pathway is a known therapeutic target in metastatic RCC, and anti-angiogenic drugs targeting VEGFR have been the foundation of ccRCC therapy in recent decades. In the past, the core of systemic therapy used to be interferon-α (INF- α) and interleukin 2 (IL-2), but the results of this treatment were unsatisfactory [171]. In 1993, the VHL gene was discovered, and then thanks to the advances in research and

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understanding of the role of HIF and VEGF, a breakthrough occurred in treatment involving the use of tyrosine kinase inhibitors, such as sorafenib, sunitinib and pozapanib, which target VEGFR [172].

A new era in the treatment of metastatic RCC came with the use of the combination of ICI and tyrosine kinase inhibitors (TKIs). Current treatment regimens are more effective because blocking VEGF-A/VEGFR-2 plays an important role in altering immunity, affecting tumour vascularization, cytokine levels, and conditioning the influx of immune cells [171]. VHL belongs to the group of tumour suppressor genes, and takes part in the regulation of cell division, cell death, and response to cellular stress. The VHL gene is located on the short arm of chromosome 3 (3p25). Almost 90% of patients with sporadic ccRCC have a confirmed loss of at least one copy of chromosome p3. Analyses of samples from different areas of renal tumours confirm that the VHL mutation and loss of one copy of 3p were present in all the examined regions and samples; whereas other mutations, such as MTOR, PTEN, SETD2 and KDM5C, showed heterogeneity within the tumour [173].

Studies of the ccRCC cell lines confirmed the relationship between the loss of VHL and ove-rexpression of VEGF protein. The main substrate for VEGF protein is HIF, which plays a critical role in the cell's response to hypoxia. This treatment is not ideal because there are still many patients with metastatic RCC who are lost due to treatment failure and disease progression. Hence the need for a broader view of possible therapeutic targets, and MMPs deserve special attention here. Thanks to the development of the knowledge of MMPs, unique proteases involved in the regulation of intracellular and extracellular processes have been discovered. Today, there is a fresh look at their role in the diagnosis and prognosis of the course of RCC and its treatment [174]. It is not surprising that the first-generation inhibitors of MMPs did not gain the expected success. Their molecules blocked the catalytic centre of the enzyme, and were admittedly potent inhibitors, but due to their lack of selectivity they caused severe complications, such as musculoskeletal syndrome, which was most severe. Because of these side-effects and lack of therapeutic success, all clinical trials were discontinued [175].

Since the first generation of MMPs inhibitors blocked multiple groups of MMPs, further research was directed toward selective MMPs inhibitors which block single enzymes. The result of this work was the development of the second-generation inhibitors with a refined selectivity profile. A selective inhibitor has been developed for a number of MMPs, but none of them has reached the market and are not used to treat cancer patients, for reasons similar to the case of the first generation, namely, lack of therapeutic efficacy and side-effects [110].

There is some hope in monoclonal antibodies developed in recent years and used to block selectively single MMPs. Unlike small-molecule drugs, monoclonal antibodies have high selectivity for their target, exhibit a longer half-life, which allows dosing at intervals, and have a different toxicity profile. There are many monoclonal antibodies with proven efficacy in *in vitro* and *in vivo* studies [176]. Such antibodies have been developed, for example, to inhibit the activity of MMP-2and MMP-14. MMP-14 plays a key role in the process of primary tumour growth, tumour invasion and neo-angiogenesis. With its ability to activate pro-MMP-2,

MMP-14 promotes proteolytic activity in the tumour micro-environment.

Devy et al. (2009) discovered a human MMP-14-blocking antibody, called DX-2400, which was found to inhibit angiogenesis by reducing VEGF-directed cell invasion and activating pro-MMP-2 in *in vitro* studies. *In vivo* experiments on mouse models of breast tumours confirmed that the blockade of MMP-14 by DX-2400 exhibits TGF-β-reducing immunosuppressive activity, which results in the shrinkage of the primary tumour and improvement of the response to radiotherapy, especially in tumours with high levels of MMP-14 expression [177].

Ingvarsen et al. (2013) also described a monoclonal antibody, known as 9E8, which blocks a single function of MMP-14 in a selective manner, which means that 9E8 inhibits the ability of MMP-14 to activate pro-MMP-2, without affecting the proteolytic activity of MMP-14 [178]. Udi et al. (2015) described an antibody called LEM-2/15, which, unlike the above agent, selectively blocks the proteolytic activity of MMP-14, without affecting the ability to activate pro-MMP-2. LEM-2/15 is responsible for inhibiting the cleavage of substrates such as gelatine and type I collagen. At some stages of the cancer development, pro-MMP-2 activation is a beneficial phenomenon, and, in such a case the proteolytic activity of MMP-14 is a target, and LEM-2/15 can serve as a potential therapeutic agent [179]. One potential application of monitoring the activity of MMPs may be to predict the patient survival and the course of metastatic RCC. Among the currently available prognostic models, however, there are none which apply the assessment of the MMPs activity. The most common prognostic model used in many recent clinical trials, the IMDC, uses six variables (Karnofsky grade, time from diagnosis to treatment inclusion, calcium concentration, haemoglobin concentration, platelet count and neutrophil percentage).

Perhaps the inclusion of MMPs activity into prognostic models may provide additional information and improve existing models, and improve the care of patients with RCC. Elderly patients with multiple comorbidities, such as frailty syndrome and small renal tumour (SRM) incidentally diagnosed, for example, have a low RCC-specific mortality rate and a significantly higher risk of death from non-cancer causes. One option for treating such patients is an active surveillance (AS) strategy of monitoring tumour size with possible delayed surgical intervention if tumour progression occurs. Before qualifying patients for this form of treatment, it is common practice to conduct a biopsy of the tumour to determine its histologic type and degree of histologic malignancy. Currently, there are no biomarkers to help qualify patients at higher risk of disease progression. There is another place where assessment of MMP activity could potentially be used: tumors with higher MMP-3 and MMP-9 activity which are associated with higher malignancy; therefore, determining the activity of MMPs could potentially help select patients for surgical treatment or active surveillance groups.

According to current scientific evidence, there is no basis for neo-adjuvant therapy for ccRCC. Until recently, adjuvant treatment was not included into daily clinical practice. A number of studies using tyrosine kinase inhibitors, such as sunitinib, sorafenib and pazopanib, have shown no effect on improving the overall survival of patients with RCC. There is some hope with the KEYNOTE-564 trial, which

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confirmed a positive effect of adjuvant immunotherapy with pembrolizumab on disease-free survival (DFS) in patients at higher risk of RCC recurrence. However, the lack of biomarker data makes it difficult to qualify patients for such treatment. Perhaps the use of monitoring the activity of MMPs in patients with RCC could improve the stratification of patients into groups at risk for increased RCC recurrence, and for which adjuvant treatment could be used. A number of randomized clinical trials are underway to determine the efficacy of adjuvant immunotherapy. Adding an assessment of MMPs activity, which often correlates with malignancy, could be helpful in selecting optimal therapy. Thanks to scientific evidence from the CARMENA and SURTIME studies, it has been observed that patients in the intermediate and poor prognosis groups according to IMDC, do not benefit from early cytoreductive nephrectomy. Patients in these groups achieve better treatment outcomes when they receive early systemic treatment based on a combination of ICIs, such as nivolumab with ipilimumab, or a combination of ICIs and TKIs, such as pembrolizumab with cabozantinib, nivolumab with cabozantinib. Systemic treatment is not perfect as it causes a relatively high number of side-effects, and should be carried out only in the highest referral centres. Median survival in the favourable, intermediate and poor prognosis groups according to IMDC, is 43.2 months, 22.5 months and 7.8 months, respectively. Research should therefore be conducted on the use of drugs from other groups. Monoclonal antibodies directed against MMP-2 and MMP-14 may be used in this role. The use of these molecules may be particularly important in tumours with strong vascularization, which undoubtedly comprise ccRCC.

SUMMARY

MMPs and TIMPs are compounds produced by stromal and tumour cells involved in a number of physiological and pathological processes, including tumorigenesis. An imbalance between TIMPs and MMPs is crucial in the process invasion and metastasis formation [180]. Patients with RCC confined to one organ have a five-year survival rate of >90%. This is the group of patients with the best prognosis. The ability of tumour cells to form metastases significantly affects the survival of patients, as in the group of patients with metastases, the five-year survival rate does not exceed 10% [181]. In patients with RCC confined only to one organ or locally advanced, the basic treatment remains surgery. RCC shows natural resistance to radiotherapy and conventional chemotherapy. Currently, the standard of care for patients with metastatic disease is immunotherapy. Although patients treated with immunotherapy achieve improved overall survival, response rates are limited and new therapeutic agents need to be identified. The hope for the better future lies in monoclonal antibodies developed in recent years to selectively block single MMPs, such as MMP-2 and MMP-14, which may be particularly important in heavily vascularized tumours, which undoubtedly include ccRCC. In addition, if assessment of the activity of selected MMPs were to become standard in the diagnosis and surveillance of patients with RCC in the future, it could potentially help select patients for surgical treatment groups or active surveillance and could also be helpful in selecting optimal therapy.

ABBREVIATIONS

APRF – factor of acute phase response; **bFGF2** – basic fibroblast growth factor 2; **BMI** – body mass index; **BMP-2** – bone morphogenetic protein 2; **β-ct** – catenin β; **CAF** – cancer-associated fibroblasts; **ccRCC** – clear cell renal cell carcinoma; **CD63-R** – CD63 receptor; **CG** – cathepsin G; **chRCC** – chromophobe renal cell carcinoma; **CLDN-1** – claudin-1; **COX-2** – cyclooxygenase 2; **CT-α** – chymotrypsin α; **DBP** – diastolic blood pressure; **ECM** – extracellular matrix; **EMMPRIN** – extracellular inducer of MMPs; **EMT** – mesenchymal-epithelial transition; **EPIC** – European Prospective Investigation into Cancer and Nutrition; **FAK** – focal adhesion kinase; **Fas-L** – ligand Fas; **FGF** – fibroblast growth factor; **GA** – glutamic acid; **GCs** – glucocorticoids; **GCTB** – giant cell tumour of bone; **HGF** – hepatocyte growth factor; **HIF** – hypoxia-inducible factor; **HO-1** – heme oxygenase-1; **ICI** – checkpoint inhibitor; **IFN-β** – interferon β; **IFN-γ** – interferon γ; **IL-10** – interleukin 10; **IL-12** – interleukin 12; **Il-1β** – interleukin 1β; **IL-8/CXCL-8** – interleukin 8; **IκBα** – nuclear factor kappa-light-chain-enhancer of activated B cells type α; **LPS** – lipopolysaccharide; **MAPK** – mitogen-activated protein kinase; **MMP** – matrix metalloptoteinase; **MNRN2** – multimerin 2; **NF-κB** – nuclear factor kappa-light-chain-enhancer of activated B cells; **NQO1** – quinone oxidoreductase-1; **NRF-2-ARE** – nuclear factor erythroid-2-related factor 2/antioxidant response element; **NSCLC** – non-small cell lung cancer; **PAR** – poly-ADP-ribose; **PDGF** – platelet-derived growth factor; **PI-3-K** – phosphoinositide 3-kinase; **PIAS** – protein-activated STAT inhibitor; **PLC** – phospholipase C; **pRCC** – papillary renal cell carcinoma; **p120-ct** – catenin p120; **RCC** – renal cell carcinoma; **SBP** – systolic blood pressure; **SOCS** – suppressor cytokine signalling proteins; **sRCC** – sarcomatoid renal cell carcinoma; **SRM** – small renal tumour; **TAM** – cancer-associated macrophag; **TC-PTP** – T-cell protein tyrosine phosphatase TC45; **TGF-β** – transforming growth factor β; **TIMP** – tissue inhibitor of matrix metalloproteinases; **TKIs** – tyrosine kinase inhibitors; **TNF** – tumor necrosis factor; **TR** – trypsin; **Tr-3-M/Tp-5-M** – γ-3-monooxygenase tyrosine/5-monooxygenase tryptophan activating protein; **uPAR** – urokinase-type plasminogen activator receptor; **VEGF** – vascular endothelial growth factor; **VEGFR** – receptor of vascular endothelial growth factor; **VHL** – von Hippel-Lindau protein

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