The relationship between renal cell carcinoma and nuclear retinoid/rexinoid receptors

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Background. Renal cell carcinoma (RCC) is a urologic malignancy with a steady rise in incidence and high mortality rate. Between 60 to 70% of patients with renal cell carcinoma can only be cured with surgery but despite advances in early diagnostis, in around 20-30% of cases there is metastasis. For these patients, chemotherapy and radiotherapy are ineffective and hence the prognosis is poor. Retinoids are biologically active compounds of either natural or synthetic origin that are involved in complex physiological and developmental processes in many tissues including cell proliferation and activation of tumour suppression genes. This article reviews the role of retinoids and their cognate nuclear retinoid/rexinoid receptors in relation to renal cell carcinoma.
Methods. A literature search using ScienceDirect and Medline with a focus on the relationship between renal cell carcinoma and nuclear retinoid/rexinoid receptors.
Results. Use of retinoids/rexinoids in the treatment of locally advanced and metastatic RCC significantly prolongs median time of tumour progression and overall survival of patients. Combination therapy with other preparations has greater efficacy than treatment with retinoids alone. Patient survival can be predicted on the basis of the expression of different all-*trans* retinoic acid receptor (RAR) and 9-*cis* retinoic acid receptor (RXR) subtypes.
Conclusions. Since nuclear retinoid receptors play a crucial role as ligand-activated, DNA binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes, retinoids might be an alternative approach for the treatment of renal cell carcinoma.

Key words: renal cell carcinoma, retinoids, rexinoids, nuclear receptors, chemoprevention

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INTRODUCTION

Renal cell carcinoma (RCC) is the most common kidney tumour. It is a urologic malignancy with a steady rise in incidence over the last 50 years, a peak in the 6th and 7th decades of life and high mortality rate. Clear cell RCC accounts for around 70% to 80% of all cases of diagnosed RCCs. About two thirds of patients with RCC can be usually only treated with surgery. The prognosis for these patients is excellent. However, despite advances in diagnostics, in about 20-30% of patients a metastatic RCC is confirmed. For these patients the prognosis is rather poor and further chemotherapy and radiotherapy treatment yields relative weak or ineffective results[2].

Retinoids are biologically active compounds of natural or synthetic origin that are involved in complex physiological and developmental processes in many tissues of higher vertebrates[3]. Today, we know more than 4000 natural and synthetic retinoids[4]. Retinoids are closely related to retinoic acids. All these compounds act through interactions with two basic types of nuclear receptors: retinoic acid receptors (RAR α, β and γ) and retinoid X receptors (RXR α, β and γ) as ligand-activated, DNA-binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes. In higher organisms, retinoids affect a broad spectrum of biochemical and molecular biological reactions[5]. However, they have both beneficial and as well as detrimental effects. Retinoids have tumour-suppressive activity (inhibit tumour cell proliferation and induce cell differentiation and apoptosis) but on the other hand, they are teratogenic[3]. A number of retinoids and rexinoids acting through their cognate nuclear receptors have been tested both *in vitro* and *in vivo*, using cell cultures or animal models for renal cell cancer.

RENAL CELL CARCINOMA

Epidemiology

Renal cell carcinoma is the most common kidney tumour. It represents around 90% of all cancers affecting the kidneys, which range from 2% to 3% of solid tumours of the adult population world wide[1]. Although RCC is a global problem, the incidence varies considerably. The highest incidence of RCC was recorded in Western, Central and Eastern Europe, North America and Australia, and

the lowest incidences in India, Japan, Africa and China. These differences are probably due to exogenous risk factors, geographic differences and varying availability of diagnostic methods[6].

However, the incidence of kidney cancer in Europe and the U.S.A in the last three decades has an upward trend that cannot be explained only by improved diagnostic methods (ultrasound, CT, MRI). Along with the incidence of early stages of RCC, there is increasing incidence of advanced disease stages, as well. The rise in incidence of RCC is greater in men than in women and in black populations[2]. The last two decades, has seen an increase in the incidence of RCC of about 2% per year. An exception to this trend is Denmark and Sweden, where a decrease in incidence of RCC was reported[7].

In 2006, there were 64,000 new cases of RCC diagnosed in Europe. Standardized incidence rates were calculated by the direct method using the World Standard Population (WSR), 14.5/100 000 for men and 6.9/100 000 for women, which in absolute numbers is 40 395 and 24 656 cases respectively[8]. An unflattering global leadership in incidence of RCC was recorded in the Czech Republic. In 2008, 1812 cases were diagnosed for RCC in men (incidence WSR 21.65/100 000) and 1029 cases for women (WSR 9.28/100 000) (ref.[9]). This "paradox" has not yet been reliably explained.

The incidence of RCC in Slovakia is comparable to that of neighbouring European countries and has an upward trend. According to data from the National Cancer Registry of the Slovak Republic in 2005, 484 RCC were diagnosed in men (crude incidence CR - 18.5/100 000, WSR - 14.6/100 000) and 299 cases in women (CR – 10.8/100 000, WSR 7.0/100 000). These data rank Slovakia among countries with a high incidence of RCC (ref.[2,10]).

Worldwide mortality for RCC in 2006 represented around 102 000 patients. The incidence for men was twice as high as for women. The total mortality approximately replicates the incidence rates of RCC. The highest numbers are in the developed countries of North America and Europe and lowest in countries of African and Asian continents[1]. In 2006, there were registered 27 326 deaths from RCC in the EU countries, which is about 2.2% of all deaths from malignant diseases – 16 798 deaths in men (WSR 5.8/100 000) and 10 528 deaths in women (WSR 2.6/100 000). Individual countries within the European Union differ in mortality for RCC. A decrease in mortality was recorded in Scandinavian countries starting from the 80's of the last century. A similar decrease was also found in France, Germany, Italy and the Netherlands from the beginning of the 90's. The countries of Eastern and Central Europe have recorded a yearly increase in mortality for RCC (ref.[1,2]).

In Slovakia, a minimal increase in mortality for RCC for the past 10 years has been recorded compared to 1994 (WSR 4.0/100 000) and 2003 (WSR 4.5/100 000). It is very likely that these data reflect improvement in diagnostics and treatment approaches to RCC in recent decades[11].

Etiologic and Risk Factors

The etiopathogenesis of renal cell carcinoma is currently unknown. According to a large number of studies, several risk factors play a role, including smoking, obesity and hypertension. Other risk factors are age, gender and socioeconomic factors[7].

Smoking has been confirmed as a risk factor for RCC by several studies that confirmed that the risk for development of RCC in male smokers is more than 54% higher than in non-smokers. In female smokers, the incidence of RCC is 22% higher than in non-smokers. Although the link between smoking and RCC is relatively low, the risk of RCC occurrence increases in proportion to the duration of smoking, accumulated dose and the number of packs per year[1,12-14]. Currently, we have only limited evidence of the size of the positive impact of smoking cessation. The risk of RCC falls by 15 to 30% after 10 to 15 years of quitting smoking and about 30 years of non-smoking the risk is about the same as for non-smokers[2,12].

Obesity is considered the next risk factor for RCC as identified in a number of studies that followed BMI – body mass index. Meta-analysis data identified a comparable risk of RCC development in males and females. This was 36% in overweight populations with and by 84% in obese people compared to those with normal weight[15]. Other studies have shown a higher risk in obese women than in men[1]. Generally, the increase in obesity may partly explain the increase in RCC incidence. RCC linked to obesity in up to 40% was recorded in the U.S.A and more than 30% in Europe[2]. The exact mechanism by which obesity increases the risk of RCC occurrence is currently not well understood. It is assumed that an obesity-induced increase in insulin-like growth factor (IGF), sex steroid estrogens and androgens and elevated concentration of peptides may contribute to RCC. Atherosclerosis and hypertension as a consequence of obesity may also markedly contribute to carcinogenesis[2,13].

There is a proven link between elevated blood pressure (uncontrolled arterial hypertension) and increased risk of RCC but there is no clear mechanism of action known as to how the high blood pressure contributes to its development. It is believed that long-term high blood pressure causes micro trauma parenchymal renal and metabolic and functional changes in the renal tubules[2,12].

The next widely accepted risk factor for RCC is advanced age. The vast majority of sporadic RCC is diagnosed in patients older than 50 years, the maximum incidence is in age of 60-70 years[7,12].

In developed countries, the incidence rates of RCC are higher in men than in women, this ratio is approximately 1.5:1 (male to female). The only exception is West Africa, where the incidence of RCC is higher in women than in men[1,7].

Familial occurrence of RCC is not significant. It accounts for about 3-4% of all diagnosed kidney cancers. Familial RCC differs from sporadic RCC in the tendency to occur in younger patients and often multifocal and bilaterally[2,13].

Another but not negligible risk factor for RCC is socio-economic conditions. Higher incidence rates and

mortality rates are found in urban areas than in rural areas. The risk of kidney cancer is not clearly linked to hazardous occupations[2]. The link between increased risk of RCC and working exposure to substances such as asbestos, gasoline, benzene fumes from burning fossil fuels, herbicides, polyvinyl chloride, cadmium, lead, trichloroethylene, perchloroethylene is significant[12,14,16].

Pathology

The World Health Organization (WHO) classification of renal tumours from 2004, defines the various histological subtypes of tumours based on their molecular and cytogenetic characteristics of variable biology and clinical behavior[1,17]. Renal cell carcinomas are malignant epithelial tumours which originate from the renal cells, particularly in the proximal tubules but also in the collecting ducts[13,17,18].

According to the WHO classification, there are 4 major histological subtypes of RCC – clear cell, papillary and chromophobe RCC and carcinoma of the collecting ducts[16]. Clear cell RCC accounts for approximately 75-80% of all histologically diagnosed kidney cancers. It has a characteristic macroscopic appearance, usually grows as a well-defined tumour, but its growth may change into expansive, with overgrowth of bushes and ingrowths into the renal vein and pelvis[12,19]. Most of these tumours grow in the kidney cortex as a solid, solitary, fibrous pseudocapsule well- circumscribed from the surrounding formation[1,20].

Papillary RCC is the second most frequently occurring cancer of the kidney, in approximately 10-15% of cases. More often than other RCCs it is multifocal (approximately 39% of cases) and on both sides (about 4% of cases) presents with numerous, small sided and papillary adenomas[13]. Papillary RCC shows as low nuclear grade with no metastasis and thus with good prognosis[12,19]. According to the morphological and histological images, papillary RCC is divided into two types[13,17,19]. Survival of patients with papillary RCC type 1 was also compared to type 2 (ref.[16]).

Chromophobe RCC accounts for about 5% of kidney cancers. It occurs most frequently in the sixth decade of life[2]. It has a significantly better prognosis than clear cell RCC[12]. Grossly, it usually presents as a solitary, well-defined and homogeneous noncapsulated tissue with a light brown surface. Rarely, it may be multifocal and bilateral. Most commonly there is sarcomatoid dedifferentiation, characterized by aggressive behaviour of cancer and a very poor prognosis[12,13,19].

Carcinoma of the collecting ducts is a rather rare tumour that accounts for less than 1% of renal malignancies. It is the most aggressive form of RCC, with a poor prognosis and frequent finding of metastases already at the time of diagnosis and unresponsive to conventional therapy[2,13].

The remaining approximately 3-5% of renal carcinomas can be classified by Heidelberg criteria into groups of unclassified RCC. This includes cancers that cannot be assigned to any of the above histological types[21].

Familial renal cell carcinoma

Kidney cancer occurs in sporadic and familial forms. Familial forms account for approximately 3-4% of the total number of diagnosed RCC and have proven inborn genetic defect. Some are due to mutation or inactivation of tumour-suppressor genes and others activation of oncogenes. All are transmitted in an autosomal dominant way[1,2]. Each of the different histological subtypes of RCC occurs as a part of a familial syndrome. All these syndromes are relatively rare and tend to occur bilaterally and multifocally, especially in younger patients[1,2,16].

The familiar RCC are classified to these four tumour syndromes:

von Hippel-Lindau syndrome

The most common familial RCC is clear cell carcinoma which is associated with the von Hippel-Lindau (VHL) syndrome. The VHL syndrome is an autosomal dominant disorder with an incidence of approximately 1/36 000 (ref.[14]). This multisystemic neoplastic syndrome manifests as the formation of clear cell RCC, pheochromocytomas, retinal angiomas, hemangioblastomas of the cerebral cortex, cerebellum and spinal cord[12,16]. Clear cell RCCs do not develop in all patients with VHL disease but only in about 40-50%. Typical is its early development in the third, fourth or fifth decades of life and a higher tendency towards bilateral and multifocal occurrence[16].

The tumour-suppressor VHL gene is located on chromosome 3p25-p26 and it plays a role in the development of sporadic and familial forms of clear cell RCC. Up to 100% of patients with VHL syndrome have the inherited mutant VHL allele but only after mutation or inhibition of the second allele can it develop clear cell RCC. For the creation of up to 60% of sporadic clear cell RCCs VHL gene defects are responsible[2,16]. The root cause of clear cell RCC lies in the failure of the VHL protein that occurs on mutation of the VHL gene which is responsible for normal production[16]. Two VHL protein isoforms have been distinguished. They have similar function in the body - act as a tumour-suppressor which prevent the formation and growth of renal cell carcinoma[20,22]. Normally, the VHL protein genes inhibit HIF (hypoxia inducible factors) ubiquitin-mediated degradation of HIF-1 and HIF-2, and thus maintain their levels at low levels. HIF-1 and HIF-2 are intracellular proteins that play an important role in regulating the cellular response to hypoxia, starvation and other stress. Inactivation or mutation of the VHL gene leads to nonregulated expression of HIF-1 and HIF-2. This subsequently leads to non-regulated expression of the angiogenesis growth factors (VEGF - vascular endothelial growth factor, PDGF - platelet derived growth factor) and cell proliferation (TGFα - transforming growth factor α) associated with clear cell RCC (ref.[2,16,20]). Growth factors HIF-1, HIF-2, VEGF and PDGF bind to specific tyrosine kinase receptors on the surface of endothelial cells of blood vessels, lymphatic cells, vascular pericytes and neurons, causing cell proliferation, migration and their survival, and thus ultimately support tumour angiogenesis[2].

Hereditary papillary RCC type 1

Hereditary papillary RCC type 1 is an autosomal dominant disorder that is associated with a congenital defect (mutation) in the c-MET proto-oncogene which is located on chromosome 7q31. C-MET proto-oncogene encodes a receptor tyrosine kinase that is normally inactive. C-MET mutation results in activation of the receptor, a growth that supports tumour activity[1,2,12,16,20,22]. In patients with this hereditary disease, type 1 papillary carcinoma occurs multifocally and bilaterally, in macroscopic and microscopic forms. Macroscopic lesions are usually diagnosed in the fifth or sixth decade of life and are usually characterized by slow growth. Until now, this disease has not been linked with diagnosed systemic involvement[1,22].

Hereditary papillary RCC type 2

The incidence of type 2 papillary carcinoma is associated with hereditary leiomyomatosis that manifests with the leiomyomas of skin and uterus. The syndrome is through autosomal dominant transmission. Although, there is not yet well known the process of malignant transformation, we know that the basis for the emergence of a mutation or deletion of fumarate hydratase gene, which is encoded on chromosome 1q42. Type 2 cancers in this syndrome are regularly aggressive, larger, occur at a younger age and are more likely to have a higher stage and significantly worse prognosis[1,14,22].

Birt-Hogg-Dubé syndrome

Birt-Hogg-Dubé syndrome is a rare autosomal dominant disorder that is associated with the development of multiple benign skin tumours (hamartomas) on the face, neck and upper chest, multiple lung cysts, spontaneous pneumothorax, intestinal polyps, medullary thyroid carcinoma and frequently bilateral and numerous renal tumours originating in the distal nephron[1,2,14,16,22]. Kidney cancer affects approximately 20-40% of patients with this syndrome[1]. The majority of renal tumours in this syndrome has limited biological aggressiveness, but it has been described also cases with metastatic behavior[1,22]. Birt-Hogg-Dubé syndrome is associated with mutations of foliculine, tumour suppressor gene on chromosome 17p11.2. Although currently it is not well known the exact function of foliculine, it is assumed its role as a tumour suppressor[1,20,22].

Classification, symptoms and treatment of RCC

For scientific and clinical purposes the TNM classification is used. This is based on a differentiated prognostic evaluation of the local extent of the primary cancer, nodal and tumour vascular promote and the incidence of distant metastases[23].

Factors affecting the prognosis of patients with RCC are divided into 4 categories: anatomy (the substance of the TNM classification), histology (nuclear grade, histological subtype of the tumour, presence of sarcomatoid features, invasive growth and tumour necrosis), clinical (characterizing the overall condition of the patient) and molecular factors that we do not use in common practice. The use of molecular factors in practice remains to be seen in the near future[23].

Most kidney cancers due to renal retroperitoneal position remain asymptomatic until late disease stage. Today, more than 50% of RCCs are detected incidentally during noninvasive imaging examinations (ultrasonography, CT, MRI) (ref.[23]). The first symptoms are usually intermittent and nonspecific. Local symptoms usually develop until the time when the tumour reaches a certain size and can move and infiltrate surrounding organs. The classic diagnostic triad of kidney tumour - hematuria, abdominal or flank pain and palpable tumour - represents a late manifestation of RCC and currently is not a frequent finding. Rare are locoregional symptoms (acute varicocele, dilated collateral veins, swelling of the legs), which are associated with tumour promotion in the inferior vena cava or the lymphatic system[2]. The presence of systemic symptoms (loss of appetite, weight loss, fatigue, weakness, subfebrile, night sweats) or symptoms of paraneoplastic syndrome are non-specific for RCC (ref.[13,14]).

With the development and increased use of diagnostic imaging methods kidney tumours are usually diagnosed in an incidental stage, when patients have a good chance of full recovery. The only curative treatment of RCC is surgical treatment. These days, the surgical treatment of renal cell carcinomas use multiple approaches from radical nephrectomy through methods of nephron sparing surgery (partial nephrectomy) or use of percutaneous and minimally invasive techniques. In operations, there are used open approaches, laparoscopic as well as robot-assisted approaches[24]. In patients with a high risk of postoperative local or distant relapse or in patients with inoperable tumours or metastatic disease systemic treatment is indicated. The most common treatment modalities in systemic treatment include immunotherapy (cytokines interferon α and interleukin-2) and in particular preparations of targeted therapy that inhibit angiogenesis and have higher efficiency[24-26].

TWO BASIC CLASSES OF RETINOIC ACID RECEPTORS

Nuclear receptors (NRs) are intranuclear receptors that bind specific lipophilic ligands and upon ligand binding, they change conformation, allowing direct binding to DNA, yielding in the gene expression. Therefore nuclear receptors are transcription factors activated by biologically active ligands. Nuclear receptors are part of the signalling pathways that regulate and control the metabolism, growth and development of the organism, cell differentiation, sexual maturation and reproduction and physiological organ function. Receptors, which are structurally and functionally similar to steroid receptor family members are collectively classified into "superfamily" of nuclear receptors. Overall 48 nuclear receptors classified into this "superfamily" have been identified in the human body[3,27-29].

Biological activities of retinoic acids and retinoids (derivatives of retinoic acid) in the target cells are mediated

through specific representatives of the NR's "superfamily" - receptors for all-*trans* retinoic acid (RAR) and receptors for 9-*cis* retinoic acid (RXR) (ref.[31]).

The variety of retinoic acids activities in target cells are determined by the existence of three subtypes of RAR (α, β and γ) and three subtypes of RXR (α, β and γ) (ref.[3,27,28,32,33]).

Retinoic acid receptors (RAR and RXR) differ in their specificity for ligand binding. All-*trans* retinoic acid (ATRA) can bind and activate only RAR receptors and all three subtypes have similar affinity. In contrast, 9-*cis* retinoic acid binds and activates all three subtypes of RAR and RXR but with a different affinity for each receptor and its subtypes. RAR and RXR are ligand-inducible transcription factors that function predominantly as RAR-RXR heterodimers, positively or negatively affecting the specific genetic expression[30,31,33]. The all RAR subtypes bind all-*trans* and 9-*cis* retinoic acid with similar affinity, whereas RXRs bind only 9-*cis* retinoic acid. RAR-RXR heterodimers bind to specific DNA sequences called "retinoic acid response elements" (RARE). In the absence of RAR ligands or in the presence of certain RAR antagonists, the DNA binds to RAR-RXR heterodimers in the presence of protein transcription corepressors[3,27-29,33].

Upon ATRA binding to its cognate receptors, corepressor proteins are separated from the nuclear receptor complex that allows a subsequent association of nuclear receptor with coactivator proteins leading to activation of gene transcription[3,28,29,34].

RETINOIDS IN CHEMOPREVENTION AND TREATMENT OF MALIGNANT DISEASES

Retinoids are biologically active compounds of natural or synthetic origin that are involved in complex physiological and developmental processes in many tissues of higher vertebrates[3]. They are used as potential chemopreventive and chemotherapeutic agents. These substances cause inhibition of tumour cell proliferation, affect cell differentiation of malignant cells, induce apoptosis of damaged and old cells and also have anti-oxidant effects[4,35]. Their molecular structure contains cyklohexenyl ring and their "parent" compound is the all-*trans* retinol, what is actually vitamin A (ref.[3,33]).

Epidemiological studies have shown that a reduced intake of vitamin A results in increased risk for development of cancer[36]. Impaired expression of retinoic acid receptors is associated with malignant transformation of tissue culture cells and animal tissues cells, as well. In addition, retinoids suppress carcinogenesis in animal models of tumours of the skin, mouth, lung, breast, bladder and prostate[4,33]. In humans, retinoids exhibit strong potential for the treatment of precancerous epithelial lesions and in the prevention of some primary solid tumours - lung, liver and breast[4,36]. Despite the positive effects of retinoids, their teratogenic effect, which limits their use in women of child-bearing period cannot be overlooked[3,31,35,37].

The positive effects of retinoids in individual cancers have been repeatedly proven. It has been demonstrated that the effects of retinoids were not equally present in the same types of malignancies, yielding different clinical responses. This led Xu and his colleagues (1994) to compare the expression pattern of RAR and RXR subtypes in cells of healthy and neoplastic tissues[38]. They compared the expression of retinoid receptors in squamous cell skin, head and neck carcinomas, dysplastic skin lesions, adjacent normal tissue and with that of corresponding intact tissue of volunteers, and found that the expression of RARβ was inhibited in malignant tissue, in dysplastic and adjacent healthy tissue compared to healthy tissue[38]. Other studies too have confirmed the unique status of RARβ in the family of retinoic acid receptors and have demonstrated that loss of RARβ2 is common for precancerous and malignant tissues and tumour cells in the skin of the head and neck, breast, lung, esophagus, pancreas, cervix and prostate[39-43]. Loss of RARβ2 expression occurs at an early stage in the development of malignancies and is associated with subsequent ongoing carcinogenesis[39-41]. In patients after treatment with 13-*cis* retinoic acid an increased expression of RARβ2 was observed, which correlated with clinical response, as well[44]. Several studies have confirmed the assumption that the RARβ2 plays an important role in the suppression of carcinogenesis[39,41-43]. For the above cancers, it has been demonstrated that the RARβ2 gene is frequently deleted or RARβ2 gene promoter is attenuated due to aberrant DNA methylation or histone repression[41]. On the basis of these findings, aberrant DNA methylation of the RARβ2 gene promoter can be used as a biomarker for early detection of malignancy or as a marker for monitoring the effect of chemopreventive compounds in active clinical trials[39].

To date, the role of RARγ in carcinogenesis is not yet fully understood. The expression of RARγ is much more limited than the expression of other subtypes of RAR. Reduced expression of RARγ in malignant tumours and especially in their later stages has also been described. At the moment, we can assume that the role of RARγ has the significant impact for growth regulation of certain tumours rather than the other RAR subtypes[34,45].

RETINOIDS AND RENAL CELL CARCINOMA

The positive effects of retinoids in chemoprevention and treatment of individual cancers have been repeatedly proven[39]. In the 1990s a number of authors tried to explain the link between retinoids and renal cell carcinoma.

In 1996, Hoffmann examined the antiproliferative effects of 13-*cis* retinoic acid (13-*cis* RA) on 12 renal cancer cell lines and correlated these findings with the basal and induced expression of RARα, β and γ. Eleven of 12 renal cancers did not express RARβ. In these cells, 13-*cis* RA treatment did not induce RARβ expression. Only 1 of 12 cell lines was inhibited by 13-*cis* RA. Expression of RARα was abundant in all 12 cell lines examined, low levels of RARγ transcripts were detectable in 6 of 10 renal cancers. Expression of RARα and RARγ was not affected by 13-*cis* RA (ref.[46]). Based on these results, the majority of renal cancer cell lines are resistant to 13-*cis* RA. It has been sug-

gested that the resistance to antiproliferative action of 13-*cis* RA correlates with repressed RARβ expression and the antiproliferative effects of 13-*cis* RA in renal cancer cells are mediated predominantly through RAR β1 (ref.[46]).

The antiproliferative effect of 13-*cis* retinoic acid was studied in a group of 23 patients suffering from advanced RCC. Berg administered monotherapy with 13-*cis* retinoic acid (1mg/kg/day) to these patients daily for a period of 12 months. After evaluation of the study, only 1 patient experienced an obvious reduction in tumour size, the others remained stable or their disease progressed. He recorded only mild side effects and confirmed that monotherapy with 13-*cis* retinoic acid did not produce the expected effect in patients with advanced disease[47].

Later, these same authors analyzed 34 tissue samples collected from patients with RCC who participated in the clinical study (administration of 13-*cis* retinoic acid and IFN α-2a). The data showed that the expression of RARβ was increased in RCC cells of patients whose clinical status was improved after retinoid medication. Retinoids, which potentially cause an increase in the expression of RARβ, should therefore be included in the treatment of patients with advanced RCC (ref.[48]).

This was followed by other studies on elucidating the effect of combined treatment with 13-*cis* retinoic acid and IFN-α-2a in patients with locally advanced or metastatic RCC and to clarify the effect of treatment depending on the expression of RARβ in renal cell carcinoma tissue. Unfortunately, only a small sample of patients was recruited[48,49]. Leung et al. (1995) administered 13-*cis* retinoic acid and IFN-α-2a to 23 patients. They recorded clinical improvement in 4 patients. They also demonstrated that this improvement was associated with increased expression of RARβ (ref.[49]).

Another promising study was a II. phase by Motzer et al. (1999), who recruited 43 patients with metastatic RCC. The patients were treated with daily administration of IFN-α-2a in the dose of 3 mU (with a gradual increase to 6 and 9 mU) and 13-*cis* retinoic acid in amounts of 1 mg/kg/day. Overall, the response to this treatment was observed in 30% of patients (in 3 total and in 10 partial response). The treatment of patients yielded a reduction the number of bone metastases and reduction in size of the primary tumour bearing. Several patients remained for 1 year without progression, as well. The study confirmed the positive antitumour activity of a combination of IFN-α-2a and 13-*cis* retinoic acid in the treatment of metastatic RCC (ref.[50]).

Clinical results even in small groups of patients have increased enthusiasm for the use of retinoids in locally advanced and metastatic RCC. This was followed by further and larger studies. 284 patients with metastatic RCC were divided into three arms from the year 2000. In the first two arms, patients took daily the combination of IFN-α-2a in a dose of 3 mU or 9 mU together with 13-*cis* retinoic acid. Patients in the third arm took daily only IFN-α-2a alone. Up to 63% of patients had two or more metastases. Unfortunately, only in 12% of patients was there observed an overall response to the combination therapy (in 5 total and in 10 partial response) and only in 6% of patients on monotherapy by IFN-α-2a (in 1 total and in 8 partial response) (ref.[51]).

A large randomized study with 320 patients was carried out from 1996 to 2001. All patients had diagnosed metastatic RCC and were divided into two arms. In the first arm, patients received a combination of IFN-α-2a with 13-cis retinoic acid and the ones in the second arm only IFN-α-2a. Patients receiving combination therapy in comparison to patients with monotherapy experienced significantly prolonged median time to progression and overall survival time. The study confirmed the positive effect of combined therapy with minimal increase in toxicity of the treatment[52].

The effect of combined therapy was also studied in group of 38 patients with locally advanced RCC with distant metastases. One group of patients received a combination of IFN-α-2a and all-*trans* retinoic acid and the second group received only all-*trans* retinoic acid alone. The authors observed increased antitumor activity in patients with combination therapy. To support the effect of IFN-α-2a, they recommended the use of ATRA in combination therapy[53].

Today, a number of researchers focus on clarifying the use of nuclear receptors expression pattern as valuable biomarkers for detecting malignant kidney tumours, their histological subtype and then determine the appropriate treatment and predict the prognosis of the patient, as well[54]. Of course, today it is only a dream but studies aimed at clarifying the connection between nuclear receptors and specific histological types of RCC anymore.

Lot of efforts have been done to predict the survival of patients with metastatic RCC on the basis of analysis of the detection of RXRα localization in tumour tissues. Buentig et al. found that patients with RXRα localized predominantly in the nucleus have significantly longer survival than patients with aberrant, outside the nucleus positioned RXRα. Although the exact RXR function remains unclear for the moment. RXRα can be used in the complex of other factors as an independent factor assessment of RCC patient survival[55].

The team around Goelden focused on the evaluation of the expression of RARβ subtypes in the different types of RCC. They have found that the expression of RARβ1 was significantly increased in chromophobe RCC when compared to the healthy kidney tissue and other types of RCC. On the other hand, they did not record any difference in expression of RARβ2 between healthy kidney tissue and RCC tissue[56].

In 2007, scientists for the first time tried to define the prognostic significance of the link between dihydroxyvitamin D_3 receptor (VDR) and RXR subtypes in RCC. They used immunohistochemistry for assessment of expression of VDR and RXR subtypes in healthy kidney and in malignant kidney tumours. They confirmed that all types of RCC are VDR negative, i.e. with zero expression of VDR. In contrast, the loss of RXRγ expression was associated with advanced RCC. The decrease of RXRγ expression was observed in 76% of patients with RCC in the I.stage, but only in 25% of patients with RCC in IV. stage[57].

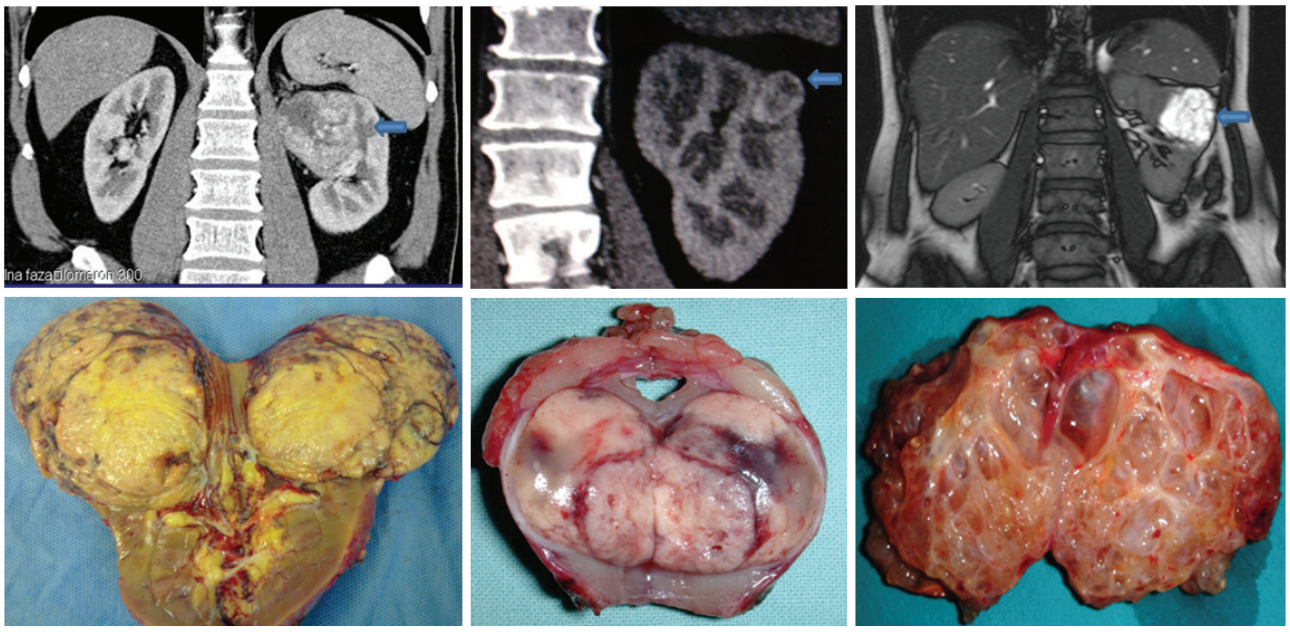

Fig. 1. Representative CTs and corresponding macroscopic views of renal mass of selected patients; a) 60 yrs old man after left sided nephrectomy because of papillary RCC type 1, pT2aG2; b) 48 yrs old man with histologically confirmed well-defined clear cell RCC, pT1a (nuclear grade has not been set), patient underwent left sided partial nephrectomy, and c) 52 yrs old woman after left sided nephrectomy because of multilocular cystic clear cell RCC, pT2aG1.

In the years ranging from 2007 to 2010, Lenko et al., analyzed RCC and healthy kidney tissues that were removed from specimen after partial or radical nephrectomy. Representative CTs and corresponding macroscopic views of renal mass of selected patients are shown in the Fig. 1. The tissue samples were analyzed by the method of reverse transcription and subsequent polymerase chain reaction (RT-PCR). The data has shown that all types of RCC cells and healthy kidney cells are capable to express all subtypes of RAR's (RARα, RARβ, RARγ), retinoid X receptors, RXRα, RXRβ, and only some of them are capable to express RXRγ. Expression of RARγ has been found to be enhanced in comparison with that of intact renal tissue[58-61].

CONCLUSION

The high incidence of renal cell carcinoma requires early detection and early target treatment of disease. Nowadays, the use of nuclear receptors as biomarkers, capable of rapid diagnosis and determination of cancer prognosis, is still not exploited in clinical oncology. Since nuclear retinoid receptors play a role as ligand - activated, DNA – binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes, retinoids might probably thus represent a possible alternative to established treatment of renal cell carcinoma. Retinoids may be used as a supportive therapy in the adjuvant treatment of locally advanced or metastatic RCCs or in combination treatment with the so-called preparations of targeted therapy for metastatic RCCs.

In conclusion, a number of novel chemical compounds, receptor selective retinoids and rexinoids, have been synthesized up to now and tested both *in vitro* and *in vivo*. In spite of that rapid progress novel synthetic retinoids and rexinoids with greater retinoid receptor selectivity, reduced teratogenic and other side effects are still highly required[3,5].

In coming time, sophisticated molecular biology approaches will open new possibilities for retinoids/rexinoids and its synthetic analogues exploitation in novel directed therapies for renal cell carcinomas. This will increase our knowledge about the link between RCC and the role of nuclear retinoid and retinoid X receptors. It will greatly help to innovate approaches for cancer chemoprevention and/or treatment in future.

ABBREVIATIONS

ATRA, All-*trans* retinoic acid; CR, Crude incidence; CT, Computed tomography; HIF, Hypoxia inducible factors; IFN-α-2a, Interferon alfa-2a; MRI, Magnetic resonance imaging; NR, Nuclear receptor; PDGF, Platelet derived growth factor; RA, Retinoic acid; RAR, All-*trans* retinoic acid receptor; RCC, Renal cell carcinoma; RXR, 9-*cis* retinoic acid receptor; TGFα, Transforming growth factor α; VDR, Dihydroxyvitamin D_3 receptor; VEGF, Vascular endothelial growth factor; VHL, von Hippel-Lindau; WHO, World Health Organization; WSR, World Standard Population.

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CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: *None declared.*

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