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Review

Malignant tumours of the kidneys are relatively rare tumours that occur in adults, although there has been a constant increase in the incidence of this cancer type in recent years. It occupies the 10th place in terms of the number of new cases of cancer in men and 14th place in women.

Considerable progress in the treatment of renal cell carcinoma over the last years has forced researchers to look for new factors of potential prognostic or predictive value in this tumour type in order to clarify the selection of patients for optimal treatment. The drugs from the group of tyrosine kinase inhibitors have played a decisive role. So far, the Motzer model, grouping the prognostic factors, has been most commonly used in clinical practice.

Based on the current research looking for new markers of prognostic or predictive value, these factors can be divided into cellular hypoxia-induced proteins and proteins regulating the cell cycle and the apoptosis process. In the second part of this study, hypoxia-inducible factors will be discussed.

Key words: kidney cancer, cellular hypoxia-inducible factors, prognostic factors, predictors.

Seeking new prognostic and predictive factors in patients with metastatic renal cell carcinoma – hypoxia-induced factors

Beata Młot, Cezary Szczylik, Piotr Rzepecki

Central Clinical Hospital, Military Institute of Medicine, Warsaw, Poland

The second group of markers that may have prognostic or predictive value in kidney cancer, still in the phase of intensive research, comprises factors induced in the hypoxia phase.

One of the proteins from this group is carbonic anhydrase IX (CAIX).

Carbonic anhydrase IX, which belongs to the family of transmembrane proteins regulating the cellular transmembranous calcium transport, seems to be one of the most promising markers that may have a predictive role in kidney cancer. The CAIX gene is induced during hypoxia. Catalysing the reversible reaction of bicarbonate ion formation from water and carbon dioxide, it stabilises the extracellular pH value in hypoxic conditions. In normoxic conditions, CAIX gene expression is blocked by the von Hippel-Lindau gene (VHL). In the clear cell type of renal cell carcinoma, the loss of VHL gene function induces CAIX gene expression [1].

Jean-Jacques Patard *et al*. studied the relationship between CAIX expression, VHL gene mutation, and clinical characteristics of this gene. The study was conducted on the material collected from 100 patients after nephrectomy performed due to renal cell carcinoma (RCC). The DNA material was isolated from frozen sections of the tumour. The polymerase chain reaction (PCR) method was used to detect the presence of VHL gene mutation. The degree of CAIX expression was evaluated using M75 monoclonal antibody and an immunohistochemical colour reaction with an appropriate marker. VHL gene mutation was detected in 58 patients (58%); high CAIX expression (> 85%) was seen in 78 samples (78%). CAIX expression was higher in the tumour tissue with detected VHL gene mutation. Based on this analysis, the following conclusions were reached: low expression and no VHL gene mutation were associated with more advanced tumours in terms of tumour size (T in TNM) and the presence of metastases; and conversely, high expression and VHL gene mutation were associated with longer progression-free time and longer survival. Based on the performed study, three prognostic groups of patients were also defined:

- with a good prognosis – VHL gene mutation and high CAIX expression (2-year survival – 86%),
- with an intermediate prognosis – VHL gene mutation or high CAIX expression (2-year survival – 69%),
- and with a poor prognosis – no VHL gene mutation and low CAIX expression (18-month survival – 45%) [2].

Kim *et al*. from the Medical University in Seoul performed studies in the years 1995–2006 seeking predictive factors in patients with metastatic kidney cancer treated with tyrosine kinase inhibitors. The material constituted tumours from 62 patients who underwent nephrectomy in which the degree of expression of carbonic anhydrase IX, cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF) was analysed. The follow-up period was 54 months. The results were referred to the clinical observations

of the course of cancer in each patient from the study group. The results were as follows: 52 patients (84%) had clear cell carcinoma, 5 had the sarcomatoid type, 3 had the papillary type, and 2 had the undifferentiated type of kidney cancer. Out of the 18 patients (83%) who responded to the causal treatment, 15 presented high expression of carbonic anhydrase, compared to 24 of 44 patients (55%) who did not respond to this treatment. A positive correlation was also seen between the degree of cyclooxygenaseitors. In addition, the adjusted calcium level ≤ 10 mg%, normal haemoglobin levels and the degree of COX-2 expression ≥ 50% were independent predictors of overall survival in patients with clear cell type of renal cell carcinoma treated with tyrosine kinase inhibitors. The researchers from Seoul confirmed that CAIX and COX-2 may be valuable predictors of response to treatment with tyrosine kinase inhibitors in patients with metastatic RCC [3].

In addition, another study showed that in a group of patients with non-metastatic kidney cancer, low CAIX expression (< 85% tumour cells) was an independent predictor of a shorter average survival time compared to the group of patients with metastatic renal cell cancer. On the other hand, in the latter group of patients, high CAIX expression (> 85% tumour cells) was associated with a longer disease-specific survival time, which was also confirmed by multivariate analysis [4].

Similar conclusions were presented by Atkins *et al.* who evaluated a group of patients with metastatic renal cell carcinoma, this time treated with IL-2. They found that high expression of CAIX in cancer cells increases the likelihood of partial or complete response to treatment with high doses of IL-2. Survival longer than 5 years was only seen in cancer patients with high CAIX expression [5].

The presented examples of analyses confirm that carbonic anhydrase can be a very helpful immunomarker of predictive value in kidney cancer.

The history of studies on vascular endothelial growth factor (VEGF) reaches back to the beginning of the 1980s. In 1983, Senger *et al.*, evaluating the process of angiogenesis in tumours, described a factor that increased vascular permeability, VPF (vascular permeability factor). A few years later, a protein with strong mitogenic properties for vascular endothelial cells was discovered and it was named VEGF. Later on, it became apparent that VPF and VEGF are the same proteins. The important role of VEGF in the physiological processes of the human body is indicated by the fact that mouse embryos deprived of only one allele of the VEGF gene died even before birth. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PlGF). VEGF-A is a glycoprotein; its gene is located on chromosome 6p21.3. The state of hypoxia is one of the strongest inducers of VEGF-A expression. The expression level of VEGF-A mRNA is inversely proportional to the partial oxygen pressure outside the cell [6].

Zhanq *et al.* assessed the degree of VEGF receptor expression in kidney tumour tissue taken from 82 patients with metastatic papillary kidney cancer; they referred the results to clinical observations. VEGFR-1 receptor expression was seen in 82.9% of specimens; the VEGFR-2 receptor was detected in 63.4% of papillary cancer cases, VEGFR-3 in 34.1%, and PCNA in 67%. Increased expression of VEGFR-2 was correlated with tumour size, grading and the number of metastases. The degree of VEGFR-3 expression was related to the degree of malignancy, the degree of lymph node involvement and the number of metastases, but it was not correlated with patient age, gender, tumour size and clinical stage by TNM classification. However, the degree of VEGFR-1 receptor expression did not correlate with any clinical or pathological factor. The authors of this study have confirmed the predictive value of two of the tested receptors, VEGFR-2 and VEGFR-3, in papillary renal cell carcinoma [7].

Another example of a cellular hypoxia-inducible factor of potential prognostic value in renal cell carcinoma is chemokine type 4 receptor CXCR-4 (CXC chemokine receptor-4).

D'Alterio *et al.* evaluated the predictive value of the CXCR-4 receptor in 240 patients with renal cell carcinoma. A low level of expression was detected in 19.1% of patients, intermediate in 20%, and high in 60.8%. Multivariate analyses have confirmed that patient age, clinical characteristics and the degree of CXCR-4 expression have a significant prognostic value in RCC [8].

Another study involving a group of 223 patients with metastatic renal cell carcinoma showed the predictive value of the CXCR-4 and CXCR-7 receptors.

In the case of the CXCR-4 receptor, a low level of expression was seen in 18.8% of patients, intermediate in 31.9%, and high in 49.3%. In the case of the CXCR-7 receptor, a low level of expression was seen in 19.8% of patients, intermediate in 29.1%, and high in 51.1%. The high level of expression was a good predictor of shorter progression-free time. Both receptors examined in this study were independent predictors of metastatic renal cell carcinoma [9].

Another protein which is worth attention in our study, belonging to the group of hypoxia-inducible factors, is insulin-like growth factor II mRNA binding protein 3 (IMP3). It plays an important role as a promoter in cell proliferation during carcinogenesis, and its expression has been reported in many tumours [10].

Hoffmann *et al.* compared 5-year and 10-year progression-free survival in a group of patients with locally confined renal cell carcinoma. In the group of subjects with positive IMP3 expression (26% of cases), there was significantly lower 5-year and 10-year progression-free survival compared to the group without IMP3 expression [11].

Also, Jiang *et al.* in a similar study involving about 5000 papillary and chromophobe renal cell carcinoma cases obtained significantly shorter average survival and metastasis-free survival in the group of patients with IMP3 expression compared to patients without expression of this protein [12].

Increased IMP3 expression is seen in approximately 17% of renal cell cancer cases, and it was shown to be statistically correlated with the stage, grade and histological type of kidney cancer [12].

Another protein which could play an important role as an independent prognostic marker in metastatic renal cell carcinoma is a hypoxia-inducible factor 1α (HIF-1α). It is a protein which regulates the metabolism, growth, angiogenesis and metastatic spread of cancer. It also regulates the cell cycle and apoptosis [13].

Under physiological normoxic conditions, HIF-1α is inactivated within the proteasome. In hypoxic conditions,

HIF-1α accumulates in the cell due to inactivation or absence of the VHL suppressor gene, becoming a transcription factor for vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor receptor (EGFR), glucose transporters (GLUT-1), insulin-like growth factor (IGF), receptors for chemokines (CXCR) and carbonic anhydrases IX (CAIX) and XII (CAXII) [8].

At the University of California, Klatte *et al*. assessed the degree of HIF-1α expression in specimens collected from 357 patients during nephrectomy due to RCC. The highest expression of this protein was seen in the clear cell type of renal cell carcinoma. It was found that patients who had a higher level of HIF-1α expression, i.e. > 35%, had worse survival compared to patients with low protein expression (≤ 35%). In the first group of patients, the median survival was 13.5 months, whereas in the other group it was 24.4 months.

The researchers also showed that in the group of patients with clear cell renal cell carcinoma there is a correlation between HIF-1α expression and the expression of anhydrases CAIX and CAXII, apoptosis-regulating factors p21 and p53, mTOR pathway proteins p27, Akt and CXCR3, and proteins of the VEGF family.

In multivariate analysis, the degree of expression of HIF-1α and CAIX has proved to be an independent prognostic factor in patients with metastatic clear cell renal cell carcinoma [14].

Another group of markers which have a distinct mechanism of action, and may also have prognostic and predictive significance in kidney cancer, is a group of proteins associated with cellular adhesion. They include, among other factors, the epithelial cell adhesion molecule (EpCAM), epithelial membrane antigen (EMA), E-cadherin, α-catenin, and cadherin 6 [12].

From among these proteins, the epithelial cell adhesion molecule will be presented in this article. EpCAM is a glycosylated transmembrane protein of the epithelial cells, also known as KSA, KS1/4. It is commonly detected in many epithelial tumours including colon cancer, lung cancer, gastric cancer, pancreatic cancer, thyroid cancer, breast cancer, ovarian cancer, cervical cancer, bladder cancer and prostate cancer. In addition to these cancers, EpCAM has also been described in healthy tissues such as glandular tissue and the transitional epithelium. There is ongoing research on the use of this molecule to confirm the epithelial nature of tumours and to detect the presence of micrometastatic cancer [15]. An assessment of EpCAM expression in tumour tissue derived from 417 patients who underwent nephrectomy due to RCC from 1985 to 2000 was performed at the Medical School of University of California. The obtained results were compared to clinical data related to the cancer course in each patient. Among the tested specimens, 341 presented clear cell carcinoma, 42 papillary type carcinoma, 9 oncocytoma, and 8 chromophobe type. The follow-up period was 28 months. The average age of patients was 61 years (age range: 27–88 years), and the men to women ratio among the study subjects was 2 : 1. 49% of patients had metastatic disease at the time of cancer diagnosis. The progress of cancer was monitored based on subjective, objective and radiological examinations performed every 6–12 months. Based on the performed analysis of the group of patients with and without EpCAM protein in their tumour tissue, the following conclusions were made: in the first group of patients, the clinical stage of tumour by TNM classification was higher, the number of organs with metastases was higher, the baseline tumour size was larger, and the ECOG performance status was higher. High expression of EpCAM protein was seen in normal epithelium of the distal nephron, where it is typically present. The expression dominated in the cell membrane, rarely in the cytoplasm. The degree of EpCAM protein expression was higher in chromophobe renal cell carcinoma and in the histological subtype referred to as collecting duct carcinoma. Both types most likely derive from the distal nephron. However, in clear cell carcinoma, in which the original site is the proximal nephron, lower levels of EpCAM expression were seen. The lowest level of EpCAM expression was reported in tumour cells of sarcomatoid cancer. The researchers have shown that EpCAM protein may serve as a new prognostic and diagnostic molecular marker in patients with RCC. The positive degree of EpCAM expression in clear cell carcinoma can become an independent prognostic and predictive factor. It is associated with a higher degree of organ involvement by the neoplastic process, and with a higher risk of tumour recurrence in patients after nephrectomy with locally confined disease [15].

In the search for factors which could have potential prognostic and predictive value in patients with renal cell carcinoma, intensive research is also ongoing at the genetic level of tumour cells. An example is the study of Yao *et al*., evaluating the gene expression profile in 33 samples containing material collected from patients during nephrectomy due to RCC, and in 9 samples containing normal kidney tissue. The tests were performed using oligonucleotide microarrays. The objective was an attempt to identify biomolecular markers which could help in early diagnosis of cancer, or serve as potential prognostic factors in therapy.

The mean gene expression level was three times higher in clear cell renal cell carcinoma compared to chromophobe cancer or to the unchanged kidney tissue. Two of the tested genes were selected for further analysis. The first one was adipose differentiation-related protein (ADFP), also known as adipophilin, and the other was nicotinamide *N*-methyltransferase (NNMT). According to the results of tests based on quantitative PCR reaction, increased concentrations of ADFP and NNMT mRNA were observed significantly more frequently in clear cell renal cell carcinoma than in other subtypes of kidney cancer. What is more, patients with higher levels of ADFP mRNA presented a higher survival rate in univariate and multivariate analyses. The process of transcription of this gene is regulated by the von Hippel-Lindau pathway. A clear cell carcinoma cell contains many lipids and cholesterol. Probably the ADFP activation process as a result of VHL gene inactivation plays a significant role in the morphological appearance of clear cell renal cell carcinoma. It can be assumed that on the basis of these reports, the degree of ADFP expression could be used as a prognostic biomolecular marker in patients with clear cell renal cell carcinoma [16].

Modern prognostic markers are currently under intensive investigation. The preliminary reports presented in this article clearly show that these factors may play a significant

role in the prognostic assessment of metastatic renal cell carcinoma, and in the selection of appropriate causal treatments for this type of cancer.

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Address for correspondence

Beata Młot MD, PhD
Central Clinial Hospital
Military Institute of Medicine
Szaserów 128
00-909 Warszawa
e-mail: beatamlot@op.pl

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