A novel prognostic criteria and targeted therapy in Sarcomatoid variant of Chromophobic Renal Cell Carcinoma ~~Sarcomatoid variant of Chromophobic Renal Cell Carcinoma and outlining of targeted therapy in it~~

Abstract

Chromophobic renal cell carcinoma (CRCC) is a rare subtype of renal cell carcinoma, accounting for only 5.9% of epithelial kidney tumors. This study reports the findings studied in chromophobic renal cell carcinoma case with sarcomatoid differentiation in a 66-year-old patient admitted in Federal State Budgetary Institution (FSBI). This study concludes that, the criteria of aggressive behavior for chromophobic renal cell carcinoma include the following characteristics: the size of the tumor more than 7.0 cm; presence of necrosis; grade III according to Paner et al classification; sarcomatoid differentiation (more than 30.0%); positive reaction with common acute lymphocytic leukemia antigen (CD10); nuclear expression of p53 in more than 80.0% of tumor cells; proliferation marker Ki67 in more than 9.0% of tumor cells. In this case, the indication for targeted therapy was sarcomatoid differentiation (in more than 10.0% of the tumor) and a strong reaction with Vascular endothelial growth factor ; 5-6 points ~~VEGF-A~~ ((VEGF-A) ~~5-6 points).~~

Introduction.

Chromophobic renal cell carcinoma (CRCC) is a rare subtype of renal cell carcinoma, accounting for only 5.9% of epithelial kidney tumors. In World Health Organisation (WHO) classification, chromophobic renal cancer was included in 2004, and sarcomatoid transformation of this tumor, which was first described by Akhtar and et al. in 1997 [1], ~~it~~ is observed only in 9.0% of all CRCC cases [2]. The aim of this case report is to study the morphological features of sarcomatoid chromophobic renal cell carcinoma and to analyze the criteria for its aggressive behavior and outlining of clue for targeted therapy based on observation in the case study and review of literature.

Materials and methods.

The left side nephrectomy with resected descending colon and retroperitoneal lymphadenectomy was the specimen which was studied. The surgery was carried out in "Russian scientific center of surgery named after academician B. V. Petrovsky".

The tumor specimen was fixed in 10% neutral formalin, which on hardening were put into the paraffin. From each paraffin block, 5-7 microns thick sections were cut. The prepared paraffin sections were stained with hematoxylin and eosin. Immunohistochemical study was performed on sections from paraffin blocks. The slides were stained in automatic mode (Bond-Max, Leica) with the following antibodies: multicytokeratin (clone AE1/AE3, Dako), cytokeratin 7 (clone RN7, Leica), epithelial-related antigen (clone E29, Dako), CD117 (clone 104D2, Dako), E-cadherin (clone NCH-38, Dako), epithelioid antigen (clone MOC-31, Dako), BerEp4 (clone Ber-EP4, Dako), RCC (clone SPM314, Dako), CD10 (Dako, clone SS2/36), S100 (clone S1/61/69, Leica), CD15 (clone Carb-3, Dako), vimentin (clone V9, Dako), Smooth muscle antigen (SMA) (clone 1A4, Dako), α-1-antitrypsin (Polyclonal clone, Dako), CD68 (clone 514H12, Leica), Nonspecific Enolase NSE (clone BBS/NC/VI-H14, Dako), CD34 (clone QBEnd/10, Leica), VEGF-A (Gene Tex, clone EP1176Y), Ki67 (clone MIB-1, Dako), p53 (clone DO-7, Dako) (for the last two markers, the percentage of the number of tumor cells with nuclear expression among 1000 cells was determined in the sarcomatoid and carcinomatous parts of the tumor).

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The method of semi-quantitative determination of VEGF-a in the cytoplasm of tumor cells was used [3,4]. At the same time, at least 10 fields of sarcomatoid and carcinomatous areas in the tumor were studied with magnification x400, the number of VEGF-positive tumor cells was calculated: 0 – no staining, 1 point (1-25% positive cells), 2 points (26-50% positive cells), 3 points (more than 50% positive cells). The intensity of VEGF receptor staining was estimated: 0- no staining, 1 point (weak staining), 2 points (moderate staining), 3 points (strong staining). Scores of the number of positive cells and staining intensity of VEGF-A are summarized. The score was divided into: 0 (negative reaction), 1-2 (weak reaction), 3 (moderate reaction), from 4 to 6 (strong reaction).

Case Report.

We report our findings studied in chromophobic renal cell carcinoma case with sarcomatoid differentiation. A 66-year-old patient was admitted in FSBI "Russian scientific center of surgery named. Acad. B. V. Petrovsky " on July 6, 2017. Since March 2017, patient noted the appearance and rapid growth of tumor formation in the left half of the abdomen. Ultrasound examination and computed tomography revealed that the patient had a volumetric heterogeneous formation with uneven contours originating from the left kidney, measuring 35.0 cm in size.

On 10 July 2017, the patient underwent left nephrectomy with resection of the descending colon and widened retroperitoneal lymphadenectomy. During exploration it was noticed that the entire left half of the abdomen was occupied by a solid tumor of the size of 35,0x35,0x20,0cm, the descending colon was sprawled on the lateral edge of the neoplasm. The upper pole of the tumor was seen to be extending from the lower edge the body and tail of the pancreas till the the spleen.

The gross specimen comprised of part of the colon of length of 20.0 cm, adipose tissue and kidney and the overall size of the mass was 45,0x35,0x18,0 cm. In the cut section, the renal tissue was found to be replaced by gray-brown mass, of the size 40,0x29,0x16,0 cm (figure 1A), with light brown patch of mass found to be extending in the renal pelvis, and the renal vein. The tumor mass showed multiple foci with necrotic changes. The maximum thickness of uninvolved renal tissue at the periphery of the tumor mass was 1.5 cm. The tumor had a soft and spongy texture, visually extending into the wall of the colon, without changing its mucous layer. Separately, para-aortic lymph nodes and fatty tissue were also received and 6 lymph nodes varying from 0.5 to 4.0 cm size were found in dissected mesentery.

On microscopic examination, the sections from tumor mass showed heterogenous areas, with alternation of epithelial and sarcomatoid differentiation (figure 1B). More than 80% of epithelioid sites comprised of large polygonal cells with light foamy cytoplasm, forming solid, trabecular and alveolar patterns. The cell membrane was clearly visible and resembled - the cells of plant origin (figure 1B). Epithelial cells were smaller in size with eosinophilic granular cytoplasm present in a small amount. The nuclei of both types of cells were hyperchromic, wrinkled, with coarse chromatin and noticeable nucleoli. In appearance, the nuclei of tumor cells were similar to raisins (raisinoid nuclei). Around the nuclei there was an area of enlightenment (perinuclear halo) (figure 1B). Mitosis in the epithelioid areas of the tumor were not determined. Adjacent to the sarcomatous area there was an increase in the nuclear-cytoplasmic ratio (nuclei enlarged 3 or more times), uneven distribution of chromatin and cell aggregation with fusion of nuclei (figure 1G). Thin and wide fibrous septa, focal infiltration by lymphocytes, macrophages and eosinophils, as well as medium-sized blood vessels with thickened walls were seen in the stroma.

Sarcomatoid component of the tumor mass occupied about 70.0% of renal tissue. Areas of the tumor infiltrating the wall of the colon (figure 1D), as well as lymph nodes with metastases (figure 1E) (4 of 6 lymph nodes) showed sarcoma-like changes showing packed spindle-shaped

cells with polymorphic or multi-lobed nuclei and large number of mitoses. There were seen double-nuclei and multinucleated cells resembling tuton cells (figure 2A). In the intervening stroma were seen the necrotic changes and focal hemorrhages (figure 2B).

Immunohistochemical study of the carcinomatous component of the tumor showed positive reaction with the following markers: cytokeratin 7 (membrane expression) (figure 2B), epithelial membrane antigen, CD117 (expression in the cytoplasm and on the cell membrane) (figure 2G),E-cadherin (figure 2D), MOC-31, BerEp4. There was a significant negative reaction seen with the following markers: RCC, CD10, vimentin, S100, CD15. Cells in sarcomatoid areas of the tumor expressed vimentin (figure 2E), SMA, CD10 (figure 2G), α-1-antitrypsin (figure 2B), CD68 (multinucleated and Tuton like cells) (figure 2i). There was found a negative reaction with the following markers: RCC, CD117, cytokeratin 7-type, NSE, CD34. Ki67 proliferation index in carcinomatous component of tumor was equal to 2.0-5.0% (figure 3A), at the border with sarcomatoid sites – 20.0-30.0% (figure 3b), in sarcomatoid component – 70.0% (figure 3b). p53 in the carcinomatous component of the tumor was found in 20.0% of cells (figure 3G), adjacent to sarcomatous area, tumor cells were 60.0% (figure 3D), in the sarcomatoid component, there were 85.0% of tumor cells (figure 3E). In carcinomatous component there was a significant negative response with VEGF-A (figure 3G), in areas adjacent to sarcomatoid areas – there was seen weak response with VEGF-A (ballroom 2) (figure 3ɜ). The sarcomatoid component showed a strong reaction with VEGF-A (score 5) (figure 3i).

Correlating the clinical data with histopathological and immunohistochemical data, the results of our study concluded that the final diagnosis of our case was chromophobic renal cell carcinoma, with sarcomatoid differentiation, infiltrating into the muscle layer of the descending colon and metastasing into 4 lymph nodes of the paranephric fat.

Discussion:

Each year, more than 40,000 new patients with renal cell carcinoma are reported in the United States [5], of which 3,000 patients have histopathological findings suggestive of chromophobic renal cell carcinoma [6]. Chromophobic renal cell carcinoma was first described by Thoenes et al. in 1985 [7].

chRCC is a distinctive type of renal neoplasm that was 1st described by in 1986.[8-12] Histologically, two variant has been described, the classical and eosinophilic variant.[9]Renal NET are extremely rare .[12] Only 62 cases of renal carcinoid tumor have been reported and primary small cell carcinoma of the kidney is even rarer .[12] Only one case of large cell neuroendocrine of the kidney has been described.[12]. Different theories suggest that NETs in the kidney may arise from primitive totipotential stem cells that subsequently differentiate in a neuroendocrine direction.[12]NET of the kidney has been reported in association with chRCC.[8- 10] The association between chRCC and neuroendocrine carcinoma was 1st reported in 2008 by Parada and Pena.[9] .Roy *et al.* in their study have reported a composite tumor of the kidney .[10] .One mass showed the histological and the immunohistochemical characteristic of chRCC while the other separate mass was a carcinoid tumor.

In comparison with other subtypes, chromophobic renal cell carcinoma ~~these cancers~~ have the best prognosis and are rarely progressive and they rarely metastasize. Distant metastases are described only in 4.0% of cases of chromophobic renal cell carcinomas [7]. 5-year survival rate of patients with CRCC is 96.0%. However, in the presence of sarcomatoid differentiation, the prognosis is only 35.0%, and 2-year survival is seen in 25.0% cases ~~[8~~13, ~~9~~14]. Renal tumors with sarcomatoid features were originally called sarcomas, a~~. A~~nd the majority of them were seen against the background of renal cell carcinoma (RCC). Therefore, such tumors were called sarcomatoid RCC, which were categorized as a separate subgroup [~~10~~15]. Most reports ~~states~~ indicate frequency of sarcomatoid renal tumors to be 1.0-9.0%, however, it varies greatly depending on the stage of renal cell cancer [~~10~~15]. In patients with stage 4, 5.0-20.0% of tumors

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has sarcomatoid differentiation, and they often metastases. The probability of metastasis is very high, if more than 30.0% of the primary tumor consists of sarcomatoid cells [~~10~~15]. The incidence of sarcomatoid differentiation also depends on the histological type of tumor. Sarcomatoid elements occur in 3.0% of papillary RCC, 8.0% of light-grade RCC and 9.0% of chromophobe type of cancers [2]. Chromophobic renal cancers with sarcomatoid differentiation are most often metastasized into lungs, subclavian lymph nodes, mediastinum, liver and pelvic bones [~~11~~16].

Most often, the sarcomatoid part of CRCC is represented by malignant fibrous histiocytoma or fibrosarcoma. However, there may be other subtypes of sarcomatous tissues like osteosarcomatous, chondromatous and rhabdomyosarcomatous types. They were first described by Hes et al. in 1999 [1]. The distribution of sarcomatoid areas in the tumor may be monomorphic or heterogeneous [~~12~~17], with sarcomatoid elements ranging from 1.0 to 100.0% CRCC (in most cases - less than 50.0%) [~~13~~18]. An important feature of chromophobe renal cell carcinoma is the mutation of the transcription factor p53 (in 32.0-42.3% of all CRCC cases), which plays an important role in the sarcomatoid transformation of the tumor [6, ~~14~~19]. Sarcomatoid component has a higher mutation rate of p53 than carcinomatous component (79.0% and 14.0%, respectively). The presence of mutation p53 can be seen with pronounced nuclear expression in more than 80.0% of tumor cells [~~14~~19, ~~15~~20]. At the same time, not only by immunohistochemical detection method but p53 expression results were also confirmed by molecular genetic studies in 85.0% of cases [13]. In our own observation, the number of tumor cells expressing p53 was 85.0% in the sarcomatoid component and 20.0% in the carcinomatous component.

Types of mutations seen typically in CRCC are: Von Hippel-Lindau (VHL) (34,6%), cyclin-dependent kinase Inhibitor 2A (CDKN2A) (26,9%), NF2 (19,2%) [12]. B-Raf Proto-Oncogene (BRAF) and Kirsten Rat Sarcoma (KRAS) gene mutations can be detected in 20.0% of cases [~~16~~21].

In addition to sarcomatoid differentiation and high frequency of p53 expression, the signs of aggressive behavior of chromophobe type of renal cell carcinomas are tumor size over 7.0 cm, necrosis [~~11~~16], proliferation index over 9.0% [3, ~~11~~16]. In our case Ki67 expression was ~~seen~~ detected in 2.0-5.0% of carcinomatous component and 70.0% of sarcomatoid component. The approximate size of the involved area was 40,0x29,0x16, 0 cm and the tumor showed marked necrotic changes.

~~In m~~Majority of cases chromophobic renal cell carcinoma, unlike clear cell carcinoma, did not expresses CD10. However, this marker was found positive in 26.0% of CRCC cases in one study (including in the tumor cells of our case), which is a sign of aggressive behavior of the tumor [~~17~~22]. At the same time, the internal control can be ~~seen~~ observed as a strong membrane staining of CD10 in the epithelium of proximal tubules and glomeruli, as well as in the Bowman's capsule [~~18~~23].

In contrast to the above sign's hyperchromatic nuclei, nuclear polymorphism, and the visualization of the nucleoli do not have a predictive value. However, based on these histopathological features according to Furman classification, 80.0% of CRCC are estimated as grade III or grade IV [~~11~~16].

In 2010, Paner et al. suggested a 3-point system for evaluation of Chromophobic type of renal cell carcinoma, which more accurately reflects the stage and outcome of the disease (table 2) [~~19~~24]. According to this classification 74,0% of Chromophobic type of renal cell carcinoma has~~ve~~ the first degree of differentiation (grade I). It is important to note that the first and second degree of differentiation of CRCC is not related to the clinical outcome of the disease. Only the

third degree of differentiation reflects a high probability of disseminated cancer or recurrence [~~19~~24].

Table 2: 3-point system for the evaluation of Chromophobic type of renal cell carcinoma (Classification by Paner et al. [~~19~~24]

Histological findings	Grade I	Grade II	Grade III
Uneven distribution of tumor cells	_	+	+
Nuclear anaplasia	Size uneven, with raisin-like surface wrinkles	Certain nuclear polymorphism	Intensive anaplasia, multilobular nuclei
Increased nuclear size in the tumor cells	_	Nuclear size increase more 3 times	Gigantic tumor cells
Heterogeneity of nuclear chromatin	_	+	+
Contact of tumor nuclei	_	+	+
Sarcomatoid tumor cells	_	_	+

The fact of presence of heterogeneous components with carcinomatous and sarcomatoid elements present in the tumor in our case is interesting. The signs of the first, second and third degree of differentiation according to the classification of Paner et al were noted [~~19~~24]. The invasive component of the tumor with lesions in the colon, as well as lymph nodes with metastases were presented exclusively in grade III.

The prognostic significance of the Paner et al [~~19~~24] classifications is questioned as it is critiqued that an additional criterion for grading of CRCC [~~20~~25] is required. According to the recommendations of International Society of Urological Pathology (ISUP 2013), CRCC is not graded yet, however, studies have concluded that the percentage of sarcomatoid elements in the tumor is necessarily considered as an essential criterion [~~20~~25]. According to the literature, it is believed that renal cell carcinoma is resistant to chemotherapy. However, sarcomatoid CRCC have highly effective targeted therapies that work by inhibiting the VEGF (vascular endothelial growth factor) [4]. Proteins belonging to the VEGF family are glycoproteins that stimulate the formation of new blood vessels and lymph vessels and increase vascular permeability. The family includes 6 growth factors: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PLGF) [~~21~~26]. VEGF-A plays an important role in pathological angiogenesis [4]. Under its influence the tumors are formed with abnormally branched blood vessels that imbalance the ratio of the number of arterioles, veins and capillaries. A wide gap ~~Between the endothelial cells~~ is formed ~~a wide gap~~ between the endothelial cells, through which the plasma flows into the tumor tissue. As a result, compression of the tumor blood vessels occurs and hypoxia develops [~~22~~27].

About 4% of RCC occur within the context of Von Hippel-Lindau disease and it is the most common cause of hereditary renal cell carcinomas [28]. The Von Hippel-Lindau tumor suppressor gene is located on the short arm of the chromosome 3, in the 3p 25-26 locus of the human genome [28,29]. VHL tumor-suppressor gene has been shown to be mutated in both, familial as well as sporadic renal cell carcinoma [29].The VHL gene encodes a protein known as VHL gene product or VHL protein (pVHL) that appears to play role in regulating several aspects of cellular function [30,31]. The pVHL protein exerts its functions through two domains that

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allow it to interact with various cellular proteins, such as elongins, fibronectin and hypoxia-inducible factor (HIF-1 [30,31,32]. Functional alterations can cause the protein to lose its tumour suppressor capacity, potentially triggering the genesis of renal cell carcinomas. One of the targets of the complex containing VHL protein (pVHL) is hypoxia-inducible factor 1(HIF-1) [32]. When VHL gene is mutated, HIF-1 level remains high, and this constitutively active protein increases the transcription and production of hypoxia-inducible factor, proangiogenic proteins such as VEGF (vascular endothelial growth factor) and TGF-alpha(Transforming growth factor-alpha) [33].Thus, both cell growth and angiogenesis are stimulated leading to formation of the VHL associated renal cell carcinomas.The VHL gene acts as a tumor-suppressor gene in both sporadic and familial renal cell carcinomas.

Based on cytogenetics, and histology, both familial and sporadic renal cell carcinomas are classified as- clear cell carcinoma, papillary carcinoma, chromophobe renal cell carcinoma and collecting duct carcinoma. The mutations of the VHL gene are associated with the development of clear cell renal cell carcinomas and chromophobe variety of renal cell carcinomas. 25 per cent of chromophobe renal cell carcinomas shows VHL gene mutation [34]. Various cytogenetic and molecular studies have been performed to detect VHL gene mutation in sporadic and familial renal cell carcinoma but there are very few studies that analysed VHL expression at the protein level by detecting the cellular localization of pVHL within human tissues [35]. Present study detected VHL protein (pVHL) in the tissue of renal cell carcinoma by using monoclonal antibodies.

CDKN2A pathway" Alterations in CDKN2A is identified as an oncogenic pathway of importance across the RCC spectrum. A variety of mechanisms were identified that could inactivate CDKN2A, including mutations in the gene and hypermethylation of the CDKN2A promoter. Among the CDKN2A-altered tumors, survival rate was found to be decreased. Thus, in both papillary and clear cell RCC, tumors with CDKN2Aalterations correlate with aggressive subtypes, therefore strategies to target CDKN2A biology may prove useful across the kidney cancer spectrum(36,37).

BRAF and KRAS belong to the RAF proto-oncogene serine / threonine-protein kinase (c-RAF) gene family and their over expression or mutations trigger abnormal cell proliferation. Kamai et al. (38) evaluated the association of KRAS in RCC. Of the 51 patients, mRNA expression of KRAS were significantly high (38). Kozma et al. (39) analyzed 36 RCC samples for KRAS amplification. The authors reported that the amplifications correlated with tumor grade and size but not with lymph node involvement. In a comprehensive analysis of 121 RCC samples, KRAS and BRAF did not reveal any mutations (40). In a multicenter study, Szymanska et al. (41) investigated the correlation between KRAS (codon 12) mutation and Von Hippel-Lindau (VHL) gene in tissue samples derived from 361 RCC (334 clear-cell carcinomas) patients. The authors observed VHL mutations. KRAS mutations were not detected in any patients. The authors concluded that KRAS mutations do not have a major contribution to RCC development, provided that the VHL gene is not inactivated (41).

It is known that the frequency and intensity of VEGF staining increases with an increase in the stage of renal cell carcinoma, with the invasion of the tumor into the pararenal fatty tissue and renal vein [4, ~~23~~38]. The concentration of VEGF reaches a maximum at 2nd and 3rd degree of differentiation according to the Furman classification, but reduced in 4th degree, especially when there is sarcomatoid differentiation seen in tumor [3]. According to other studies, the 4th degree of tumor differentiation by Furman is accompanied by an increase in VEGF expression [4, ~~24~~39]. In targeted therapy, VEGF suppression is overwhelming when sarcomatoid CRCC therapy include bevacizumab (a monoclonal antibody to VEGF-A) and sunitinib (which belongs to the tyrosine kinase inhibitors, drug is the 1st line drug therapy for CRCC) [~~12~~17, ~~25~~40]. Anti-VEGF drugs block the growth of abnormal blood vessels, reduce their density~~,~~ and ~~reduce~~ the size of gaps between endothelial cells [~~22~~27]. At the same time, the concentration of the targeted

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drug is very important, as well as its ratio to the amount of VEGF. With a high concentration of the drug or a low content of VEGF, excessive "pruning" of blood vessels occurs, which leads to hypoxia in the tumor and dissemination of the cancer cells [~~26~~41]. It is known that in cases of CRCC with sarcomatoid differentiation, when treatment is done with sunitinib in combination with gemcitabine, 63.0% of cases showed a complete response or stabilization of the disease [~~26~~41, ~~27~~42].

It is studied that the number of sarcomatoid cells is important for determining the treatment protocol. Chemotherapy with tyrosine kinase inhibitors should be performed only in cases when sarcomatoid elements are more than 10.0% in these tumor [~~27, 28~~42].

Currently, in CRRC with sarcomatoid differentiation, renal cell carcinoma has a correlation between the degree of expression of VEGF and the effectiveness of anti-VEGF targeted drugs. According to some studies before chemotherapy it is very important to assess the level of expression of VEGF-A. Only a strong expression of VEGF-A (5-6 points) has prognostic value and hence it is a marker of treatment efficacy for targeted drugs [4]. Ano~~O~~ther study states that the degree of response to treatment with bevacizumab does not correlate with the expression level of VEGF-A [~~29~~43]. It is possible that such contradictory results are responsible for ~~making it impossible~~the impossibility ~~to use~~of using Furman classification for Chromophobe renal cell carcinomas.

Treatment with tyrosine kinase inhibitors sometimes leads to necrosis and cavitation in the tumor without changing its size. As a result, when computed tomography is done, an erroneous conclusion about the lack of effectiveness of therapy is interpreted. Keeping it in mind, attempts are being made to use an alternative method like immunohistochemical expression of VEGF to assess the therapeutic response in sarcomatoid variant of chromophobic renal cell carcinoma [~~30~~44]. In our study due to presence of sarcomatoid differentiation (grade III), a ~~-~~strong reaction with VEGF-A (score 5) was observed. Hence, the patient was referred to Cancer institution for anti-VEGF therapy.

Table 3. Expression levels of VEGF, p53 and Ki67 in chromophobic renal cell carcinoma with sarcomatoid features in areas according to the classification of Paner et al. [~~19~~24]. (in our study is as follows)

Stage of differentiation	VEGF-A			P53, %	Ki67, %
	Number of positive cells (scores)	Color intensity, (scores)	Summary of scores		
I	0	0	0	20	2-5
II	1	1	2	60	20-30
III	3	2	5	85	70

Clear expression of prognostic markers based according to the classification of Paner et al. indicates its important role in evaluating the effectiveness of treatment with tyrosine kinase inhibitors and bevacizumab.

In summary, we describe an interesting case of chRCC with an aggressive component and suggest the use of a modified adjuvant therapy.

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Conclusion:

Thus our study conclude that, the criteria of aggressive behavior for chromophobic renal cell carcinoma– include the following characteristics: the size of the tumor more than 7.0 cm; presence of necrosis; grade III according to Paner et al classification; sarcomatoid differentiation (more than 30.0%); positive reaction with CD10; nuclear expression of p53 in more than 80.0% of tumor cells; Ki67 in more than 9.0% of tumor cells. In our case, the indication for targeted therapy was sarcomatoid differentiation (in more than 10.0% of the tumor) and a strong reaction with VEGF-A (5-6 points).

References:

1. Quiroga-Garza G, Khurana H, Shen S, Ayala AG, Ro JY. Sarcomatoid chromophobe is renal cell carcinoma with heterologous sarcomatoid elements. A case report and review of the literature. Arch Pathol Lab Med. 2009;133 (11):1857-1860. https://doi:10.1043/1543-2165-133.11.1857

2. de Peralta-Venturina M, Moch H, Amin M, Tamboli P, Hailemariam S, Mihatsch m, Javidan J, Stricker H, Ro JY, Amin MB. Sarcomatoid differentiation in renal cell carcinoma: a study of 101 cases. Am J Surg Pathol. 2001; 25(3):275-284. The link is active on 12.01.2018. https://www.ncbi.nlm.nih.gov/pubmed/?term=Sarcomatoid+differentiation+in+renal+cell+carcinoma%3A+a+study+of+101+cases

3. Ebru T, Fulya OP, Hakan a, Vuslat YC, Necdet S, Nuray C, Filiz O Analysis of various potential prognostic markets and survival data in clear cell renal cell carcinoma. Int Braz J Urol. 2017;43 (3):440-454.
https://doi: 10.1590 / S1677-5538.IBJU.2015.0521

4. Osman WM, Youssef NS. Combined use of COX-1 and VEGF immunohistochemistry refines the carried out a histopathologic prognosis of renal cell carcinoma. Int J Clin Exp Pathol. 2015; 8(7)8165-8177.
The link is active on 12.01.2018. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4555713/

5. Chowdhury S, Matrana MR, Tsang C, Atkinson B, Choueiri TK, Tannir NM. Systematic therapy for metastatic non-clear cell renal cell carcinoma: recent progress and future directions. Hematol Oncol Clin North Am. 2011;25 (4):853-869. https://doi: 10.1016/j.hoc.2011.05.003

6. Yang Y, Vocke CD, rickets CJ, Wei D, Padilla-Nash HM, Lang M, Sourbier C, Killian JK, Boyle SL, Worrell R,Meltzer PS, reed T, Merino MJ, Metwalli AR, Linehan WM. Genomic and metabolic characterization of chromophobe is a renal cell carcinoma cell line model (UOK276). Genes Chromosomes Cancer. 2017;56 (10):719-729. https://doi: 10.1002 / gcc.Twenty-two thousand four hundred seventy six

7. Zhang Z, Min J, Yu D, Shi H, Xie D. Renal collision tumour of papillary cell carcinoma and chromophobe cell carcinoma with sarcomatoid transformation: a case report and review of the literature. Can Urol Assoc J. 2014; 8(7-8):E536 -- 9. https://doi: 10.5489 / cuaj.One thousand eight hundred eleven

8. Kuroda N, Tamura M, Hes O, Michal M, Gatalica Z. Chromophobe renal cell carcinoma with neuroendocrine differentiation and sarcomatoid change. Pathol Int. 2011;61:552–4.

9. Parada DD, Peña KB. Chromophobe renal cell carcinoma with neuroendocrine differentiation. APMIS. 2008;116:859–65.

10. Roy S, Hooda S, Huang GJ, Pantanowitz L, Parwani AV. A novel case of concurrent renal tumors: Chromophobe renal cell carcinoma and carcinoid tumor of the kidney with brief review of renal neuroendocrine tumors. Int J Surg Pathol. 2012;20:531–5.

11. Nagashima Y. Chromophobe renal cell carcinoma: Clinical, pathological and molecular biological aspects. Pathol Int. 2000;50:872–8.

12. Lane BR, Jour G, Zhou M. Renal neuroendocrine tumors. Indian J Urol. 2009;25:155–60.

813. Urological Surgical Pathology. - 3rd ed./edited by David G. Bostwick, Liang Cheng. Philadelphia: Elsevier; 2014.
914. Tanaka Y, Koie T, Hatakeyama S, Hashimoto Y, Ohyama C. chromophobe is renal cell carcinoma with concomitant sarcomatoid transformation and osseous metaplasia: a case report. BMC Urol. 2013;13: 72. https://doi: 10.1186/1471-2490-13-72
1015. Shuch B, Said J, LaRochelle JC, Zhou Y, Li G, Klatte T, Pouliot F, Kabbinavar FF, Belldegrun as, Pantuck AJ. Histologic evaluation of metastases in renal cell carcinoma with sarcomatoid transformation and its implications for systemic therapy. Cancer. 2010;116 (3):616-624. https://doi: 10.1002 / cncr.24768 19998348
1116. Practical renal pathology: a diagnostic approach/ edited by Donna J. Lager, Neil A. Abrahams. Philadelphia: Elsevier; 2013.
1217. Zhang t, Gong J, Maia MC, Pal SK.Systematic Therapy for Non-Clear Cell Renal Cell Carcinoma. Am Soc Clin Oncol Educ Book. 2017; 37:337-342. https://doi: 10.14694/
1318. Shuch B, Bratslavsky G, Linehan WM, Srinivasan R. Sarcomatoid renal cell carcinoma: a comprehensive review of the biology and current treatment strategies. Oncologist. 2012;17 (1):46-54. https: / / doi: 10 .1634 / theoncologist .2011-0227
1419. Oda H, Nakatsuru Y, Ishikawa T. Mutations of the p53 gene and p53 protein overexpression are associated with sarcomatoid transformation in renal cell carcinomas. Cancer Res. 1995; 55(3):658-662. The link is active on 12.01.2018. https://www.ncbi.nlm.nih.gov/pubmed/?term=7834636
1520. Cserni G, Kovács BR, Tarján m, Sápi Z, Domján Z, Szabó Z. Sarcomatoid renal cell carcinoma with foci of chromophobe carcinoma. Pathol Oncol Res. 2002; 8(2):142-144. https://doi: PAOR.2002.8.2.0142
1621. Wu J, Joseph SO, Muggia FM. Targeted therapy: its status and promise in selected solid tumors part I: areas of major impact. Oncology (Williston Park). 2012;26 (10):936-943. The link is active on 12.01.2018. https: / / www. ncbi.nlm.nih.gov/pubmed/?term=23176005
1722. Kobayashi N, Suzuki K, Murakami H, Kagawa E, Aoki I, Nagashima Y. chromophobe is renal cell carcinoma with sarcomatoid transformation in a dog. J Vet Diagn Invest. 2010;22 (6):983-987. https://doi: 10. 1177 /10406387002200624
1823. Martignoni G, Pea M, Brunelli M, Chilosi M, Zamó a, Bertaso M, Cossu-Rocca P, Eble JN, Mikuz G, Puppa G, Badoual C,Ficarra V, Novella G, Bonetti F. CD10 is expressed in a subset of chromophobe renal cell carcinomas. Mod Pathol. 2004; 17(12):1455-1463. https://doi:10.1038/modpathol.Three million eight hundred thousand two hundred thirty six
1924. Paner GP, Amin MB, Alvarado-Cabrero I, Young an, Stricker HJ, Moch H, et al. A novel tumor grading scheme for chromophobe renal cell carcinoma: prognostic utility and comparison with Fuhrman nuclear grade. Am J Surg Pathol. 2010; 34: 1233-1240. https://doi: 10.1097 / PAS.0b013e3181e96f2a
2025. New approaches to the classification, gradation and forecast pochernkletocny cancer Moskvina L. V., Andreeva Yu. Yu., Malkov P. G., Frank G. A. New approaches to the classification, gradation and forecast pochernkletocny cancer. Archives of pathology. 2014; (76)2: c.60-70. The link is active on 12.01.2018. [Moskvina L. V., Andreeva Yu.Yu. , Malkov P. G., Frank G. A. New approaches to the classification, grading, and prognosis of renal cell carcinoma. Arkhiv patologii. 2014; (76)2: p.3.60-70. (InRuss.).] https: / / www. mediasphera.ru /issues /arkhiv-patologii/2014/2/downloads/EN/030004-19552014214
2126. Nefedova N. A. Davydov S. Yu., the Role of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) in tumor angiogenesis. Modern problems of science and education. 2015;3: c.51-64. The link is active on 12.01.2018. [Nefedova N. A., Davydova S. Yu. The role of vascular endothelial growth factor and hypoxia-inducible factor in tumor ' s angiogenesis. Sovremennye problem nauki I obrazovaniya. 2015;3: c.51-64.(InRuss.)] https://science-education. EN/ EN/ article / view?id=17924

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~~22~~27. Varlamov A.V., Paltseva E. M., Sekacheva M. I., Skipenko O. G., Fedorov D. N. The influence of preoperative drug therapy on the expression of angiogenesis markers in colorectal cancer metastases in the liver. Archives of pathology. 2017;79 (1):c.36-42. ~~https://doi: 10.17116 / patol201779136-42 [Varlamov AV, PAL'tseva EM, Sekacheva MI, Skipenko OG, Fedorov DN Impact of preoperative drug therapy on the expression of angiogenesis markers in colorectal liver metastases. Arkhiv patologii. 2017; (79) 1: p.3.36-42. (InRuss.)]. https://doi: 10.17116 / patol201779136-42~~

28. Iliopoulos O, Eng C. Genetic and clinical aspects of familial renal neoplasms. Semin Oncol. 2000 Apr; 27(2):138-49. [PubMed]

29. Rohan SM, Xiao Y, Liang Y, Dudas ME, Al-Ahmadie HA, Fine SW, Gopalan A, Reuter VE, Rosenblum MK, Russo P, Tickoo SK. Clear-cell papillary renal cell carcinoma: molecular and immunohistochemical analysis with emphasis on the von Hippel-Lindau gene and hypoxia-inducible factor pathway-related proteins. Mod Pathol. 2011 Sep; 24(9):1207-20. doi: 10.1038/modpathol.2011.80. Epub 2011 May 20.

30. Giménez-Bachs JM, Salinas-Sánchez AS, Sánchez-Sánchez F, Lorenzo-Romero JG, Donate-Moreno MJ, Pastor-Navarro H, García-Olmo DC, Escribano-Martínez J, Virseda-Rodríguez JA. Determination of vhl gene mutations in sporadic renal cell carcinoma. Eur Urol. 2006 Jun; 49(6):1051-7. Epub 2005 Dec 20.

31. Duan DR1, Humphrey JS, Chen DY, Weng Y, Sukegawa J, Lee S, Gnarra JR, Linehan WM, Klausner RD. Characterization of the VHL tumor suppressor gene product: localization, complex formation, and the effect of natural inactivating mutations. Proc Natl Acad Sci U S A. 1995 Jul 3; 92(14):6459-63.

32. Turner KJ, Moore JW, Jones A, Taylor CF, Cuthbert-Heavens D, Han C, Leek RD, Gatter KC, Maxwell PH, Ratcliffe PJ, Cranston D, Harris AL. Expression of hypoxia-inducible factors in human renal cancer: relationship to angiogenesis and to the von Hippel-Lindau gene mutation. Cancer Res. 2002 May 15; 62(10):2957-61.

33. Linehan WM, Rubin JS, Bottaro DP. VHL loss of function and its impact on oncogenic signaling networks in clear cell renal cell carcinoma. Int J Biochem Cell Biol. 2009 Apr; 41(4):753-6. doi: 10.1016/j.biocel.2008.09.024. Epub 2008 Oct 2.

34. Kenck C, Wilhelm M, Bugert P, Staehler G, Kovacs G. Mutation of the VHL gene is associated exclusively with the development of non-papillary renal cell carcinomas. J Pathol. 1996 Jun; 179(2):157-61.

35. Corless CL, Kibel AS, Iliopoulos O, Kaelin WG Jr. Immunostaining of the von Hippel-Lindau gene product in normal and neoplastic human tissues. Hum Pathol. 1997 Apr; 28(4):459-64.

36. Brannon AR, Reddy A, Seiler M, Arreola A, Moore DT, Pruthi RS, et al. Molecular Stratification of Clear Cell Renal Cell Carcinoma by Consensus Clustering Reveals Distinct Subtypes and Survival Patterns. Genes Cancer. 2010;1(2):152–63.

37. Brannon AR, Haake SM, Hacker KE, Pruthi RS, Wallen EM, Nielsen ME, et al. Meta-analysis of clear cell renal cell carcinoma gene expression defines a variant subgroup and identifies gender influences on tumor biology. European urology. 2012;61(2):258–68.

~~23~~38. Song SH, Jeong IG, you D, Hong JH, Hong B, Song C, Jung WY, Cho YM, Ahn H, Kim CS.VEGF/VEGFR2 and PDGF-b / PDGFR-β expression in nonmetastatic renal cell carcinoma: a retrospective study in 1,091 conservative patients. Int J Clin Exp Pathol. 2014; 7(11):7681-7689. ~~The link is active on 12.01.2018. https: / / www. ncbi. nlm. nih. Gov /pubmed/?term=VEGF%2FVEGFR2+and+PDGF-B%2FPDGFR--% CE% B2+ expression+in + non-metastatic +renal +cellcarcinoma %3Aa+retrospective+study+in+1%2C091+constructive+patients~~

~~24~~39. Minardi d, Santoni M, Lucarini G, Mazzucchelli R, Burattini l, Conti a, Bianconi M, Scartozzi M, Milanese G,Primio RD, Montironi R, Cascinu S, Muzzonigro G. Tumor VEGF expression correlates with tumor stage and identities prognostically different groups in patients

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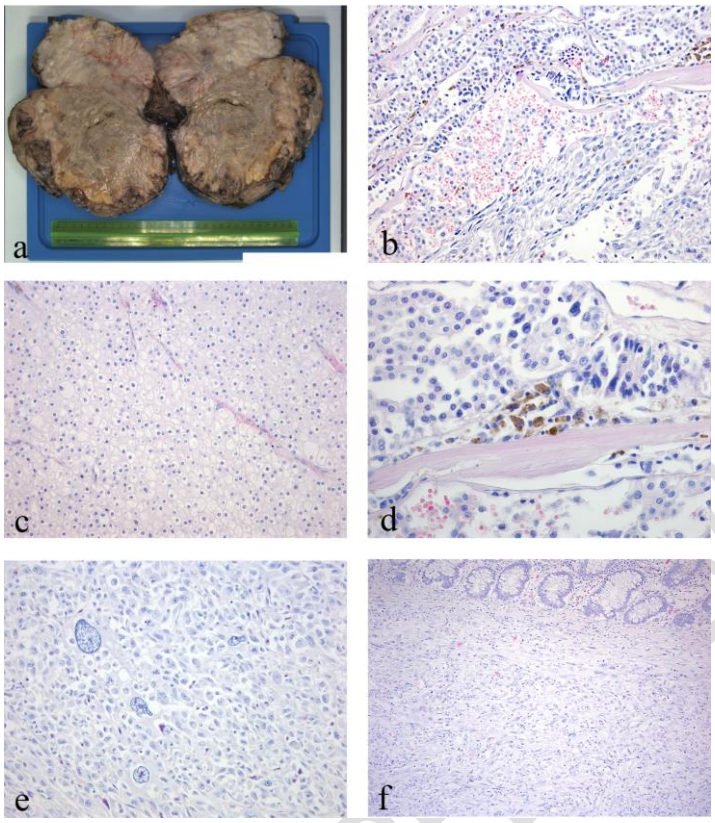
with clear cell renal cell. Urol Oncol. 2015; 33 (3):113.e1-7. https://doi: 10.1016 / j. urolonc. 2014.06.014
~~25~~40. Stubbs C, Bardoli AD, Afshar M, Pirrie S, Miscoria M, Wheeley I, Porfiri E. a Study of Angiogenesis Markers in Patients with Renal Cell Carcinoma Undergoing Therapy with Sunitinib. Anticancer Res. 2017;37 (1):253-259. https://10.21873/anticanres.Eleven thousand three hundred fifteen
~~26~~41. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011; 473 (7347):298-307. ~~https://doi: 10.1038 /nature 10144~~
~~27~~42. Michaelson MD, McKay RR, Werner L, Atkins MB, Van Allen EM, Olivier KM, Song J, Signoretti S, McDermott DF, Choueiri TK. Phase 2 trial of sunitinib and gemcitabine in patients with sarcomatoid and/or poor-risk metastatic renal cell carcinoma. Cancer. 2015;121 (19):3435-3443. ~~https://doi: 10.1002 / cncr.Twenty nine thousand five hundred three~~
~~28. Saylor PJ, Michaelson MD. New treatments for renal cell carcinoma: targeted therapies. J Natl Compr Canc Netw. 2009;7 (6):645-656. The link is active on 1.02.2018. https://www.ncbi.nlm.nih.gov/pubmed/?term=19555586~~
~~29~~43. Baumgarten P, Blank AE, Franz K, Hattingen E, Dunst m, Zeiner P, Hoffmann K, Bähr O, Mäder L, Goeppert b, Machein M, Seifert V, Steinbach JP, Plate Kh, Harter PN, Mittelbronn M. Differential expression of vascular endothelial growth factor a, its receptors, VEGFR-1, and3- and co-receptors neuropilin-1 and -2 does not predict bevacizumab response in human astrocytomas. Neuro Oncol. 2016;18 (2):173-183. https://doi: 10.1093/ neuonc / no30. Desar IM, Stillebroer AB, Oosterwijk E, Leenders WP, van Herpen CM, van der Graaf WT, Boerman OC, Mulders PF, Oyen WJ. 111In-bevacizumab imaging of renal cell cancer and evaluation of neoadjuvant treatment with the vascular endothelial growth factor receptor inhibitor sorafenib. J ~~Nucl Med. 2010; 51 (11):1707-1715. https://doi: 10.2967 / jnumed.110.078030 20956472~~
~~30~~44. Desar IM, Stillebroer AB, Oosterwijk E, Leenders WP, van Herpen CM, van der Graaf WT, Boerman OC, Mulders PF, Oyen WJ. 111In-bevacizumab imaging of renal cell cancer and evaluation of neoadjuvant treatment with the vascular endothelial growth factor receptor inhibitor sorafenib. J Nucl Med. 2010; 51 (11):1707-1715~~. https://doi: 10.2967 / jnumed.110.078030 20956472~~

Figures

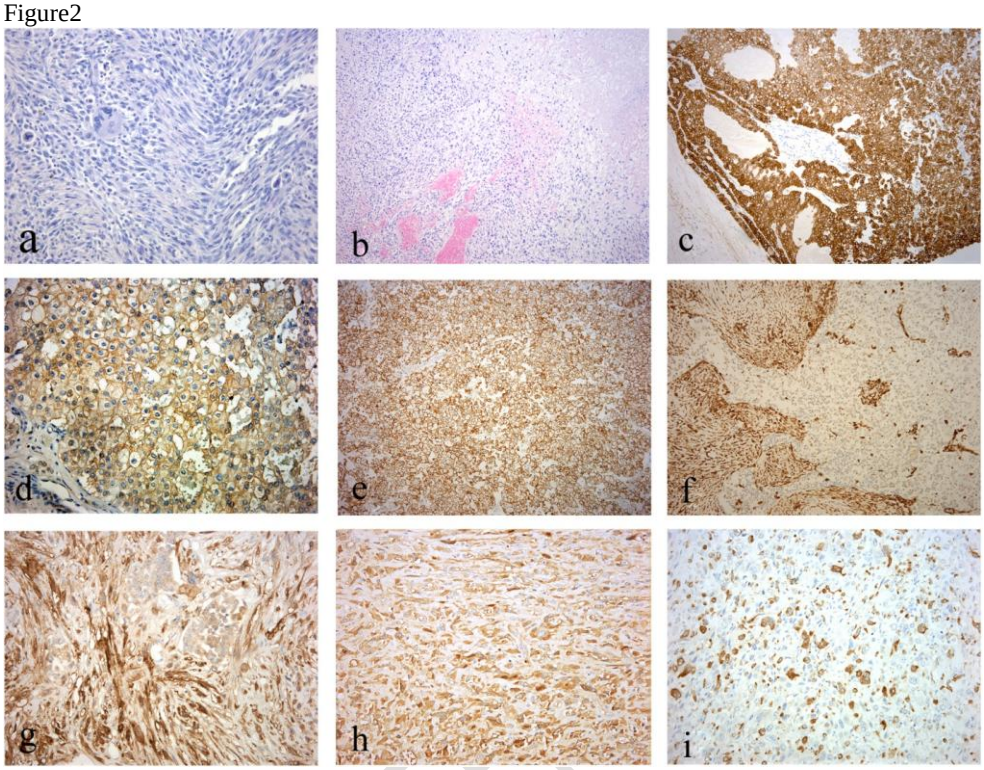
Sarcomatoid chromophobe renal cell carcinoma (Figure 1)



Chromophobic renal cell carcinoma with sarcomatoid differentiation:

a – Gross specimen shows capsulation and gray-brown coloration and tumor size is 40,0x29,0x16,0 cm with partial replacement of the renal tissue;
b - tumor tissues shows alternating epithelioid (right) and sarcomatoid (left) differentiation;
c- epithelioid areas are represented by bright polygonal cells , hyperchromatic nucleus, with prominent nucleoli and perinuclear halos (grade I according to Paner et al. classification).
d- adjoining tissue with sarcoma-like areas with marked increase in nuclear-cytoplasmic ratio and aggregation of cells with fusion of nuclei (grade II according to Paner et al. classification).
e- the sarcomatoid components of the tumor appeared as tightly packed cells with spindle shape or polymorphic forms or multinuclear type of cells (grade III according to Paner et al. classification)
f- sarcomatoid type of tumor areas are infiltrating into the wall of the colon;
b - f – sections stained with hematoxylin and eosin; b,c,e – X200; d – X400; f-X100.

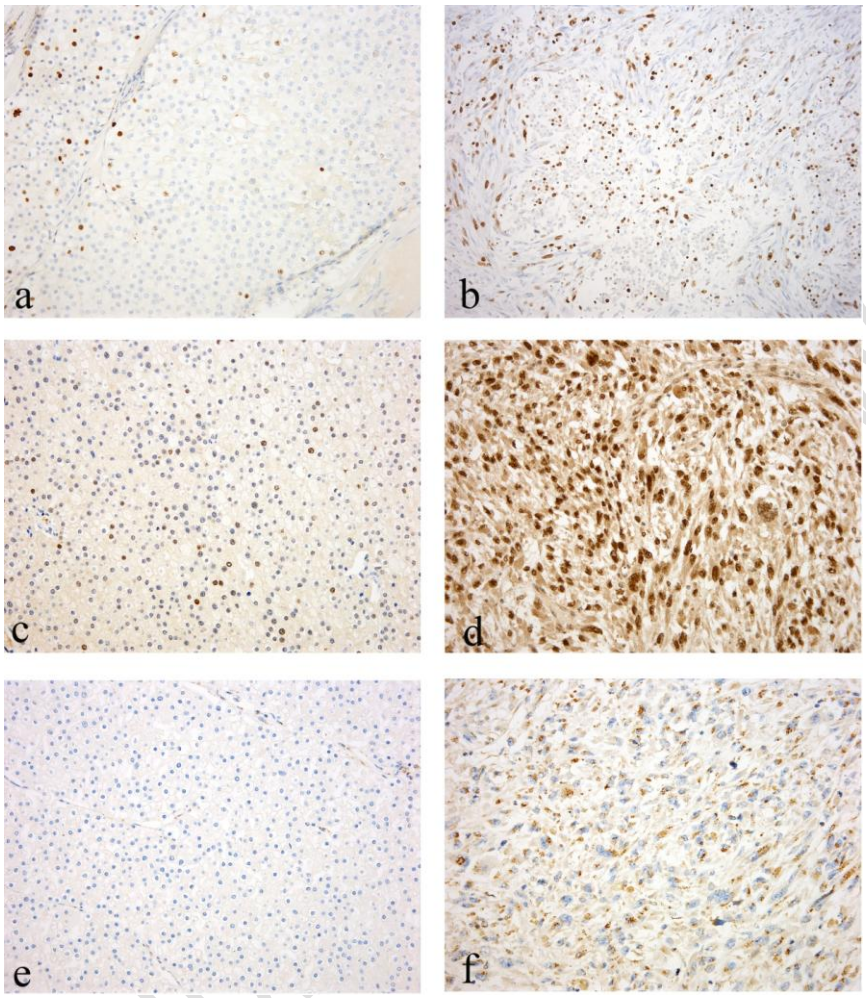
Figure2



Sarcomatoid chromophobe renal cell carcinoma:

a-sarcomatoid component of the tumor is represented by densely packed cells of fusiform and polymorphic shape along with multi-nucleated cells;
b-shows tumor showing necrotic changes (in the upper right corner) and focal hemorrhages;
c-shows positive reaction with cytokeratin 7 in the carcinomatous component in the tumor;
d - CD117 expression in the cytoplasm and cell membrane of tumor cells in the carcinoma component in the tumor;
e - positive reaction with cytokeratin 7 in the carcinomatous component; with expression of E-cadherin in carcinomatous component;
f-tumor cells of the sarcomatoid component (left) showing expression of vimentin, the expression of vimentin in the carcinomatous component (right) is negative;
g-shows positive expression with CD10 in the sarcomatoid component;
h- sarcomatoid component of tumor cells showing expression of α-1-antitrypsin;
i-shows multi-nucleated and tuton-like cells in the sarcomatoid component is determined by a positive reaction with CD68;
a, b, hematoxylinand eosin; c-i, immunohistochemical reaction; b – X 100, the rest – X200.

Figure 3



Expression of prognostic markers according to Paner et al. classification in Chromophobic renal cell carcinoma with varying degrees of differentiation (our case study). The proliferation index of Ki67 in the carcinomatous component (grade I) (a) is 2.0%, in the sarcomatoid component (grade III) (b) is 70.0%; p53 in the carcinomatous component of the tumor (grade I) (c) is expressed in 20.0% of cells, the sarcomatoid component (grade III) (d) is seen in 85.0% of tumor cells; in the carcinomatous component (grade I) (e), there is a negative expression with VEGF-a, in the sarcomatoid component (grade III) (f) there is a strong expression VEGF-a (score 5);a – f - immunohistochemical reaction; X200.