Analysis of Insulin-Like Growth Factors and Insulin-Like Growth Factor I Receptor Expression in Renal Cell Carcinoma

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Abstract

*The expression of insulin-like growth factors I (IGF-I) and II (IGF-II) and insulin-like growth factor I receptor (IGF-IR) was studied in 137 clear cell, 23 chromophobe, and 20 papillary renal cell carcinomas (RCCs) using a tissue microarray technique. IGF-I immunoreactivity was detected in 110 (82.1%) of 134 clear cell, 8 (36%) of 22 chromophobe, and 3 (15%) of 20 papillary RCCs (*P < .001*). IGF-IR immunoreactivity was detected in 39 (29.5%) of 132 clear cell, 9 (41%) of 22 chromophobe, and 19 (95%) of 20 papillary RCCs (*P < .001*). In contrast, all tumors lacked IGF-II expression. Expression of IGF-I and IGF-IR was not related to tumor stage, grade, or prognosis. The IGF system is expressed differentially among different tumor types. The expression of IGF-I together with its receptor, IGF-IR, provides evidence for the existence of an autocrine-paracrine loop of tumor cell stimulation in RCC and makes this type of cancer a candidate for therapeutic strategies aimed to interfere with the IGF pathway.*

Renal cell carcinoma (RCC) accounts for about 2% of cancers worldwide. It has been increasing in incidence in North America and northern Europe, with rates increasing at about 3% per year in the United States. Survival has improved, with the 5-year relative survival rate increasing from 30% to 40% in the 1960s to between 50% and 60% in the 1990s.[1] As for the majority of cancers, tumor stage at diagnosis and histologic tumor grade are the principal prognostic factors.[2] Prognosis also is related to histologic subtype, because patients with clear cell RCCs have poorer cancer-specific survival than patients with papillary or chromophobe tumors.[3] However, the underlying basis for the unfavorable prognosis of the clear cell subtype is unclear.

The insulin-like growth factor (IGF) system has a critical role in the growth and development of the organism and also has been implicated in tumorigenesis.[4-6] The IGF system is formed by the IGF ligands (IGF-I and IGF-II); cell surface receptors, including the IGF-I receptor (IGF-IR) and the IGF-II receptor (IGF-IIR), which mediate the biologic effects of the ligands and are known to form heterodimers with the insulin receptor; and 6 types of IGF binding proteins (numbered 1-6), which are known to modulate IGF function.[4-6] IGF-I and IGF-II are single-chain polypeptides that have 62% homology in their amino acid sequences and share structural similarities with proinsulin.[4] In humans, IGF-I and IGF-II are produced in multiple human tissues throughout life.[6] Both IGF-I and IGF-II interact with IGF-IR, a transmembrane tyrosine kinase that is related structurally and functionally to insulin receptor. IGF-I, however, has a 2- to 15-fold higher binding affinity to IGF-IR than IGF-II.[4] IGF-II also can bind to IGF-IIR, which has no tyrosine kinase activity and functions as a clearance receptor by endocytosis and intracellular

degradation of its ligand, thereby influencing extracellular IGF-II levels.[6]

The binding of IGFs to the extracellular subunit of IGF-IR activates the receptor's tyrosine kinase activity and leads to activation of mitogen-activated protein kinase and phosphoinositol-3-kinase cascades, thus mediating mitogenic, differentiative, and antiapoptotic effects.[6] In addition, IGF-IR signaling is supposed to be involved in cell transformation and in the maintenance of the transformed phenotype by modulating cancer cell motility[7] and adhesion[8] and angiogenesis.[9] High levels of circulating IGF-I have been reported to be associated with increased risks of breast,[10] prostate,[11] lung,[12] and colorectal[13] cancer development. Regulation of IGF-IR expression is associated closely with the function of several tumor suppressor genes and oncogenes. Thus, expression of wild-type p53 inhibits IGF-IR expression, whereas mutant *p53* has been shown to up-regulate IGF-IR expression.[14] Overexpression of IGF-IR has been noted in several cancers.[15] The simultaneous expression of both ligand (IGF-I and/or IGF-II) and receptor (IGF-IR) in the same tumor provides evidence for an autocrine-paracrine loop of cancer cell stimulation.[16]

With respect to renal cancer, only a few studies investigating the IGF system exist. They were restricted to the clear cell subtype and showed conflicting results: varying degrees of IGF-IR expression[15,17-19] or complete absence of IGF-IR and its ligand IGF-I.[20] Recently, Parker et al[18,19] reported a poorer outcome in patients with clear cell RCCs immunoreactive for IGF-IR compared with patients with IGF-IR–negative clear cell tumors.[18,19] A systematic analysis regarding the expression of IGF-IR and its ligands, however, is lacking. Therefore, we performed a systematic immunohistochemical analysis of a large series of RCCs, including all histologic subtypes, to investigate the expression of IGF-I, IGF-II, and IGF-IR with respect to association with tumor stage, grade, histologic subtype, and prognosis.

Materials and Methods

Case Selection

Paraffin-embedded specimens of RCC from 180 patients (109 men, 71 women) who underwent operation between January 1995 and June 2002 in our department (Department of Urology, Medical University of Graz, Graz, Austria) were chosen for analysis. The mean and median age of patients at operation were 62.3 and 62.9 years, respectively (range, 28-85 years). All specimens were reevaluated with respect to tumor stage, grade, and histologic subtype by two of us (C.L. and M.R.). Tumor stages were adjusted according to the International Union Against Cancer 2002 TNM system,[21] and nuclear grading was performed according to the Fuhrman grading system.[22] Histologic subtypes were assessed according to the World Health Organization guidelines[23]: clear cell (n = 137 [76.1%], including 9 tumors with small foci of sarcomatoid change and 4 with predominant sarcomatoid morphologic features and only small residual foci of preexisting clear cell tumor), papillary (n = 20 [11.1%], including 12 type 1 and 8 type 2 tumors), and chromophobe (n = 23 [12.8%]). Details regarding pT stage and grade related to histologic subtype are listed in **Table 1**. Two specimens of nonneoplastic renal tissue were included for comparison.

Immunohistochemical Analysis

For immunohistochemical evaluation, a tissue microarray (TMA) technique was used that permitted staining of a large number of specimens on 1 slide. TMAs were constructed using a manual tissue arraying instrument (Beecher, Silver Spring, MD). The details of this technique have been described previously.[24] With respect to the well-known heterogeneity of RCC, 3 cylindrical core biopsy specimens, 0.6 mm in diameter, were obtained from different sites of each tumor, which had been selected on the original tumor slides to include all patterns of differentiation. Four-micrometer TMA sections were mounted on Superfrost slides (Menzel-Gläser, Braunschweig, Germany) for immunohistochemical analysis using an automated immunostainer (DAKO Autostainer, Universal Staining System, DAKO, Glostrup, Denmark).

Briefly, TMA sections were deparaffinized, rehydrated in graded alcohols, and treated for 5 minutes with 1% hydrogen peroxide. For the detection of IGF-I and IGF-II, sections then were treated for antigen retrieval (1% trypsin for 20 minutes at room temperature) and subsequently were incubated with the following monoclonal antibodies: IGF-I (clone I-5C9, dilution 1:500; Linaris, Wertheim, Germany) and IGF-II (clone W2-H1, dilution 1:500; Linaris). Binding of the primary antibodies was assessed by using the DAKO EnVision+ System

Table 1
Tumor Stage and Grade Related to Histologic Subtype of Renal Cell Carcinoma*

	Clear Cell (n = 137)	Chromophobe (n = 23)	Papillary (n = 20)
Stage			
pT1a	50 (36.5)	6 (26)	7 (35)
pT1b	21 (15.3)	3 (13)	4 (20)
pT2	5 (3.6)	3 (13)	3 (15)
pT3a	27 (19.7)	6 (26)	4 (20)
pT3b	34 (24.8)	5 (22)	2 (10)
Grade			
1	18 (13.1)	0 (0)	1 (5)
2	71 (51.8)	14 (61)	13 (65)
3	44 (32.1)	9 (39)	6 (30)
4	4 (2.9)	0 (0)	0 (0)

* Data are given as number (percentage).

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detection kit. For the detection of IGF-IR, sections were submitted to microwave antigen retrieval (30 minutes, 160 W, EDTA, pH 8.0) and subsequently were incubated for 30 minutes with a mouse monoclonal antibody directed against the α subunit of IGF-IR (clone 24-31, dilution 1:50; NeoMarkers, Fremont, CA). Binding of the IGF-IR primary antibody was assessed by using the DAKO ChemMate detection kit.

Immunohistochemical Evaluation and Control Samples

Immunoreactivity was assessed independently in a semiquantitative manner by two of us (C.L. and M.R.) who were blinded to the clinicopathologic data, especially pT stage and patient outcome. Discrepancies were resolved by simultaneous reexamination of the slides by both investigators using a double-headed microscope. Immunoreactivity was assessed considering the average positivity of the core biopsy specimens and documented in categories as follows: no reactivity; weak, fewer than 10% of cancer cells positive; moderate, 10% to 50% of cancer cells positive; strong, more than 50% of cancer cells positive. Sections of a colon carcinoma known to be positive for IGF-I, IGF-II, and IGF-IR served as positive control samples. Negative control samples included omission of the primary antibody and incubation with DAKO ChemMate Antibody Diluent (code No. S 2022).

Statistical Analysis

Subgroups according to pT stage, grade, and histologic subtype were compared for possible differences in immunoreactivity using the χ^2 test or the Fisher exact test. Regarding prognosis, only the clear cell subtype was analyzed because samples of the other histologic subtypes were too small for a separate analysis. Disease-free survival was studied by using the Kaplan-Meier method and compared by using the log-rank test. For multivariate testing, a Cox proportional hazards regression model for pT stage (pT3 vs pT1/pT2), tumor grade (3-4 vs 1-2), and IGF-I expression (positive vs negative) was performed.

Results

Immunohistochemical Analysis

Cancer tissue allowing a reliable evaluation of immunoreactivity of IGF-I, IGF-II, and IGF-IR was present in 176 (97.8%), 176 (97.8%), and 174 (96.7%) of 180 cases, respectively.

IGF-I immunostaining yielded a distinct finely granular cytoplasmic reactivity, sometimes accompanied by a moderate membranous accentuation **Image 1**. Overall, positivity was noted in 110 (82.1%) of 134 clear cell, 8 (36%) of 22 chromophobe, and 3 (15%) of 20 papillary RCCs ($P < .001$; χ^2). The 3 weakly positive papillary tumors were type 1 tumors (type 1, n = 12), whereas all 8 type 2 tumors lacked IGF-I immunoreactivity ($P = .2$; Fisher exact test). Strong IGF-I expression in more than 50% of cancer cells was detected in 38 (28.4%) of 134 clear cell RCCs, whereas all IGF-I–positive chromophobe and papillary tumors showed staining of less than 50% of cancer cells ($P < .001$; χ^2).

Regarding tumor stage, IGF-I immunoreactivity tended to be more common in low-stage clear cell RCCs (64 [88%] of 73 pT1/pT2 vs 46 [75%] of 61 pT3; $P = .07$; Fisher exact test), whereas for chromophobe (3 [27%] of 11 pT1/pT2 vs 5 [45%] of 11 pT3; $P = .7$; Fisher exact test) and papillary (3 [21%] of 14 pT1/pT2 vs 0 [0%] of 6 pT3; $P = .5$; Fisher exact test) tumors, no trend was noted **Table 2**. Although not significant, IGF-I expression seemed to be more common in high-grade chromophobe cancers (3 [23%] of 13 grade 2 vs 5 [56%] of 9 grade 3; $P = .2$; Fisher exact test), but in contrast, was found more often in low-grade papillary cancers (3 [21%] of 14 grade 1 or 2 vs 0 [0%] of 6 grade 3; $P = .5$; Fisher exact test). Regarding clear cell RCCs, no relation to tumor grade was found (73 [85%] of 86 grade 1 or 2 vs 37 [77%] of 48 grade 3 or 4; $P = .3$; Fisher exact test; Table 2). No differences in immunoreactivity were noted between different patterns of differentiation within the same tumors included in the core biopsy specimens.

IGF-II expression was not detected in any of the tumors. The case of colon cancer that served as the positive control sample yielded specific immunoreactivity.

Overall, IGF-IR expression was detected in 39 (29.5%) of 132 clear cell, 9 (41%) of 22 chromophobe, and 19 (95%) of 20 papillary RCCs ($P < .001$; χ^2). Regarding papillary RCCs, IGF-IR again was seen predominantly in type 1 tumors: 9 (75%) of 12 type 1 tumors showed staining of more than 75% of cancer cells compared with 0 (0%) of 8 type 2 tumors ($P = .001$; Fisher exact test).

Regarding staining patterns, distinct membranous labeling was seen in clear cell tumors **Image 2A**, whereas in papillary tumors, cytoplasmic reactivity was found **Image 2B**. Chromophobe tumors showed membranous reactivity of large, pale cells and cytoplasmic staining of small, eosinophilic cells **Image 2C**. No associations between IGF-IR and tumor stage or grade were found for any histologic subtype. No associations between IGF-I and IGF-IR expression were found: 27 (73%) of 37 IGF-IR–positive clear cell RCCs showed immunoreactivity for IGF-I compared with 79 (85%) of 93 IGF-IR–negative clear cell RCCs ($P = .1$; Fisher exact test). Nonneoplastic renal tissue, including the 2 separately studied specimens and nonneoplastic tissue contained in the core biopsy specimens, was negative for IGF-I and IGF-II, whereas distinct granular cytoplasmic immunostaining for IGF-IR was seen in samples of distal tubule and collecting duct epithelium.

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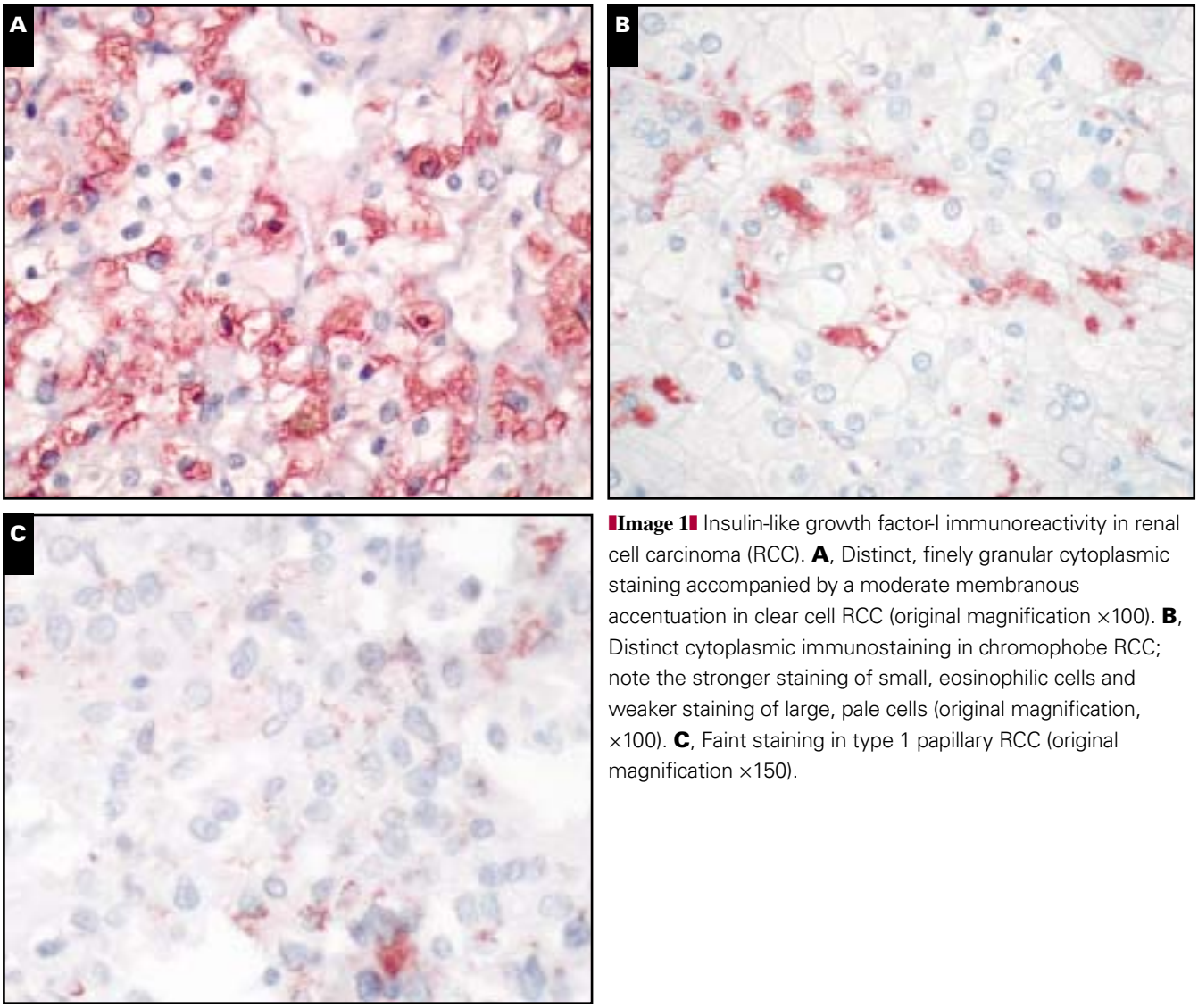

Image 1 Insulin-like growth factor-I immunoreactivity in renal cell carcinoma (RCC). **A**, Distinct, finely granular cytoplasmic staining accompanied by a moderate membranous accentuation in clear cell RCC (original magnification ×100). **B**, Distinct cytoplasmic immunostaining in chromophobe RCC; note the stronger staining of small, eosinophilic cells and weaker staining of large, pale cells (original magnification, ×100). **C**, Faint staining in type 1 papillary RCC (original magnification ×150).

Table 2
Overall Immunoreactivity Related to pT Stage and Tumor Grade Among Histologic Subtypes*

	IGF-I			IGF-IR		
	Clear Cell (n = 134)	Chromophobe (n = 22)	Papillary (n = 20)	Clear Cell (n = 132)	Chromophobe (n = 22)	Papillary (n = 20)
Positivity						
Negative	24 (17.9)	14 (64)	17 (85)	93 (70.5)	13 (59)	1 (5)
Positive						
<10%	30 (22.4)	5 (23)	0 (0)	18 (13.6)	5 (23)	2 (10)
10%-50%	42 (31.3)	3 (14)	3 (15)	13 (9.8)	4 (18)	3 (15)
>50%	38 (28.4)	0 (0)	0 (0)	8 (6.1)	0 (0)	14 (70)
Overall	110 (82.1)	8 (36)	3 (15)	39 (29.5)	9 (41)	19 (95)
Stage						
pT1a	41/47 (87)	1/5 (20)	1/7 (14)	14/46 (30)	4/5 (80)	7/7 (100)
pT1b	18/21 (86)	1/3 (33)	1/4 (25)	7/20 (35)	1/3 (33)	4/4 (100)
pT2	5/5 (100)	1/3 (33)	1/3 (33)	0/5 (0)	0/3 (0)	2/3 (67)
pT3a	21/27 (78)	2/6 (33)	0/4 (0)	7/27 (26)	3/6 (50)	4/4 (100)
pT3b	25/34 (74)	3/5 (60)	0/2 (0)	11/34 (32)	1/5 (20)	2/2 (100)
Grade						
1	16/17 (94)	—	0/1 (0)	9/17 (53)	—	1/1 (100)
2	57/69 (83)	3/13 (23)	3/13 (23)	14/68 (21)	6/13 (46)	12/13 (92)
3	36/44 (82)	5/9 (56)	0/6 (0)	12/43 (28)	3/9 (33)	6/6 (100)
4	1/4 (25)	—	—	4/4 (100)	—	—

IGF-I, insulin-like growth factor-I; IGF-IR, insulin-like growth factor-I receptor.
* Expression of IGF-I and IGF-IR is associated with histologic subtypes of renal cell carcinoma ($P < .0001$; χ^2). Data are given as number (percentage) or number/total number of cases tested (percentage).

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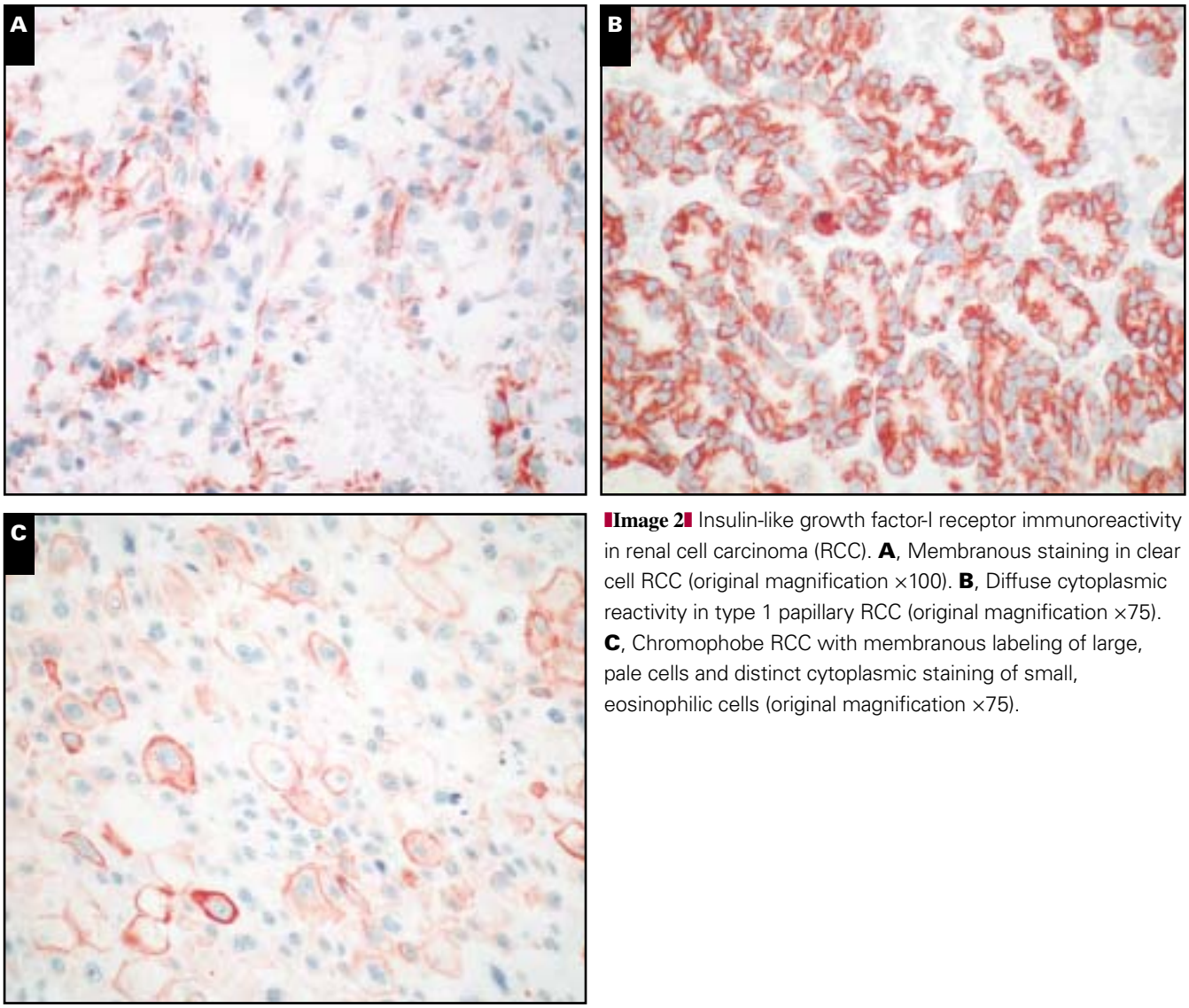

Image 2 Insulin-like growth factor-I receptor immunoreactivity in renal cell carcinoma (RCC). **A**, Membranous staining in clear cell RCC (original magnification ×100). **B**, Diffuse cytoplasmic reactivity in type 1 papillary RCC (original magnification ×75). **C**, Chromophobe RCC with membranous labeling of large, pale cells and distinct cytoplasmic staining of small, eosinophilic cells (original magnification ×75).

Survival Analysis

Follow-up data were available for 131 (97.8%) of 134 patients with immunohistochemically evaluable clear cell RCCs. After mean and median follow-up periods of 26 and 24 months, respectively, progressive disease was observed in 29 (22.1%) of 131 patients, including 15 who died of cancer and 14 patients who were alive with metastatic disease. Three patients died of causes unrelated to RCC. Regarding patients with clear cell RCCs evaluable for IGF-I immunoreactivity, progressive disease occurred in 8 (33%) of 24 patients with IGF-I–negative compared with 21 (19.8%) of 106 patients with IGF-I–positive tumors ($P = .05$; log-rank test). However, multivariate analysis proved independent prognostic significance only for tumor stage of more than 2 (risk ratio [RR], 4.5; 95% confidence interval [CI], 1.8-10.9; $P = .001$) and tumor grade of more than 2 (RR, 6.0; 95% CI, 2.3-15.8; $P = .0003$), whereas for IGF-I (RR, 1.1; 95% CI, 0.6-2.3; $P = .7$), no independent impact on outcome was found. Regarding patients with clear cell RCCs evaluable for IGF-IR immunoreactivity, progressive disease occurred in 10 (28%) of 36 patients with IGF-IR–positive compared with 19 (21%) of 90 patients with IGF-IR–negative tumors ($P = .3$; log-rank test).

Discussion

Both IGF-I and IGF-II are implicated in the growth regulation of the kidney during embryogenesis and development. In postnatal life, IGFs have a role in kidney physiology (eg, glomerular filtration rate, renal plasma flow) and pathophysiology (eg, diabetic renal hypertrophy, chronic renal failure). Immunoreactivity data for IGF-I and IGF-II have been reported for fetal human kidney and fetal and adult rat kidneys.[25]

With regard to cancer, the impact of the IGF axis on tumorigenesis is well documented for several tumors.[15,16,26-31] The association of the IGF system and RCCs, however, has been

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investigated rarely: Pekonen et al[32] were the first to identify IGF-I binding sites in renal cancer tissue. Kellerer et al[17] analyzed IGF-IR tyrosine kinase activities in RCCs and normal renal tissue and reported a 2-fold higher activity in RCCs compared with nonneoplastic tissue. In contrast, Ramp et al[20] failed to detect expression of IGF-IR and its ligand IGF-I in renal cancer cell lines by Northern blot analysis. Ouban et al[15] noted IGF-IR immunoreactivity in 2 (14%) of 14 RCCs, and, recently, Parker et al[18,19] reported significantly decreased cancer-specific survival in patients with clear cell RCCs expressing IGF-IR compared with patients with tumors without IGF-IR expression.

To the best of our knowledge, our study is the first to report a systematic immunohistochemical analysis of IGF-I, IGF-II, and IGF-IR expression in normal adult human kidneys and RCCs. IGF-I immunostaining frequently was seen in renal cancer tissue, with clear cell tumors demonstrating significantly higher expression than the other histologic subtypes. The association with the clear cell subtype may be related to the common alterations of the von Hippel-Lindau (*VHL*) tumor suppressor gene in this type of renal cancer.[33] Because IGF-I–mediated cell proliferation is inhibited in the presence of the wild-type *VHL* gene,[34] it is tempting to speculate that high IGF-I expression might be related indirectly to *VHL* gene alterations.

Interestingly, the 3 IGF-I–positive papillary cancers belonged to the type 1 category, consistent with the work by Delahunt and Eble[35] and Delahunt et al,[36] whereas all type 2 tumors lacked IGF-I immunoreactivity. Owing to the small sample, however, results lacked statistical significance, and the question of whether the IGF axis is expressed differentially in different types of papillary RCCs has to be addressed in larger series. No significant associations of IGF-I expression with tumor stages or grades were found. The consistent lack of IGF-I immunostaining in nonneoplastic renal tissue suggests that the IGF-system is involved in the pathogenesis of renal cancer. This seems to happen as an early event, because IGF-I immunostaining can be found in low-stage and low-grade tumors.

The consistent lack of IGF-II immunoreactivity in the renal cancer tissue samples in our study indicates cancer-specific expression of IGF-IR ligands. It supports the findings of an in vitro study by Bennington et al,[37] who reported an expression of 300- to 800-fold higher levels of IGF-I messenger RNA than IGF-II messenger RNA in renal cancer cells. Two case reports,[38,39] however, reported severe hypoglycemia in renal cancer patients due to secretion of an aberrant form of IGF-II that might not be recognized by the IGF-II antibody used in our study.

The expression of IGF-IR in almost 30% of clear cell RCCs in our study is comparable to data in the literature reporting IGF-IR immunoreactivity ranging from 14% to 54%.[15,18,19] However, IGF-IR expression has not been analyzed before in the other types of renal cancer. According to our data, IGF-IR immunoreactivity prevailed in papillary tumors, which supports the concept of differential expression of the IGF system in different types of renal cancer.

The expression of the ligand (IGF-I) and its receptor (IGF-IR) within the same tumor provides evidence of the existence of an autocrine-paracrine loop of tumor cell stimulation. In general, however, the expression of IGF-IR was not found to be related to the expression of its ligands, which was obvious in papillary cancer but also was shown for clear cell tumors. Therefore, IGF signaling in RCCs (particularly in the papillary subtype) might be mediated mainly by circulating ligand proteins (IGF-I and IGF-II) from sources other than cancer tissue itself.

With respect to prognosis, neither IGF-I nor IGF-IR expression demonstrated independent significance in our series. These data seem to be at variance with the studies by Parker et al[18,19] demonstrating a poor prognosis for patients with high expression of IGF-IR. However, observations similar to ours have been reported; some authors noticed dependence on the IGF system mainly in early-stage tumors and found significantly reduced levels of IGF-IR and/or IGF-I in advanced and/or dedifferentiated states, particularly in breast and thyroid cancers.[27-29] Thus, our data might indicate that the IGF system, although mediating mitogenic and antiapoptotic effects, has only a minor influence on patient outcome in renal cancer.

In conclusion, the expression of IGF-I and IGF-IR was frequent in renal cancers, with clear cell tumors showing strong IGF-I and papillary tumors showing strong IGF-IR immunoreactivity, suggesting differential expression of the IGF system in different tumor types. Neither marker was related to tumor stage or grade, and no independent impact on survival was found. However, the expression of IGF-I together with its receptor, IGF-IR, provides evidence for the existence of an autocrine-paracrine loop of tumor cell stimulation in RCC and makes this type of cancer a candidate for therapeutic strategies aimed at interfering with the IGF pathway.

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