

Membranous Expression and Prognostic Implications of Epidermal Growth Factor Receptor Protein in Human Renal Cell Cancer

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Abstract. *Background:* It has been indicated that altered expression of the epidermal growth factor receptor (EGFR) promotes the invasive and metastatic potential of a variety of human malignancies. Therefore, the aim of the present study was to determine EGFR expression in clear cell renal cell carcinomas (RCC) to evaluate its prognostic relevance for the clinical course of the disease. *Materials and Methods:* EGFR protein expression, detected by immunohistochemistry and tissue microarray analysis (TMA), was investigated in a cohort of 149 randomly selected patients subjected to tumor nephrectomy for RCC. *Results:* The tumor cells preferably exhibited a homogeneous membrane-bound reactivity for EGFR; EGFR overexpression was detected in 70 (47%) of the primary tumor specimens, but in only 12 (9%) of the benign tissue samples ($p < 0.0001$; Fisher's *t*-test). Tumor-associated EGFR staining was stratified into three groups: I: low staining score ($n=75$, 50%); II: intense expression ($n=56$, 38%); and III: strong overexpression ($n=18$, 12%). Strong reactivity for EGFR was identified as predicting the patients' survival both during uni- and multivariate analysis ($p=0.03$). Interestingly, the overall survival of the intense expression group surpassed even the low expression group ($p=0.023$). *Conclusion:* The observation that primary RCC specimens exhibit EGFR at higher levels when compared with benign renal parenchyma indicates its role in tumor development and progression. The availability of more refined prognostic factors would assist decision making in terms of the value of more aggressive

treatment options for prognostically defined subgroups of patients. Additionally, if overexpression of EGFR identifies RCC with a more aggressive biological behavior, the latter receptor might serve as a novel target for a more effective therapeutic approach to RCC.

In Western countries an estimated 35,000 individuals are newly diagnosed with renal cell cancer (RCC) and 12,000 patients are reported to die from this disease every year (1). There is considerable variance in survival times of RCC patients, despite comparable tumor stages, grading and lymph node status, suggesting that there are biologically different types of RCC with different clinical behavior, or that the currently available prognostic factors are not sufficient to predict the aggressiveness of an individual tumor.

About 30 – 40% of renal cell cancer patients present with metastasized disease at the time of first diagnosis and another 30 – 50% with initially organ-confined tumors will develop systemic tumor dissemination regardless of an initially curative surgical approach. Whereas localized RCC (T1 or T2) are considered curable by resection of the tumor-bearing kidney, achieving a long-term survival rate of about 80%, no effective treatment option for metastasized RCC patients is currently available (2, 3). Therefore, new therapeutic approaches, that promise to reveal a higher clinical efficacy when compared with established treatment modalities, the latter including the systemic application of cytokines such as interferon or interleukin, are urgently needed (4).

For RCC, a prognostic factor, which would allow the prediction of the clinical course of an individual patient in addition to established prognostic parameters, such as histopathological growth pattern, tumor stage, grade and lymph node status, is still lacking. In addition, a better understanding of the processes involved in the development and progression of this malignancy would help in the

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Table I. Evaluation of EGFR expression and pathological parameters by univariate Chi-square analysis of 149 renal cell cancer patients, using cut-off 50 for overexpression and cut-off 200 for strong EGFR protein overexpression. *According to the 2002 UICC classification.

Variable	No. Pts.	No. EGFR malign		p-value (Chi-square test)	No. EGFR malign		p-value (Chi-square test)
		(≤50)	(>50)		(≤200)	(>200)	
Total	149	79 (53.0 %)	70 (47.0 %)		131 (87.9%)	18 (12.1%)	
Gender				0.38			0.88
female	52	25 (48.1 %)	27 (51.9 %)		46 (88.5 %)	6 (11.5 %)	
male	97	54 (55.7 %)	43 (44.3 %)		85 (87.6 %)	12 (12.4 %)	
Pathological stage*				0.90			0.43
pT1	49	24 (49.0 %)	25 (51.0 %)		45 (91.8 %)	4 (8.2 %)	
pT2	56	31 (55.4 %)	25 (44.6 %)		49 (87.5 %)	7 (12.5 %)	
pT3	41	23 (56.1 %)	18 (43.9 %)		35 (85.4 %)	6 (14.6 %)	
pT4	2	1 (50.0 %)	1 (50.0 %)		1 (50 %)	1 (50 %)	
missing	1						
Tumor grade				0.46			0.56
G1	39	24 (61.5 %)	15 (38.5 %)		36 (92.3 %)	3 (7.7 %)	
G2	98	49 (50.0 %)	49 (50.0 %)		85 (86.7 %)	13 (13.3 %)	
G3	12	6 (50.0 %)	6 (50.0 %)		10 (83.3 %)	2 (16.7 %)	
Lymph node status				0.26			0.92
N0	103	55 (53.4 %)	48 (46.6 %)		89 (86.4 %)	14 (13.6 %)	
N1	5	2 (40.0 %)	3 (60.0 %)		4 (80 %)	1 (20 %)	
N2	8	2 (25.0 %)	6 (75.0 %)		7 (87.5 %)	1 (12.5 %)	
unknown	33						
Adjuvant treatment				0.69			0.28
Yes	34	17 (50.0 %)	17 (50.0 %)		28 (82.4 %)	6 (17.7 %)	
No	115	62 (53.9 %)	53 (46.1 %)		103 (89.6 %)	12 (10.4 %)	
Primary metastasis				0.158			0.25
M0	88	50 (56.8 %)	38 (43.2 %)		79 (89.7 %)	9 (10.2 %)	
M1	33	14 (42.4 %)	19 (57.6 %)		27 (81.8 %)	6 (18.2 %)	
unknown	28						
Tumor recurrence				0.53			0.45
Yes	31	18 (58.1 %)	13 (41.9 %)		26 (83.9 %)	5 (16.1 %)	
No	118	61 (51.7 %)	57 (48.3 %)		105 (89 %)	13 (11 %)	
Tumor-specific death				0.41			0.21
Yes	32	19 (59.4 %)	13 (40.6 %)		26 (81.3 %)	6 (18.8 %)	
No	117	60 (51.3 %)	57 (48.7 %)		105 (89.7 %)	12 (10.3 %)	

development of novel therapeutic approaches of higher clinical efficacy than the current approaches.

EGFR is a transmembrane glycoprotein with an extracellular ligand-binding and an intracellular domain with tyrosine kinase activity. In a variety of human malignancies, altered EGFR regulation and disturbed expression have been identified as enhancing invasive potential and metastatic behavior due to an inhibition of apoptotic pathways, as well as stimulation of processes involved in the promotion of the mammalian cell cycle, for example (16).

Although the increased expression of EGFR in RCC compared with normal renal parenchyma has been previously described (5-9), its prognostic value for surgically-treated RCC patients is a subject of controversial discussions (8, 10-15).

However, the role of EGFR as a molecular target for novel therapeutic approaches in RCC is rapidly evolving (16). Recognizing the highly variable response rates that have been reported after administration of substances revealing a negative regulatory influence on EGFR, the

subgroup of patients that promise to derive the highest clinical benefit from the latter treatment modality still remains to be identified (17-20).

The aim of the present immunohistochemical study, utilizing the high-throughput tool of tissue microarray analysis, was to further elucidate the expression of EGFR in RCC when compared with benign renal parenchyma and to determine its prognostic value for 149 randomly selected patients for whom sufficient follow-up information was available.

Materials and Methods

Patients. The present study included 149 randomly selected patients subjected to surgical treatment for RCC at Tuebingen University Hospital, Germany, between 1990 and 2002. Several patient and tumor characteristics, including tumor stage and grade, the administration of adjuvant systemic therapy after surgery, as well as the clinical course during further follow-up, were obtained for each case. Data were collected by physicians and data managers and subsequently maintained in a data base. Patients who did not return to our institution, and for whom no follow-up information was available, were personally contacted. Clinical tumor stage was classified according to the TNM classification system (2002) and nuclear grade was assessed according to the Fuhrman grading system of malignant tumors. All tumors were conventional clear RCC. The median postoperative follow-up for the entire cohort of patients (mean age: 63 years) was 50 (2-146) months. During the further course of the disease, 36 patients died from systemic tumor progression. Tumor stages, according to the TNM classification system, were, in detail: pT1, n=49 (33.1%), pT2, n=56 (37.8%), pT3, n=41 (27.7%), pT4, n=2 (1.4%). Regional lymph node and distant metastases, already present at the time of surgery, were diagnosed in 13 and 33 cases, respectively. An adjuvant systemic treatment in the form of immuno-(chemo-)therapy was administered in 34 cases. Patient characteristics are shown in Table I.

Tissue microarrays. Tissue specimens obtained from the primary tumors and benign corresponding renal parenchyma were cut in square sections 6 mm in length, formalin-fixed (pH 7, Roti-Histofix, Fa. Roth, Karlsruhe, Germany), dehydrated and paraffin-embedded. For optimal tissue microarray (TMA) planning, representative paraffin-embedded tissue areas were selected by primary evaluation of HE (Hematoxylin & Eosin)-stained slides. The TMA contained 447 samples, including 149 benign tissue samples that represented non-malignant regions within the tumor-bearing kidneys. To guarantee adequate representation of the primary tumors, 2 tumor cores were obtained from each sample site, resulting in 6 TMA slides with corresponding benign kidney tissue. The TMA slides were constructed as previously described (21-23).

Immunohistochemistry. The 5- μ m paraffin sections were deparaffinized, rehydrated and immersed in 3% hydrogen peroxide in Aqua dest. to block endogenous peroxidase activity. Antigen retrieval was accomplished by proteinases K (Dako Cytomation, S3020) pretreatment for 10 minutes. EGFR was immunohistochemically detected by a commercially available anti-EGFR antibody (monoclonal mouse antibody, Dako Cytomation, clone E30). The optimal dilution of the monoclonal anti-EGFR antibody

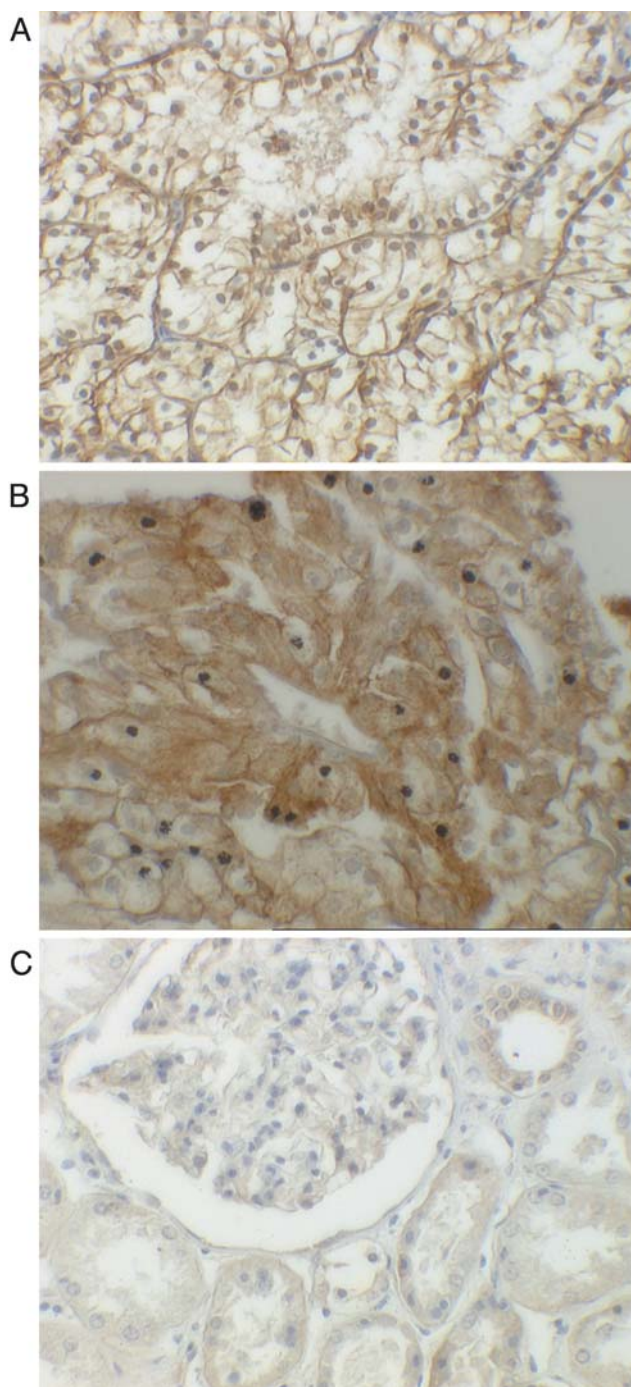


Figure 1. A) Representative positively-stained clear cell renal cancer tissue showing strong EGFR membrane protein expression (160 x magnification). B) Representative positively-stained EGFR cytoplasmatic expression in renal clear cell carcinoma (160 x magnification). C) Benign renal tissue with no staining reactivity for EGFR (160 x magnification)

was 1:100 in Dako background reducing diluent (Dako Cytomation, S3022). After 12 hours of incubation, the sections were washed in TBS and incubated with a secondary biotinylated

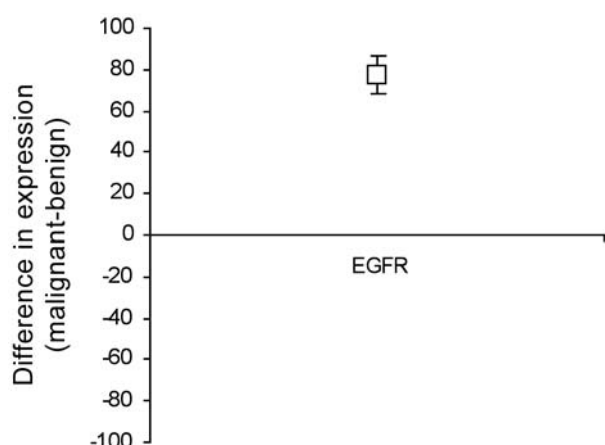


Figure 2. Difference in EGFR expression between benign and tumor tissue by Fisher's *t*-test: $p < 0.0001$ (Mean 77.1; $n = 117$; std. dev. 91.5).

anti-mouse IgG antibody (Vectastatin Elite ABC Kit, Vector Laboratories, Inc., Burlingame, CA, USA) for 60 minutes. The DAB system (Vector, SK-4100) was used for visualization according to the manufacturer's instructions. Sections were briefly rinsed in tap water, counterstained with Mayer's Hematoxylin solution and mounted. For negative control, the primary anti-EGFR antibody was replaced by non-immune mouse serum.

All TMA slides were evaluated by two independent investigators (A.S.M. and J.H.), both completely blinded to patient data and follow-up. For statistical analysis, the staining reaction was classified according to a semiquantitative IHC reference scale ranging from 0–3+, depending on the intensity of the membrane-bound expression of the EGFR protein. The relative amount of tumor cells stained positive for EGFR, together with the rating of the staining intensity, resulted in a EGFR staining score from 0–300, as previously described (24). When cytoplasmatic staining occurred, the results were documented separately according to the same rating scale. For statistical analysis and according to the immunohistochemical staining pattern observed, tissue samples were stratified into three groups: I: score <50, low level EGFR expression; II: score 50–200, intense EGFR expression level; III: >200, strong EGFR overexpression level.

Statistical analysis. Chi-square testing was used to correlate membranous EGFR expression with further patient and tumor characteristics, e.g. gender, age, tumor stage and grade, the presence of regional lymph node or distant metastases, the development of recurrent disease, as well as the administration of systemic adjuvant treatment (Table I). The impact of the aforementioned variables on the duration of the long-term survival, the latter defined as the time-period between the surgical intervention and either death from disease or end of follow-up, was calculated according to the Kaplan-Meier method. The Mantel-Cox log-rank test was used for comparison of the survival curves.

A multivariate analysis by a Cox fit proportional hazard model was applied to disclose whether any of the factors tested (age, sex, tumor stage, lymph node status, histological grade and immunoreactivity for membranous EGFR) within the primary RCC

Table II. Distribution of cytoplasmatic EGFR expression and corresponding membranous EGFR staining.

Cytoplasmatic EGFR expression	Corresponding membranous EGFR
8	low staining
5	overexpression
4	strong overexpression
n=17	

specimens could be identified as an independent prognostic factor regarding the patients' long-term survival following tumor nephrectomy. JMP (SAS Inc.) software was used for all analyses.

Results

Immunohistochemical detection of EGFR by tissue microarray analysis. In the case of positive staining for EGFR, tumor cells predominantly exhibited a membrane-bound protein expression (Figure 1A). In contrast, EGFR expression, preferably localized within the cytoplasm, was observed in only 16 (10.7%) of the tumor tissue samples investigated (Figure 1B).

Utilizing the high-throughput tool of tissue microarray analysis, EGFR overexpression (scored 50–300) was detected in 79 (53.0%), whereas low EGFR expression (scored 0–50) was detected in 70 (47.0%) of the primary RCC specimens, respectively.

In contrast, only 7 of the tissue samples obtained from normal renal parenchyma exhibited a distinctly positive reaction for EGFR. Benign tissue showed predominantly none or rare staining (Figure 1C). Accordingly, during the statistical evaluation by contingency analysis, comparison of the benign and corresponding tumor tissue by paired *t*-test analysis revealed EGFR expression at a significantly higher level within the tumor tissue samples ($p < 0.001$, cut-off 50). When strong EGFR overexpression values were analyzed separately, this observation retained the level of statistical significance ($p < 0.0001$, cut-off 200). Additionally, the difference between EGFR expression levels within the benign tissue when subtracted from that within the tumor specimens showed a statistically significant shift towards increased expression of EGFR within the tumorous tissue (Figure 2, *t*-test $p < 0.0001$, mean 77.1; $n = 117$; std. dev. 91.5). The correlation between different EGFR expression levels of membrane-bound staining and several patient and tumor characteristics is demonstrated in Table I. However, both EGFR overexpression levels (cut-off 50 and 200) did not

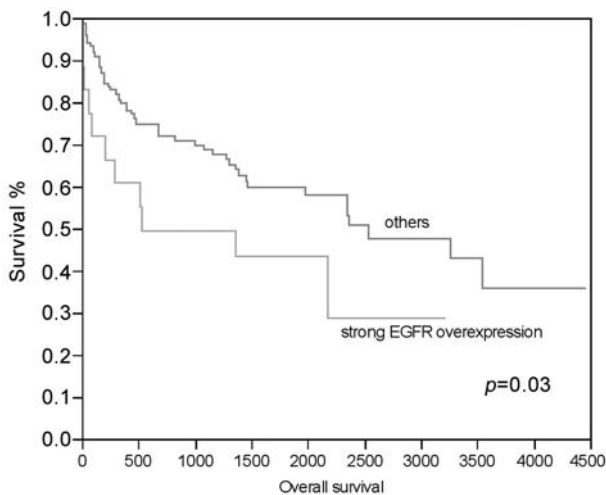


Figure 3. Kaplan-Meier overall survival curve of 149 curatively resected renal cell cancer patients according to the detection of strong membranous EGFR overexpression (cut-off 200, log-rank: $p=0.03$).

correlate with further patient or tumor characteristics, including tumor stage and grade.

Cytoplasmic expression did not reveal any statistical significance when analyzed for clinical parameters. A tendency for cytoplasmic staining accompanied by low membranous staining was observed (Table II).

Statistical analyses of overall survival. To evaluate the prognostic value of EGFR expression for RCC patients, 149 patients were classified into the aforementioned subgroups according to the EGFR staining index. The median follow-up period of the 149 patients with sufficient IHC results was 50 months (range: 2–146 months).

When low EGFR expression was compared to overexpression (cut-off >50), there was no significant reduction in overall survival time for patients ($p=0.49$). However, patients with strong EGFR overexpression (cut-off level >200) showed a significantly reduced overall survival when compared with patients revealing lower protein expression (median 17.4 months, 1–107 months, vs. median 84.7 months, 1–149, respectively, $p=0.03$; log-rank test, Figure 3).

On stratification into three groups: I: low staining (cut-off <50 , $n=75$, 50.3%), II: intense expression (cut-off 50–200, $n=56$, 37.6%), III: strong EGFR overexpression (cut-off >200 , $n=18$, 12.1%), the univariate Kaplan-Meier analyses for survival revealed predominantly strong EGFR staining associated with unfavorable prognosis. Interestingly, the overall survival of the intense expression group surpassed the low expression group ($p=0.023$, Figure 4).

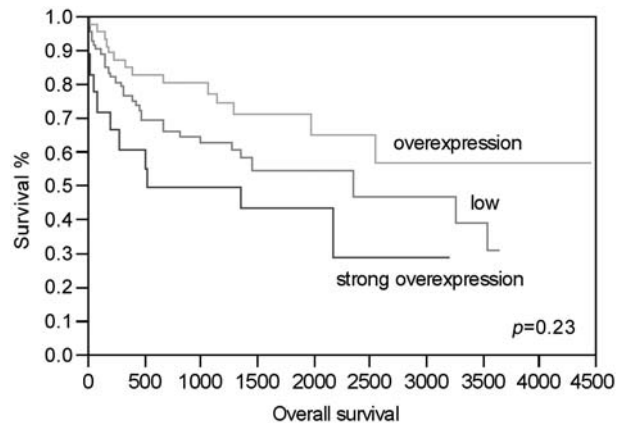


Figure 4. Kaplan-Meier overall survival curves of 149 curatively resected renal cell cancer patients according to the detection of membranous EGFR staining grouped by low (0-50), overexpression (51-200) and strong overexpression (201-300), (log-rank: $p=0.035$).

Multivariate Cox fit proportional hazard analysis was performed to correct univariate prognostic associations of the parameters investigated. For overall survival in a multivariate analysis, the EGFR overexpression at cut-off 50 had no independent prognostic significance, whereas only tumor grading ($p=0.02$), tumor stage ($p=0.05$) and lymph node status ($p=0.02$) remained independent predictors of survival after final stepwise analysis. However, when strong EGFR overexpression was considered, EGFR status ($p=0.03$), grading ($p=0.02$), age ($p=0.04$) and lymph node status ($p=0.01$) remained statistically relevant prognostic factors. Cytoplasmic expression did not reveal any statistical significance when analyzed for survival.

Discussion

EGFR, a transmembrane glycoprotein with an extracellular ligand binding domain and an intracellular domain with tyrosine kinase activity involved in signal transduction, is one of the most important factors for tumor-promoting activities. EGFR activation elicits a cascade to promote the malignant progression of neoplastic disease due to several biological activities that include the regulation of apoptotic pathways, as well as stimulatory effects towards cellular proliferation and angiogenesis. EGFR seems to participate substantially in processes responsible for local tumor growth and metastatic behavior of a variety of human malignancies *e.g.* mammary, bladder, prostate and colorectal cancer (7, 25-28).

Several immunohistochemical studies have shown EGFR overexpression and association with cell proliferation and tumorigenesis in renal cell cancer (8, 10, 11, 15). In

concordance with other investigations, we studied EGFR in both malignant and benign tissue by high-throughput TMA analysis. The relatively low expression in normal kidney tissue supports the involvement of this biomarker in pathways of carcinogenesis. Thus, EGFR serum levels may serve as an additive diagnostic marker for RCC and might help to better differentiate between solid renal masses of benign or malignant histological differentiation.

However, diverging results are evident for the role of EGFR as a prognostic factor for patients with RCC (8, 10, 14, 15). Hofmockel and colleagues studied the members of the EGFR family with regards to their prognostic significance (10). In contrast to our study, prognostic information for the clinical use of EGFR status in the case of RCC was not evident. One explanation for the conflicting results of EGFR protein expression in RCC could be the use of different IHC methods for evaluating EGFR protein expression. To our knowledge, no standardized criteria for immunohistochemical evaluation of EGFR protein in RCC exists (14). Our observation implicates the need for precise and standardized IHC evaluation for biomarker expression values for prognostic statements. Our findings of contrary prognostic results for EGFR overexpression on the one hand, and strong overexpression on the other hand, compared to low expressions (Figure 4), implicate a more complex, non-linear relationship to survival and also to clinical data.

In the immunohistochemical study by Kallio *et al.* (15), differentiation between cytoplasmic and membranous staining was performed with a semiquantitative approach. Membrane-bound EGFR expression was significantly correlated with increased long-term survival, while cytoplasmic staining was related to reduced survival. Interestingly, we observed a tendency for cytoplasmic staining accompanied by low membranous staining. Whereas the reduced survival of our low-staining group compared to the intermediate group is in line with the findings of Kallio *et al.*, we controversially found a strong membrane-stained group with a significantly reduced survival rate.

Uhlman *et al.* (8) associated membranous EGFR expression with poor disease-specific survival. This result, together with the concordant data obtained from the present investigation, indicates that strong overexpression of EGFR might participate in mechanisms such as cellular differentiation and proliferation, thus being involved in the development and further progression of RCC. Interestingly, when further separation into the aforementioned distinct staining modalities was performed, intermediate membranous EGFR expression was correlated with best survival. It can be speculated that alternative pathways in renal cell carcinogenesis exist. EGFR-dependent pathways leading to challenging possibilities for precise molecular targeting for these patient groups and, in addition, the existence of non EGFR-overexpressing tumors, might indicate EGFR-independent pathways leading to tumor

growth and differentiation in RCC. Low membranous tumor staining in patients with decreased survival might be further explained by a possible translocation of EGFR into the cytoplasm. This event may characterize a distinct group of patients with RCC, having a varying clinical course.

Other related parameters should be investigated to clarify signaling and deregulation in pathways, including EGFR. More knowledge about such pathway deregulations might lead to prognostically relevant co-expression profiles of the involved parameters, identifying individual molecular tumor growth patterns. Additionally, this might provide a molecular basis for novel approaches in RCC, based on precise identification of individual patient biomarker profiles for possible targets of pharmacological inhibitors.

In a study by Ozogul *et al.* (29), the distribution of EGFR in different cell types and cellular compartments was analyzed in mouse tissue. The collecting tubule cells of the kidney showed membranous reactivity, whereas the tubular cells exhibited predominantly cytoplasmic reactivity. The latter study exemplifies an approach that may enable a better understanding of individual tumor origins in RCC and should be subject to further evaluation in human renal tissue.

In the present investigation, of a large cohort of patients, strong EGFR expression assessed by TMA was a significant factor for decreased overall survival in univariate and multivariate analysis. Significant correlations between the degree of EGFR expression and further patient or tumor characteristics such as age, gender, histological grade, tumor stage, lymph node status, tumor recurrence, adjuvant treatment and tumor-specific death, were not evident. Different expression grades seem to be distributed throughout the aforementioned histopathological variables.

In conclusion, the strong overexpression of EGFR found in clear RCC is a prognostic factor, whereas expression levels might function as an additional diagnostic tool for decision-making in molecular-targeted therapy. However, further studies are needed to clarify the clinical use of EGFR to improve the survival of patients with RCC.

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References

- 1 Seer N: Cancer Statistics Review, 1975-2001. <http://seer.cancer.gov>, 2004.
- 2 Fuhrman SA, Lasky LC and Limas C: Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol* 6(7): 655-663, 1982.

- 3 Kontak JA and Campbell SC: Prognostic factors in renal cell carcinoma. *Urol Clin North Am* 30(3): 467-480, 2003.
- 4 Ljungberg B: Prognostic factors in renal cell carcinoma. *Scand J Surg* 93(2): 118-125, 2004.
- 5 Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield MD, Ullrich A and Schlessinger J: Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumors of glial origin. *Nature* 313(5998): 144-147, 1985.
- 6 Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield MD, Ullrich A and Schlessinger J: Amplification and overexpression of the EGF receptor gene in primary human glioblastomas. *J Cell Sci Suppl* 3: 161-172, 1985.
- 7 Neal DE, Sharples L, Smith K, Fennelly J, Hall RR and Harris AL: The epidermal growth factor receptor and the prognosis of bladder cancer. *Cancer* 65(7): 1619-1625, 1990.
- 8 Uhlman DL, Nguyen P, Manivel JC, Zhang G, Hagen K, Fraley E, Aeppli D and Niehans GA: Epidermal growth factor receptor and transforming growth factor alpha expression in papillary and nonpapillary renal cell carcinoma: correlation with metastatic behavior and prognosis. *Clin Cancer Res* 1(8): 913-920, 1995.
- 9 Thomasson M, Hedman H, Guo D, Ljungberg B and Henriksson R: LRIG1 and epidermal growth factor receptor in renal cell carcinoma: a quantitative RT-PCR and immunohistochemical analysis. *Br J Cancer* 89(7): 1285-1289, 2003.
- 10 Hofmoeckel G, Riess S, Bassukas ID and Dammrich J: Epidermal growth factor family and renal cell carcinoma: expression and prognostic impact. *Eur Urol* 31(4): 478-484, 1997.
- 11 Moch H, Sauter G, Buchholz N, Gasser TC, Bubendorf L, Waldman FM and Mihatsch MJ: Epidermal growth factor receptor expression is associated with rapid tumor cell proliferation in renal cell carcinoma. *Hum Pathol* 28(11): 1255-1259, 1997.
- 12 Moch H, Sauter G, Gasser TC, Bubendorf L, Richter J, Presti JC Jr, Waldman FM and Mihatsch MJ: EGF-r gene copy number changes in renal cell carcinoma detected by fluorescence *in situ* hybridization. *J Pathol* 184(4): 424-429, 1998.
- 13 Lager DJ, Slagel DD and Palechek PL: The expression of epidermal growth factor receptor and transforming growth factor alpha in renal cell carcinoma. *Mod Pathol* 7(5): 544-548, 1994.
- 14 Langner C, Ratschek M, Rehak P, Schips L and Zigeuner R: Are heterogenous results of EGFR immunoreactivity in renal cell carcinoma related to non-standardised criteria for staining evaluation? *J Clin Pathol* 57(7): 773-775, 2004.
- 15 Kallio JP, Hirvikoski P, Helin H, Kellokumpu-Lehtinen P, Luukkaala T, Tammela TL and Martikainen PM: Membranous location of EGFR immunostaining is associated with good prognosis in renal cell carcinoma. *Br J Cancer* 89(7): 1266-1269, 2003.
- 16 Mendelsohn J: Antibody-mediated EGF receptor blockade as an anticancer therapy: from the laboratory to the clinic. *Cancer Immunol Immunother* 52(5): 342-346, 2003.
- 17 Rowinsky EK, Schwartz GH, Gollob JA, Thompson JA, Vogelzang NJ, Figlin R, Bukowski R, Haas N, Lockbaum P, Li YP, Arends R, Foon KA, Schwab G and Dutcher J: Safety, pharmacokinetics, and activity of ABX-EGF, a fully human anti-epidermal growth factor receptor monoclonal antibody in patients with metastatic renal cell cancer. *J Clin Oncol* 22(15): 3003-3015, 2004.
- 18 Linehan WM and Zbar B: Focus on kidney cancer. *Cancer Cell* 6(3): 223-228, 2004.
- 19 Dancey JE: Epidermal growth factor receptor and epidermal growth factor receptor therapies in renal cell carcinoma: do we need a better mouse trap? *J Clin Oncol* 22(15): 2975-2977, 2004.
- 20 Petty WJ, Dragnev KH, Memoli VA, Ma Y, Desai NB, Biddle A, Davis TH, Nugent WC, Memoli N, Hamilton M, Iwata KK, Rigas JR and Dmitrovsky E: Epidermal growth factor receptor tyrosine kinase inhibition represses cyclin D1 in aerodigestive tract cancers. *Clin Cancer Res* 10(22): 7547-7554, 2004.
- 21 Kuefer R, Hofer MD, Gschwend JE and Rubin MA: Tissue microarrays. High-throughput procedures to verify potential biomarkers. *Urologe A* 43(6): 659-667, 2004.
- 22 Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G and Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 4(7): 844-847, 1998.
- 23 Merseburger AS, Kuczyk MA, Serth J, Bokemeyer C, Young DY, Sun L, Connelly RR, McLeod DG, Mostofi FK, Srivastava SK, Stenzl A, Moul JW and Sesterhenn IA: Limitations of tissue microarrays in the evaluation of focal alterations of bcl-2 and p53 in whole mount derived prostate tissues. *Oncol Rep* 10(1): 223-228, 2003.
- 24 Theodorescu D, Broder SR, Boyd JC, Mills SE and Frierson HF Jr: Cathepsin D and chromogranin A as predictors of long term disease specific survival after radical prostatectomy for localized carcinoma of the prostate. *Cancer* 80(11): 2109-2119, 1997.
- 25 Fernbro E, Bendahl PO, Dictor M, Persson A, Ferno M and Nilbert M: Immunohistochemical patterns in rectal cancer: application of tissue microarray with prognostic correlations. *Int J Cancer* 111(6): 921-928, 2004.
- 26 Abd El-Rehim DM, Pinder SE, Paish CE, Bell JA, Rampaul RS, Blamey RW, Robertson JF, Nicholson RI and Ellis IO: Expression and co-expression of the members of the epidermal growth factor receptor (EGFR) family in invasive breast carcinoma. *Br J Cancer* 91(8): 1532-1542, 2004.
- 27 Kim H and Muller WJ: The role of the epidermal growth factor receptor family in mammary tumorigenesis and metastasis. *Exp Cell Res* 253(1): 78-87, 1999.
- 28 Kim HG, Kassis J, Souto JC, Turner T and Wells A: EGF receptor signaling in prostate morphogenesis and tumorigenesis. *Histol Histopathol* 14(4): 1175-1182, 1999.
- 29 Ozogul C, Karaoz E, Erdogan D and Akyol G: Immunohistochemical localization of the epidermal growth factor receptor in normal mouse tissues. *Acta Physiol Hung* 85(2): 113-120, 1997.

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