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Expression of Nectin-4 in Chromophobe Renal Cell Carcinoma in a Multicenter Cohort: Early Prognostic and Therapeutic Considerations

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Keywords
Renal cell carcinoma · Nectin-4 · Chromophobe histology · Survival

Abstract
Introduction: Nectin-4 is a member of the nectin family and a calcium-independent immunoglobulin-like transmembrane protein that contributes to tumor growth and angiogenesis in malignant tumors. A nectin-4-directed antibody drug conjugate, enfortumab vedotin-ejf, has recently been approved for treatment in urothelial cancer and is currently under investigation in other tumor entities such as breast, lung, and prostate cancer. In non-clear cell renal cell carcinoma (RCC), vascular endothelial growth factor (VEGF)-directed tyrosine kinase inhibitors and checkpoint inhibitors are currently treatments of choice. However, due to the rarity of disease treatment recommendations for chromophobe RCC (chRCC) are limited and new therapeutic agents urgently needed. In this study, we investigated the expression and prognostic impact of nectin-4 in a large cohort of chRCC. ***Methods:*** Patients who underwent renal surgery due to chRCC were recruited. Clinical data were retrospectively evaluated. Tumor specimen was analyzed for nectin-4 expression by immunohistochemistry. ***Results:*** Eighty-one chRCC patients were eligible for analysis. In 15 (18.5%) samples, tumors were positive for nectin-4. No significant associations were found for nectin-4 expression and clinical attributes in patients with chRCC. Kaplan-Meier analysis disclosed a 5-year overall survival for nectin-4-negative and nectin-4-positive tumors of 91.8% versus 100.0% ($p = 0.316$, log rank). ***Conclusions:*** In chRCC, a small subset of tumors expresses nectin-4 potentially amenable to nectin-4-directed treatment. Expression of nectin-4 is not associated with parameters of aggressiveness or survival. Due to the rare incidence of chRCC, further studies with larger cohorts are warranted.

Marie Mikuteit, Stefanie Zschäbitz, Sandra Steffens, and Franziska Erlmeier equally contributed to this work.

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Introduction

Chromophobe RCC (chRCC) is the third most common renal cell carcinoma (RCC) subtype and constitutes 5–7% of all RCC cases [1]. Because of distinct nuclear atypia, chRCC should not be graded according to the WHO/ISUP grading system [2]. Moreover, up to now no other established prognostic grading systems or biomarkers exist. Compared to other RCC subtypes, this subtype has a favorable prognosis. The 5-year survival rate is reported 78–100%. Nevertheless, some patients show dismal clinical courses with large tumors and ultimately develop metastasis. Once metastasized, treatment options are very limited.

Due to the rare occurrence of metastatic chRCC, few clinical trials have investigated systemic treatment approaches for patients with chRCC. Current guidelines recommend tyrosine kinase monotherapies, but overall response rates (ORRs) are low [3, 4]. Inferior disease control rates compared to other non-ccRCC subtypes have been reported from phase 2 trials for checkpoint inhibitors in monotherapy as well as combination therapy such as PD-1 inhibitors pembrolizumab [5] or nivolumab plus cabozantinib [6] or lenvatinib in combination with pembrolizumab [6]. The SUNIFORECAST trial is a randomized two-arm phase 2 trial that is ongoing [7]. Here, nivolumab in combination with ipilimumab was tested against physicians' choice treatment with results pending to date.

An emerging therapeutic target in oncology is nectin-4. The nectin transmembrane protein family consists of four nectins (nectin-1–4) and five nectin-like molecules (Necl-1–5). They are calcium-independent immunoglobulin-like proteins that play a role in the regulation of intercellular junctions and tissue morphogenesis [8]. Other than nectins-1–3, nectin-4 (or poliovirus receptor like 4 (PVRL4)) is primarily expressed on malignant cells [9] and is involved in tumor angiogenesis, proliferation, and lymphangiogenesis in vitro and in vivo [10, 11]. Nectin-4 expression is a prognostic biomarker in several types of cancers such as breast, esophageal, lung, colorectal, and pancreatic cancer [10, 12–15]. We have recently reported data on nectin-4 in papillary RCC (pRCC) [16]. While we could not confirm nectin-4 as a prognostic marker in pRCC, its high abundance of up to 48% on surgical specimens raised the question of being a potential target for therapeutical approaches. Nectin-4 has come into focus since the FDA and EMA approval of enfortumab vedotin-ejf (EV) for metastatic platinum and checkpoint inhibitor refractory urothelial cancer (UC). Nectin-4 is expressed on the surface of almost all UC and variant bladder cancer cells (with the exception of neuroendocrine bladder cancer) [17]. EV is an antibody drug conjugate that consists of an antibody directed against nectin-4 and the payload monomethyl auristatin E (MMAE). MMAE is released upon cellular uptake of the antibody drug conjugate complex and lysosomal degradation. MMAE binds to β1-tubulin and causes G2/M phase cell cycle arrest [18].

Within the pivotal trial EV-301, EV was tested against standard-of-care treatment and superior efficacy (overall survival [OS], progression-free survival, and ORR) in platinum and immunotherapy refractory bladder cancer patients was demonstrated [19]. EV is currently investigated within several clinical trials and primarily but not exclusively in UC patients of different stages and treatment lines. To date and to our knowledge, information on $n = 46$ patients with non-UC that received treatment within the dose escalation/expansion phase 1 trial EV-101 has not been reported [20]. However, while data on efficacy of EV in nectin-4-positive non-urothelial tumors are missing nectin-4-directed agents offer at least a theoretical rationale for its usage.

Therefore, the aim of this study was to evaluate the prevalence of nectin-4 expression as well as the prognostic impact of nectin-4 in chRCC. To the best of our knowledge, this is the first study which analyzed this aspect in this RCC subtype.

Methods

Patients and Tumor Characteristics

We identified 81 patients, who underwent surgical resection of a chRCC primary tumor between 1996 and 2014 through the electronic pathology register. Relevant clinical information including tumor stage and histological subtype according to the UICC 2010 TNM tumor staging system was collected for each tissue sample. Only patients with a full data set were included. Suitable specimens and tissue microarrays (TMAs) were selected by an experienced pathologist (F.E.). Pathological samples were processed from the primary tumor as previously described [21–23]. A second uropathologist (A.H.) confirmed diagnosis. Accordingly, patient data were retrieved from electronic patient charts, including follow-up data regarding OS. Date of death was confirmed by the Munich Cancer Registry of the Munich Tumor Centre. The study was conducted according to the latest version of the Declaration of Helsinki. This study protocol was reviewed and approved by institutional ethics committee of the Technical University of Munich (approval number, 384/13).

Procedures

We used immunohistochemistry (IHC) to determine the expression of nectin-4. The process was conducted as previously described [21–23]. 2-µm TMA slides were stained for nectin-4 (Anti-Nectin-4 antibody, Abcam, ab192033, dilution

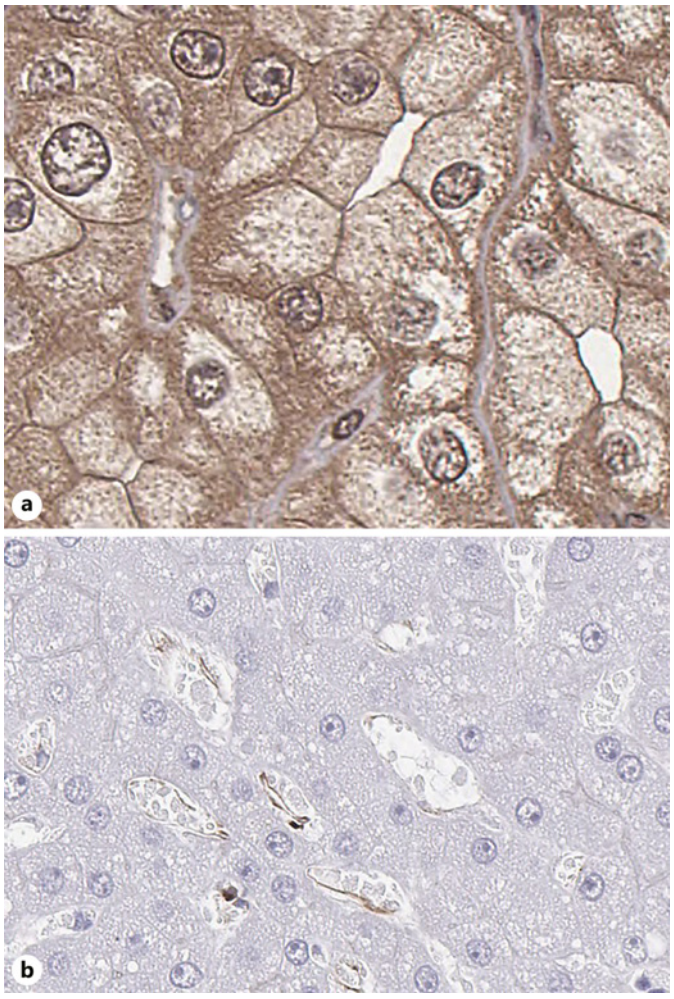

Fig. 1. Immunohistochemical staining of nectin-4 in chRCC specimen. **a** Positive (×40 magnification). **b** Negative (×40 magnification).

1:100). First, we performed heat pretreatment at 120°C for 5 min with Tris-EDTA buffer pH 9 and peroxidase blocking (Dako, Hamburg, Germany); then the antibody was applied for 30 min. We incubated the probes with a horseradish peroxidase-labeled secondary antibody polymer (EnVision, Dako) for 30 min; then we added a diaminobenzidine substrate chromogen solution (Dako) for 10 min and counterstained for 1 min with hematoxylin (Merck, Darmstadt, Germany). All procedure concerning incubation was performed at room temperature. For each staining experiment, we included positive controls (paraffin-embedded human colorectal cancer tissue) as well as negative control slides without the addition of primary antibody. A pathologist (F.E.) assessed all stained tissue samples in a blind way. Leitz ARISTOPLAN light microscope (Leica Microsystems, Germany) with a ×10 eyepiece, a 22-mm field of view, and ×40 objective lens (Plan Fluotar x40/0.70) served as tool for the evaluation.

The staining reaction was classified according to a semi-quantitative IHC reference scale previously described [24, 25]. Nectin-4 was localized primarily on the membrane and partly in the cytoplasm of tumor cells.

The staining intensity was scored from 0 to 3 (0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining) according to the H-score as already described (Fig. 1) [26, 27]. The area of staining was evaluated in percent (0–100%); a staining intensity score was defined by multiplying the score with the stained area. Values in our patient collective were dichotomized using the median of observed distribution as the binary cutoff because of the absence of normative data on cell membrane or cell cytoplasm staining intensity in the literature and the limited number of cases. A nectin-4 staining lower or equal to the median was defined as nectin-4 low, and a staining higher than the median was defined as nectin-4 high.

Statistical Analysis

We compared patient/tumor characteristics between the nectin-4 low and high group with using tests dependent upon the nature of variable (χ^2, Fisher's exact tests, Mann-Whitney U test, and independent *t* test). The duration of follow-up was calculated from the date of surgery to the date of death or last known follow-up. In the absence of death, survival was censored at the last date of follow-up. Kaplan-Meier survival times were estimated, with subgroups being compared using the log-rank test. SPSS 27.0 (USA) was used for statistical analysis. Two-sided *p* values below 0.05 were considered statistically significant.

Results

For 81 patients, full data sets were available and thus included in the analysis.

Characteristics of Patients and Expression of Nectin-4

The median age of the herein reported cohort was 59.8 (range: 31–79) years. Sixty patients (74.1%), 14 (17.3%), and 7 (8.6%) presented with pT1, pT2, and pT3 tumors, respectively. 86.4% of the patients had AJCC stage I/II disease. Furthermore, 3 (3.7%) of all patients presented with synchronous distant metastasis. Nectin-4 expression was found in 15 (18.5%) of the chRCC TMA specimens (Fig. 1). No associations between nectin-4 expression and patient or tumor characteristics were identified (Table 1).

Nectin-4 Expression and Clinical Course

Median follow-up was 40.5 (IQR: 10.8–109.3) months. At the time of last follow-up, 46 (56.8%) patients were alive, 9 (11.1%) patients had died, and 26 (32.1%) patients were lost to follow-up. Within the group of patients with tumors positive for nectin-4 expression, 9 (60.0%) patients were alive, 0 (0.0%) had died, and 6 (40.0%) were lost to follow-up (χ^2, p = 0.487). Kaplan-Meier analysis

Table 1. Characteristics of patients with chRCC in dependence of nectin-4 expression

Variable	All chRCC, n = 81 (100%)	Nectin-4 neg, n = 66 (81.5%)	Nectin-4 pos, n = 15 (18.5%)	p value
Age, median (IQR), years	59.8 (52.9–69.1)	63.4 (52.6–69.9)	56.2 (45.7–65.7)	0.064[a]
Sex				0.113[b]
Female	23 (28.4)	16 (24.2)	7 (46.7)	
Male	58 (71.6)	50 (75.8)	8 (53.3)	
Stage (TNM 2010)				0.710[c]
pT1	60 (74.1)	50 (75.8)	10 (66.7)	
pT2	14 (17.3)	11 (16.7)	3 (20.0)	
pT3	7 (8.6)	5 (7.6)	2 (13.3)	
Cancer stage (AJCC)				0.801[c]
Stage I	56 (69.1)	46 (69.7)	10 (69.1)	
Stage II	14 (17.3)	11 (16.7)	3 (20.0)	
Stage III	8 (9.9)	6 (9.1)	2 (13.3)	
Stage IV	3 (3.7)	3 (4.5)	0 (0)	
LN metastasis[#]				0.464[b]
N–	78 (96.3)	64 (97.0)	14 (93.3)	
N+	3 (3.7)	2 (3.0)	1 (6.7)	
Metastasis[#]				1.0[b]
M–	78 (96.3)	63 (95.5)	15 (100.0)	
M+	3 (3.7)	3 (4.5)	0 (0.0)	
Disease status				1.0[b]
Localized*	70 (86.4)	57 (86.7)	13 (86.7)	
Advanced[$]	11 (13.6)	9 (13.6)	2 (13.3)	

AJCC, American Joint Committee on Cancer; IQR, interquartile range; NE, not evaluable; N–, lymph node status unknown or tumor cells absent from regional lymph nodes; N+, regional lymph node metastasis present; neg, negative; pos, positive. [#]At time of renal surgery; *localized disease = pT1/2 N0/M0. [$]Advanced disease = pT3/4 and/or N+ and/or M+. [a]Mann-Whitney-U test. [b]Fisher's exact test. [c]χ^2 test.

disclosed a 5-year OS for nectin-4-negative compared to Nectin-4-positive tumors of 91.8% versus 100.0% (p = 0.316, log rank) (Fig. 2).

Discussion

The aim of our study was to evaluate the expression and prognostic associations of nectin-4 with clinical parameters, tumor aggressiveness and survival in patients with chRCC. We showed that 15 of 81 samples were positive for nectin-4 expression. Our results indicated no associations between tumor stage and nectin-4 expression neither in grading nor in stage. Furthermore, we detected no correlation between nectin-4 expression and OS.

Although metastatic disease is rare in chRCC, once it occurs treatment options are sparse, responses to standard RCC therapies (i.e., tyrosine kinase inhibitors and MTOR inhibitors) are limited, and therefore novel treatment strategies are of utmost importance. Lee et al. [6] reported no objective response to Nivolumab/Cabozantinib in their cohort of 7 patients with chRCC. Within the Keynote-B61 trial, 158 patients with non-clear cell RCC received lenvatinib in combination with the PD-1 inhibitor pembrolizumab. Of those, 29 patients (18%) had a chRCC. ORR in the trial was 49% (95% confidence interval 41–57%), however for patient with chRCC only 29% [6]. Koh et al. [28] reported data of a phase 2 trial of everolimus with 49 patients with non-ccRCC of which 8 patients had chRCC. Two patients showed objective response to everolimus and patients with chRCC had longer progression-free survival compared to patients with non-ccRCC/non-chRCC. Hutson et al. [29] conducted a single-center phase 2 trial of lenvatinib/everolimus in patients with non-ccRCC. Nine patients with chRCC, 20 with papillary RCC, and 2 patients with unclassified tumors were included. Of 8 patients that showed a partial response, 4 had a chRCC. McDermott et al. [5] analyzed efficacy of pembrolizumab in 165 patients with non-ccRCC. Of those, 12.7% had chRCC. While ORR was reported 26.7% for the overall cohort, subgroup analysis indicated inferior efficacy in chRCC (ORR = 9.5%).

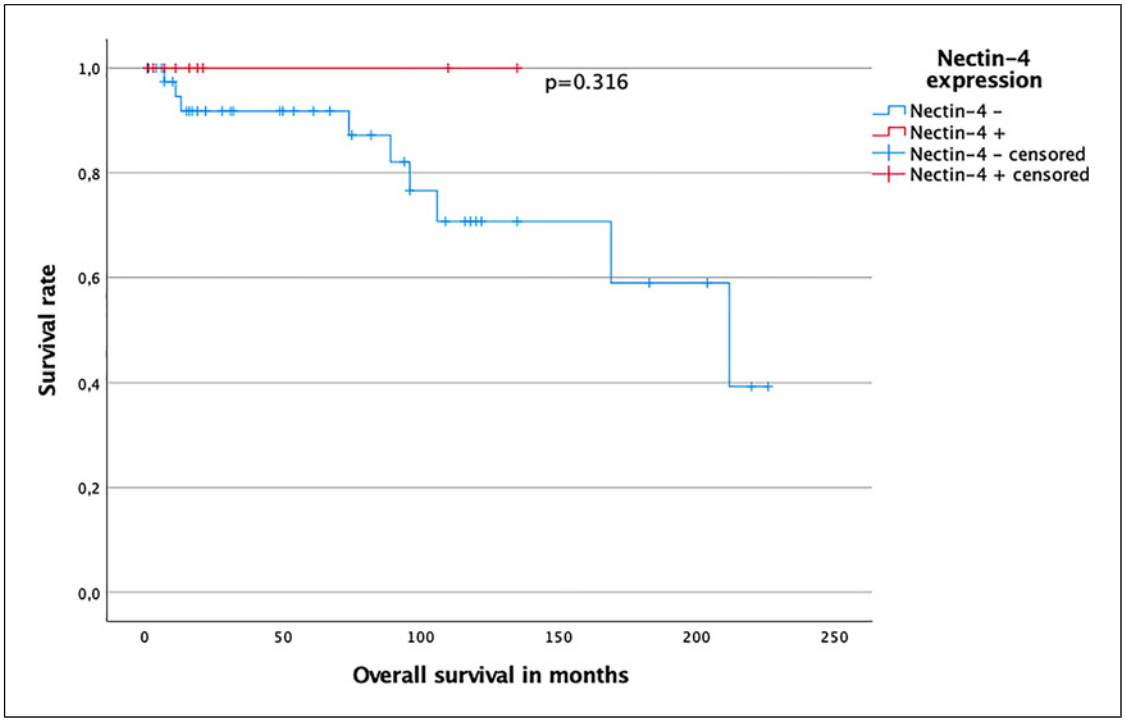

Fig. 2. 5-year OS for patients with chRCC in dependence of nectin-4 expression.

The wide availability of next-generation sequencing has allowed for a broader understanding of tumor biology and identified novel therapeutic options for a subset of patients. In metastatic chRCC, mutations in PTEN and TP53 have been identified as a common event [30–32]. Further, PTEN loss is associated with reduced T-cell infiltration, an immune suppressive phenotype, and resistance to immunotherapy [33]. Basket trials for patients with tumors that show PTEN loss are ongoing and could offer therapeutic options for patients with refractory chRCC (NCI Match, NCT02465060, CRAFT, NCT04551521 [34, 35]). In addition, the results of our investigation show expression of nectin-4 on a subset of chRCC specimen and nectin-4-directed treatment could add a further treatment strategy for those patients stratified by biological markers.

Of course, our study has several limitations, including the methodology of IHC, the scoring system, the use of TMAs, the relatively low number of cases and early stages, as well as the retrospective analysis. Hence, our study can give an initial indication that nectin-4 might not be a suitable prognostic marker in RCC but could offer potential tumor agnostic treatment option for patients with nectin-4-positive tumors that should further be explored.

Within our cohort of chRCC specimen, a small subset of tumors was positive for nectin-4 staining. Expression of nectin-4 was not associated with parameters of aggressiveness or survival. Further studies are warranted to explore the expression of nectin-4 with larger cohorts in this very rare disease for which novel treatment options are urgently needed. Also, the potential role for nectin-4-directed treatment for chRCC and other tumor entities should be investigated.

Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of the Technical University of Munich (approval number, 384/13). Informed written consent was assessed and was obtained from participants to participate in the study. Details that disclose the identity of the subjects under study were omitted.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.M., F.E., S.Z., and S.S. participated in the data interpretation and drafting of the manuscript. M.M. and S.S. performed the statistical analysis. F.E. carried out the data acquisition. M.A., W.W., and A.H. revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author M.M. upon reasonable request.

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