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Laboratory-Kidney cancer

sE-cadherin is upregulated in serum of patients with renal cell carcinoma and promotes tumor cell dissemination in vitro

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Abstract

Introduction: Cadherin family proteins are involved in the tumorigenesis of several malignancies. However, their significance in renal cell carcinoma (RCC) has not been extensively investigated. The current study investigates the potential of several cadherins to perform as biomarkers for tumor detection and exert functional RCC activity.

Methods: Pre- and postoperative concentrations of sE-cadherin, cadherin-6, N-cadherin, cadherin-11, cadherin-17, and cadherin-5 were measured in serum of patients undergoing surgery for RCC and correlated to clinical and histopathological parameters. Control serum was obtained from healthy volunteers. A498 and Caki-1 cells were incubated with sE-cadherin and assessed for cell growth, adhesion, and chemotaxis.

Results: sE-cadherin was significantly upregulated in RCC patients, as compared to controls, and discriminated them with striking accuracy (area under the curve value 0.83). Serum levels remained stable several days after surgery. Treating A498 and Caki-1 cancer cells with various concentrations of sE-cadherin attenuated cell growth and adhesion, while chemotaxis was augmented.

Conclusions: sE-cadherin is overexpressed in serum of RCC patients and provides a functional cellular switch from sessility to aggressive dissemination. While sE-cadherin is not tumor-specific and thus inappropriate for population-based screening, further studies are warranted to investigate its role in monitoring RCC and employing it as a therapeutic target. © 2019 Elsevier Inc. All rights reserved.

Keywords: Renal cell cancer; Biomarker; sE-cadherin; Tumor growth; Tumor invasion

1. Introduction

Compared to other malignancies, the incidence of renal cell cancer (RCC) at present demonstrates the highest growth rate in all ages and racial groups in the world [1,2]. Since RCC is unresponsive to chemo- or radiotherapy [3], partial or standard nephrectomy remains the standard curative care. Although up to one-third of patients are diagnosed with a primary metastatic RCC (mRCC) and one-third of initially cured patients later experience systemic progression, tumor-specific mortality is decreasing [1,3]. Many agents have recently been approved for mRCC treatment [4,5]. Yet, individualized medicine is challenging due to tumor heterogeneity, each subtype having its own specific prognosis and treatment response [6]. Furthermore, clinically relevant diagnostic, prognostic, and predictive biomarkers are lacking.

Biomarkers are quantifiable characteristics of biological processes that measure a physiological state and may be used as surrogates to diagnose cancer, predict outcomes, and provide individualized treatment [6]. Regarding mRCC, several molecular candidates have recently been tested as

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biomarkers [6,7]. Although some promising results have been reported, for example,. for osteopontin or carbonicanhydrase IX and CRP [8,9], none of these markers has improved the predictive accuracy of the current clinically based prognostic systems and hence been recommended for clinical routine [7]. Thus, a need exists to discover novel biomarkers to aid in tailoring mRCC management.

Adhesion molecules of the cadherin family are transmembrane glycoproteins that mediate specific cell-cell adhesions in a calcium-dependent manner [10] and are therefore crucial for maintaining tissue integrity and homeostasis [11]. Furthermore, changes in the cadherin expression profile are involved in cell differentiation and organ formation. It is of note, that cadherins are associated with tumorigenesis, since tumor cells demonstrate deregulated cadherin expression and inappropriate switching among family members [11]. In addition, decreased cadherin expression or altered function might facilitate invasive properties of cancer cells [12]. In the current study, the potential of a number of selected cadherins to act as biomarkers in RCC patients and exert an effect on the biological properties of tumor cells was assessed.

2. Material and methods

2.1. Clinical conditions

The study was approved by the local ethics committee of Frankfurt University Hospital, (project number SUG_01-2014). A signed informed consent form was obtained from each participant before study enrollment. Patients undergoing partial or radical nephrectomy due to suspected RCC in the Department of Urology at Frankfurt Goethe University Hospital, Germany, were prospectively enrolled between May 2012 and December 2016. Patients with histologically confirmed RCC were included in the final assessment. The tumor stage was classified according to the seventh edition of the Tumor Node Metastasis (TNM) classification system [13] and TNM supplement 2012 [14]. Tumor grading was performed according to the Fuhrman system [15]. Clinical parameters, as well as pathology reports, were collected from the patient medical charts. Blood samples for trial purposes were retrieved before, and at day 5 to 7, after surgery. Age and gender-matched blood samples of healthy volunteers ($n = 25$) were received from the blood donor center of Frankfurt Goethe University Hospital and utilized as a control group.

2.2. Blood samples

Blood samples (10 ml) were centrifuged at 3,000 rpm at 4°C (39.2°F) for 10 minutes. The serum supernatant was stored at −80°C (−112°F).

2.3. Cadherin quantification

To quantify the cadherin concentrations, 7 validated commercial ELISA kits were utilized according to the manufacturer's instructions (Table 1). The particular test procedures varied marginally between the kits. Briefly, for the soluble(s) E-cadherin ELISA, diluted serum was pipetted onto 96-well plates and incubated at 37°C for 90 minutes. The plate was then washed and followed by incubation with biotinylated anti-human E-Cadherin antibody for 60 minutes. Avidin-Biotin-Peroxidase-Complex was then added to each well after extensive well washing. Finally, TMP color development substrate was added after a 5-time well washing. After 15 minutes incubation, a stop solution was added. Optical density was measured spectrophotometrically using a microplate reader (Infinite series M200, Tecan, Crailsheim, Germany). The concentration was calculated from a standard curve using a 4-parameter curve fit (Magellan software, Tecan).

Table 1
Cadherin kits used for ELISA.

Kit	Manufacturer
Cadherin-6	EIAAB Science CO., Wuhan, China
N-Cadherin	Boster Biological Technology Co., Ltd., Pleasanton, USA
sE-Cadherin	Boster Biological Technology Co., Ltd., Pleasanton, USA
Cadherin-11	Wuxi Donglin Sci & Tech Development Co., Ltd., Jiangsu, China
Cadherin-17	Cloud-Clone Corp., Houston, USA
Cadherin-5	Abnova, Taipeh, Taiwan/ R&D Systems VE Quantiquin

2.4. Cell cultures

To assess the biological effects of sE-cadherin on cancer cells, the human RCC tumor cell lines, nonmetastatic A498 and metastatic Caki1 [16], purchased from Cell Lines Service (Heidelberg, Germany) or LGC Promochem (Wesel, Germany), respectively, were utilized. Tumor cells were grown and subcultured in RPMI 1640 (Gibco/Invitrogen; Karlsruhe, Germany) containing 10% fetal calf serum, 2% HydroxyEthyl-PiperazineEthane-Sulfonic acid buffer (HEPES-buffer) (1M, pH 7.4), 1% glutamax and 1% penicillin/streptomycin (all: Gibco/Invitrogen; Karlsruhe, Germany) at 37°C in a humidified, 5% CO_2 incubator. Subcultures from passages 5 to 24 were employed.

2.5. sE-cadherin

Human sE-cadherin was derived from Cell Systems, Troisdorf, Germany, and added to the RCC cell cultures at a concentration of 0.1, 0.5, 1.0, or 5.0 μg/ml. In all experiments, sE-cadherin-treated tumor cells were compared to the controls treated with cell culture medium alone. The incubation time of sE-cadherin depended on the experiment and is shown in respective results. To exclude toxic effects of sE-cadherin, cell viability was determined by trypan blue (Gibco/Invitrogen).

2.6. Cell viability and growth

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye reduction assay (Roche Diagnostics, Penzberg, Germany) according to the manufacturer's instruction. Tumor cells (100 μl, 1×10^4 cells/well) were seeded onto 96-well plates. After 24, 48, and 72 hours, 10 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg/ml) was added and further incubated for 4 hours. Formazan crystals, formed after 4 hours, were solubilized with a buffer containing 10% SDS in 0.01 M HCl. Plates were allowed to stand overnight at 37°C, 5% CO_2 and measured the following day in a microplate reader (Infinite series M200 Tecan, Crailsheim, Germany) at 570 nm. Each experiment was done in triplicate. After subtracting background absorbance, the corrected absorbance was interpolated from a standard curve of known cell concentration. Results were expressed as mean cell number.

Evaluation of cell growth was done by the clonogenic growth assay. Tumor cells (treated vs. nontreated) were transferred to 6-well plates at 500 cells per well. Following 10 days incubation, cell colonies were fixed and counted. Clones of at least 50 cells were counted as one colony.

2.7. Cell attachment to immobilized collagen

Six-well plates were coated with collagen G (extracted from calfskin, 90% collagen type I and 10% collagen type III; Seromed; diluted to 400 μg/ml in Phosphate-Buffered-Saline (PBS)) overnight. Uncoated wells served as the background control. Plates were washed with 1% bovine serum albumin (BSA) in PBS to block nonspecific cell adhesion. Thereafter, 0.5×10^6 tumor cells were added to each well for 60 minutes. Subsequently, nonadherent tumor cells were washed off, the remaining adherent cells were fixed with 1% glutaraldehyde and counted microscopically. The mean cellular adhesion rate, defined by adherent cells$_{\text{coated well}}$ − adherent cells$_{\text{background}}$, was calculated from 5 different observation fields.

2.8. Measurement of tumor cell migration (chemotaxis)

Serum induced chemotactic movement was examined using 6-well Transwell chambers (Greiner, Frickenhausen, Germany) with 8-μm pores. About 0.5×10^6 tumor cells/ml were placed in the upper chamber in serum-free medium. The lower chamber contained 10% serum. After 24 hours incubation the upper surface of the Transwell membrane was gently wiped with a cotton swab to remove nonmigrating cells. Cells which had moved to the lower surface of the membrane were stained using hematoxylin and counted microscopically. The mean chemotaxis rate was calculated from 5 different observation fields.

2.9. Epithelial-to-mesenchymal transition markers

Tumor cells (treated with 5 μg/ml sE-cadherin for 24 hours vs. nontreated) were washed in blocking solution (PBS, 0.5% BSA) and then incubated for 60 minutes at 4°C with the following phycoerythrin-conjugated monoclonal antibodies: anti-cytokeratin 18 (CK18, IgG; clone EPR17347, Abcam, Cambridge, UK), anti-vimentin (IgG; clone D21H3; Cell Signaling, Frankfurt, Germany), anti-Twist-1 (IgG1; clone 927403; R&D Systems, Wiesbaden, Germany).

To allow intracellular detection, cells were fixed with 100 μl fixation medium (Fix & Perm; Biozol, Eching, Germany) and washed twice in blocking solution (PBS, 0.5% BSA). Subsequently, they were incubated for 60 minutes at 4°C with 100 μl permeabilization medium (Fix & Perm) before the antibody was added.

Protein expression was measured using FACScalibur (BD Biosciences, Heidelberg, Germany; FL-2H (log) channel histogram analysis; 1×10^4 cells per scan) and expressed as mean fluorescence units. A mouse IgG1-phycoerythrin (MOPC-21; BD Biosciences) was used as an isotype control.

2.10. Statistics

Statistical analysis was conducted using the statistical software BiAS for Windows (Version 9.11, Dr. rer. nat. Hanns Ackermann, epsilon-publishers, Frankfurt, Germany). Descriptive statistics were used for patient characteristics. Spearman's rank correlation was utilized to analyze association of cadherins with clinical and histological variables. Cadherin expression was compared between the control and experimental groups using the Mann-Whitney U test. Receiver Operation Characteristic analysis was performed for discrimination of the diagnostic sensitivity and specificity for each cadherin. The difference between pre- and postoperative cadherin expression was assessed by the Wilcoxon-matched-pairs test. Multivariate logistic or linear regression analyses were conducted to investigate the ability of cadherins to independently predict clinical and histopathological outcome. A P value < 0.05 was considered to indicate a significant difference.

3. Results

Clinical and pathological characteristics of 74 patients included in the final analysis are shown in Table 2. Median age at tumor diagnosis was 65 years, 82.4% of patients were males. Cytoreductive nephrectomy was performed in 11 patients (14.9%) with distant metastases, nine of whom (81.8%) experienced bone and/or liver disease.

Out of all the cadherins assessed in serum, only sE-cadherin was significantly overexpressed in tumor patients compared to controls (median 125.5 vs. 91.4 ng/ml; Fig. 1A). In addition, sE-cadherin demonstrated a significant accuracy

Table 2
Epidemiologic, clinical, and pathological characteristics of the study cohort.

Parameters	Value
Sample size (*n*)	74
Age (y)	65 (43–82)
Gender	
Male	61 (82.4%)
Female	13 (17.6%)
Tumor size (cm)	4.5 (0.6–20)
Tumor staging	
pT1a	33 (44.6%)
pT1b	19 (25.7%)
pT2a	3 (4.1%)
pT2b	5 (6.8%)
pT3a	11 (14.9%)
pT3b	0
pT3c	0
pT4	3 (4.1%)
Metastasis	
No	63 (85.1%)
Yes	11 (14.9%)
Bone	2 (18.2%)
Liver	2 (18.2%)
Lung	1 (9.1%)
Intestinal	1 (9.1%)
Bone + liver	**3 (27.3%)**
Bone + liver + lung	**2 (18.2%)**
Lymph node metastases	
0	70 (94.6%)
≥1	4 (5.4%)
Grade	
1	16 (21.6%)
2	50 (67.6%)
3	8 (10.8%)
Subtype	
Clear cell	54 (73.0%)
Papillary	18 (24.3%)
Chromophobe	2 (2.7%)

Values expressed as median with range or number (%) as appropriate.
These numbers depict patients with synchronous bone-liver or bone-liver-lung metastasis as opposed to the other metastasized patients with solitary organs affected.

for discriminating tumor patients with an area under the curve (AUC) value of 0.83 (optimal cutoff 114.9 ng/ml, misclassification rate 19.3%; Fig. 1B). There was no difference in the serum concentration of sE-cadherin before and after surgery (day 5–7). Notably, sE-cadherin serum levels were virtually higher in metastatic, compared to nonmetastatic, patients. However, this difference did not reach statistical significance. None of the candidate cadherins were independently associated with age, lab values, tumor size, tumor subtype, tumor stage, tumor grade, lymphatic, or visceral metastasis.

The physiologic relevance of sE-cadherin was evaluated by in vitro assay. To analyze cell number and clonogenic growth, tumor cells were treated with sE-cadherin and the experimental assays started in parallel. To explore cell adhesion and chemotaxis, tumor cells were pretreated with sE-cadherin for 24 hours and then subjected to the adhesion and chemotaxis

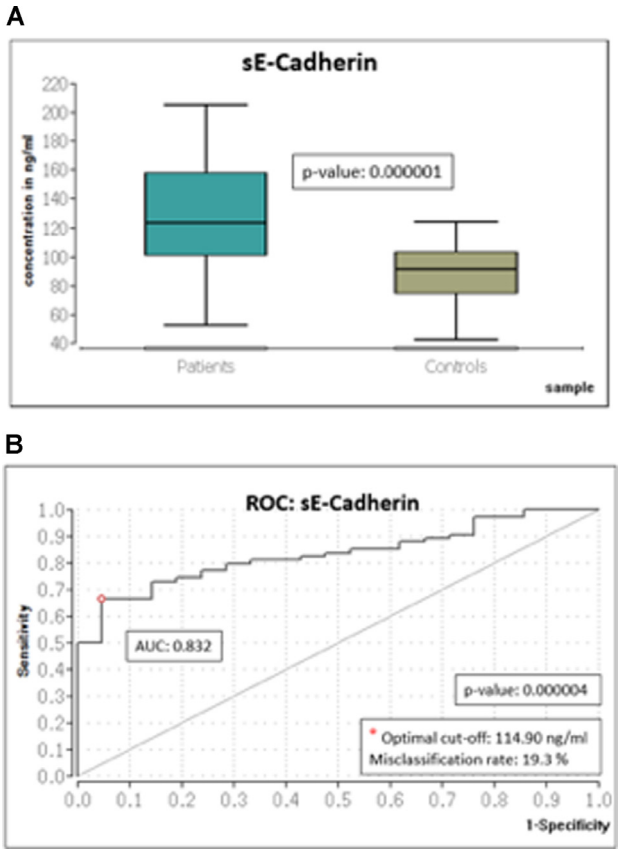

Fig. 1. A: Concentration of sE-cadherin in serum of tumor patients and controls. Y-axis: Concentration in ng/ml; X-axis: Serum samples of tumor patients and controls. Box: Lower line – quartile Q1 (25%-quantile); middle line – median; upper line – quartile Q3 (75%-quantile); aerials – extreme values. B: Receiver operating characteristics curve of sE-cadherin. Patients < optimal cutoff are defined as healthy. Patients > optimal cutoff are defined as diseased. AUC = area under the curve. *P* value determined by Wilcoxon-Mann-Whitney Test.

assays. Treatment of A498 and Caki1 cell lines with 0.1, 0.5, 1.0, and 5.0 μg/ml of sE-cadherin significantly attenuated the cell number (Fig. 2A). Clonogenic growth was also distinctly suppressed by 5 μg/ml sE-cadherin (Fig. 2B). Cell growth reduction was not caused by toxic effects, as demonstrated by the trypan dye exclusion test. Adhesion of A498 cells to collagen was significantly reduced, independently from the sE-cadherin concentration, whereas Caki1 exhibited decreased adhesion only with 5.0 μg/ml sE-cadherin (Fig. 3A). Chemotaxis of both Caki1 and A498 cells was augmented following sE-cadherin treatment (Fig. 3B).

Evaluation of the epithelial-to-mesenchymal transition (EMT) markers CK18, vimentin, and Twist revealed a significant increase of all proteins in the presence of sE-cadherin (Fig. 4).

4. Discussion

Despite extensive research, no molecular marker has yet been established as a screening or diagnostic tool to aid in detecting RCC. In advanced disease, the Metastatic Renal

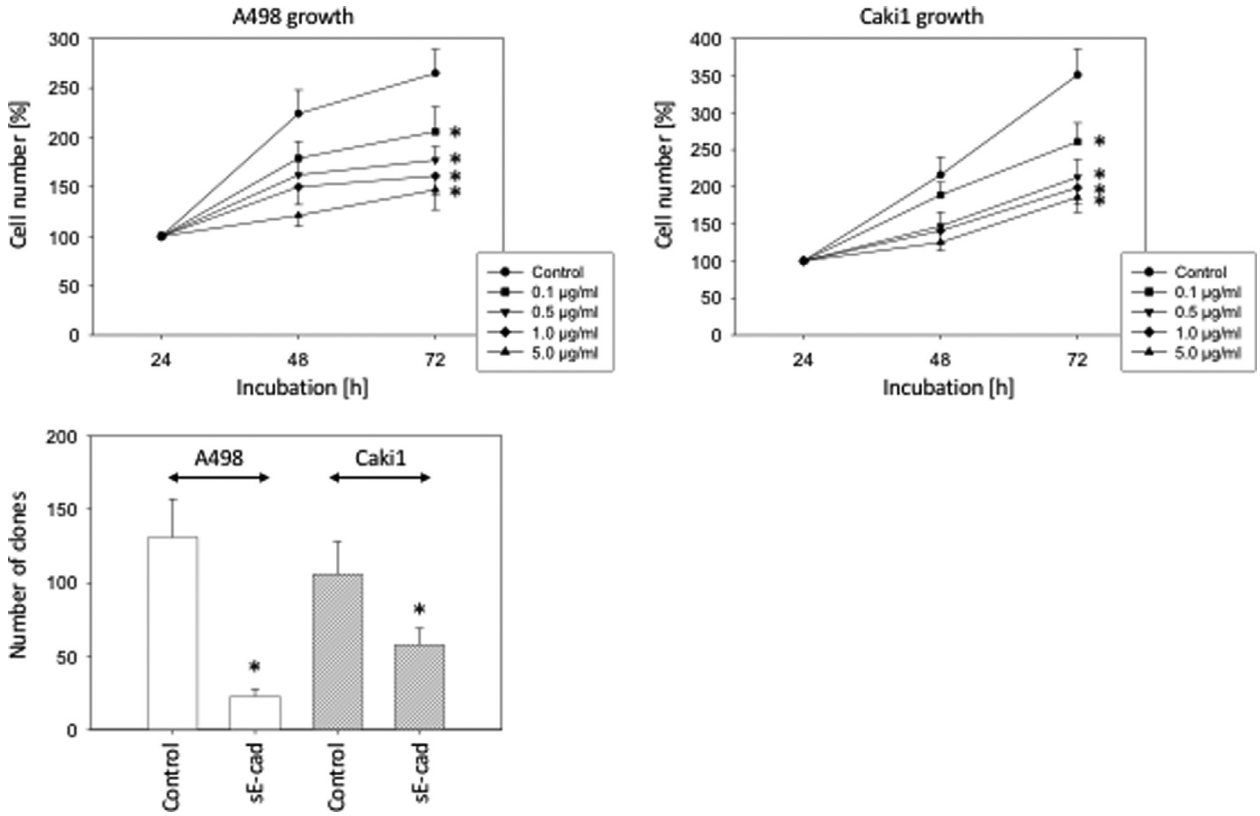

Fig. 2. Cell number and growth of sE-cadherin-stimulated compared to control A498 and Caki1 cells. A: Cell number of sE-cadherin-stimulated compared to control A498 and Caki1 cells. Mean values were calculated from 5 different observational fields. One representative of 6 experiments is shown. B: Clonogenic cell growth. Clones of at least 50 cells were counted as one colony. Experiments were done 3 times. * = significant difference to controls.

Cancer Database Consortium risk model based on performance status, conventional lab values, and time from diagnosis to treatment has been implemented in the European Association of Urology Guideline recommendations to treat metastasized clear-cell RCC [4,17]. In a localized setting, a number of multivariable prognostic models including the University of California Integrated Staging System or a nomogram by Kattan et al. have been introduced based on clinical characteristics, performance status, as well as postoperative factors such as tumor stage, grade, or subtype [18–20]. Nonetheless, there is still a poor evidence level for routine application, despite being recommended by the European Association of Urology Guideline Panel to serve as a basis for risk-adapted postoperative surveillance [4,18]. Thus, discovery of novel molecular markers is crucial, not only for better disease characterization and classification, but also for a deeper understanding of tumorigenic processes and involved mechanisms, which might potentially serve as therapeutic targets.

sE-cadherin is a soluble 80 kDa ectodomain of the transmembrane glycoprotein E-cadherin, which mediates cell-cell adhesion and is crucial for epithelial cell properties and tissue morphogenesis and remodeling [21]. In epithelial cells, transcriptional E-cadherin reprogramming is known to attenuate adhesion and promote migration and invasion during cancer progression and EMT [22]. Tumor cells are characterized by a decreased number of cadherin-mediated intercellular adhesions, since most epithelial cancers lose E-cadherin through mutation, epigenetic silencing, or expression of nonepithelial cadherins [21,23–25]. sE-cadherin emerges from proteolytic ectodomain cleavage, leading to its release into the extracellular space and hence its appearance in blood and urine [26,27].

In the present investigation, sE-cadherin exceeded other candidate molecules in discriminating RCC patients from healthy volunteers with an impressive AUC value of 0.83. In concordance, a recent study by Bodnar et al. has demonstrated an elevated concentration of sE-cadherin in the serum of patients with a mRCC undergoing systemic treatment with everolimus, while high levels of sE-cadherin were associated with a worse treatment response [28]. Also in line with these observations, another recent study demonstrated an overexpression of sE-cadherin in the serum of prostate cancer patients, compared to controls, and its correlation with unfavorable pathological outcomes after radical prostatectomy [29]. Interestingly, there might be an association of sE-cadherin expression with adverse clinical outcomes in further malignancies, as well. Okugawa et al. has delineated an overexpression of the molecule in sera of patients with colorectal carcinoma and a predictive value for poor prognosis [30]. Weiss et al. has also showed an increase of the sE-cadherin serum level in patients with advanced stage colorectal carcinoma and a potential to

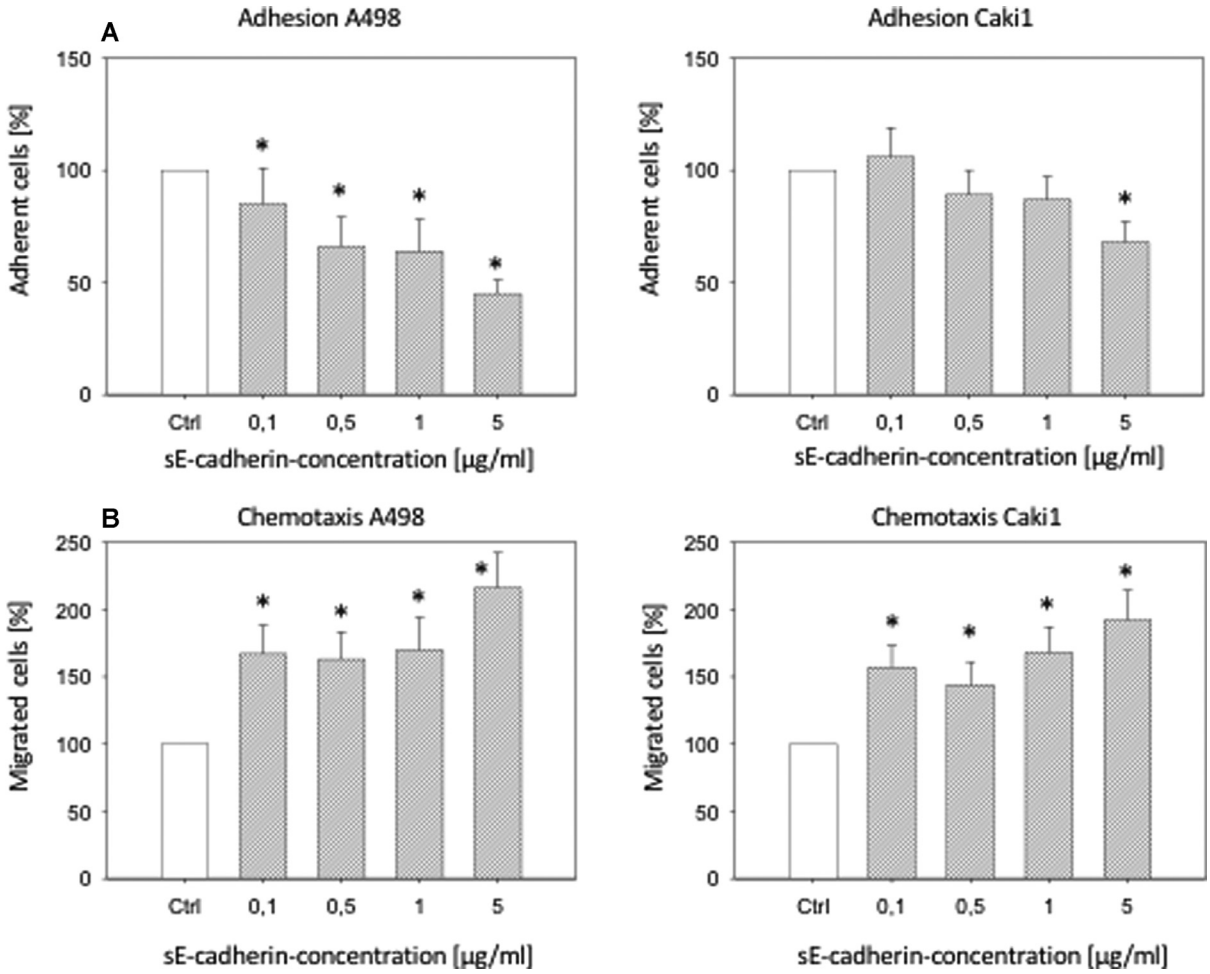

Fig. 3. A: Cell-matrix (Collagen) interaction of sE-cadherin-stimulated cells compared to control A498 and Caki1 cells. sE-cadherin-stimulated or control cells were added to immobilized collagen for 60 min. Mean values were calculated from 5 different observational fields. One representative of 6 experiments is shown. B: A498 and Caki1 cell migration. Tumor cells were seeded in the upper chamber in serum-free medium and 10% FCS, as the chemoattractant, was placed in the lower well. Cells moving to the lower membrane surface were counted. Mean values were calculated from 5 different observational fields. One representative of 6 experiments is shown. * indicates significant difference to controls.

monitor disease in some cases [31]. Shariat et al. demonstrated an AUC value of 0.72 for urinary sE-cadherin to detect bladder cancer, whereas higher levels were associated with muscle invasive disease [32]. Similarly, Matsumoto et al. described an increase of plasma sE-cadherin in bladder cancer patients, compared to healthy volunteers, and its correlation with lymphatic metastases and disease progression [33]. Moreover, in patients with gastric cancer, hepatocellular carcinoma, and lung cancer, sE-cadherin was overexpressed compared to controls, and associated with inferior disease characteristics [34–36]. Gofuku et al. has also reported an augmented concentration of sE-cadherin in the sera of 81 gastric cancer patients [37]. Still, elevation of sE-cadherin level has not been associated with clinicopathological factors. Our investigation did not reveal a significant correlation between sE-cadherin serum concentration and tumor stage and tumor grade. This finding might be attributable to the limited sample size of both cohorts, precluding a statistically significant difference. Alternatively, elevation of the marker serum concentration might reflect an intrinsically augmented turnover and activity of tumor cells in these RCC patients without being specific or sensible enough to further discriminate for tumor-specific properties.

A difference in the concentration of sE-cadherin before and on day 5 to 7 after surgery was not apparent. This is in line with the study by Yang et al. yielding no difference for sE-cadherin expression in preoperative and postoperative (day 5) samples of patients with RCC undergoing radical nephrectomy [38]. To precisely estimate the half life of sE-cadherin, its concentration should be measured over a longer time period in future studies. This would allow practical conclusions about its feasibility as a marker for treatment response, since a relatively limited half-life is required for timely adjustments in the further course of treatment.

Another aim of the present investigation was to elucidate whether shedding of sE-cadherin might be an epiphenomenon of the tumor-related enzymatic cleavage, or if the molecule may additionally exert an influence on metastatic tumor progression. It is worthy to note that cell growth and

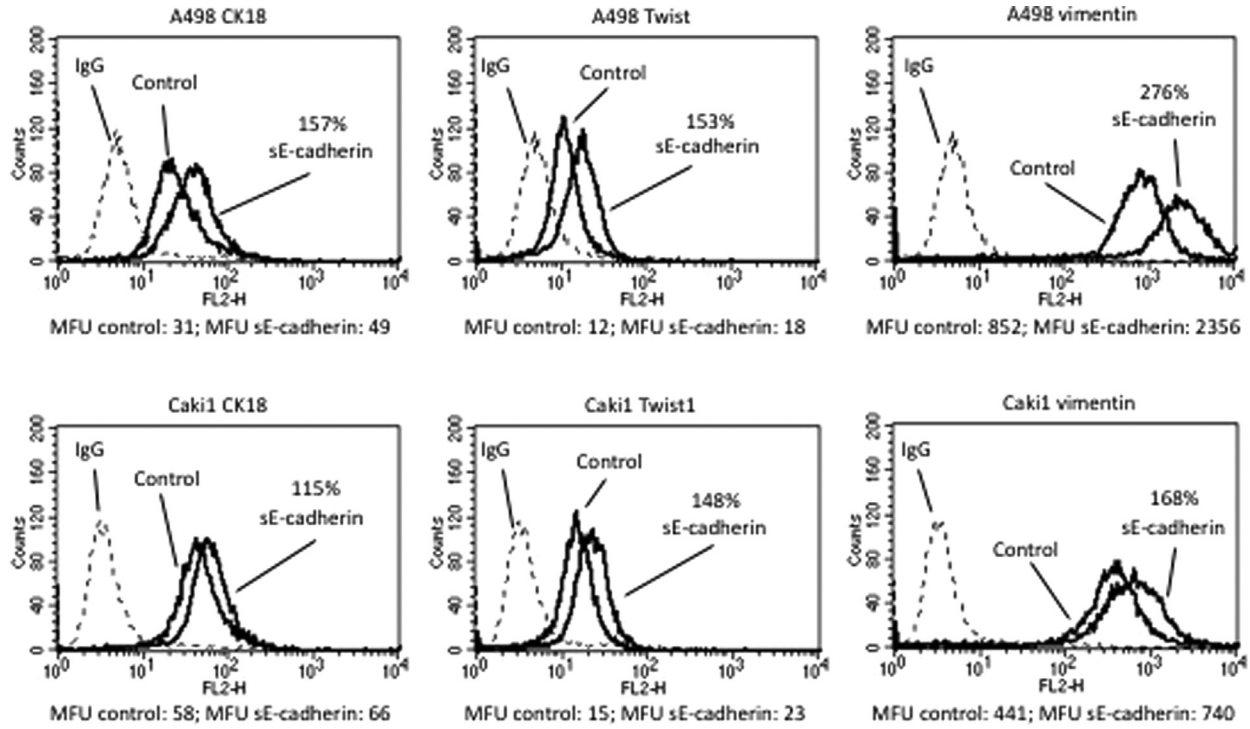

Fig. 4. Influence of sE-cadherin on CK18, Twist, and vimentin expression in A498 and Caki1 cells. Cells were stimulated with 5 μg/ml sE-cadherin for 24 h or remained untreated. Percentage expression, compared to the 100% control, as well as mean fluorescence values (MFU) of treated (sE-cadherin) vs. non-treated (control) cells is depicted. One representative of 3 experiments is shown.

adhesion capacity were significantly downregulated by exposing RCC cells to sE-cadherin. However, RCC cell motility was enhanced by sE-cadherin. This finding indicates that tumor cells downregulate their growth activity, lose firm contact to the matrix, and may so become primed for invasion. Obviously, sE-cadherin triggers a functional switch of RCC cells from being sessile and proliferative to gaining spreading properties. In fact, sE-cadherin caused a strong elevation of the EMT-markers vimentin, CK18, and Twist, which are closely involved in tumor cell dissemination.

It is unclear which receptors or molecular mechanisms are exactly responsible for this behavioral alteration of RCC cells. In a 3-dimensional coculture system comprising normal vs. cancerous canine kidney epithelial cells, sE-cadherin has been reported to induce EMT through activation of EGFR and its downstream ERK and AKT signaling pathways [39]. Interestingly, since the source of sE-cadherin elevation in cancer patients is unknown, this study demonstrated that carcinoma cells produce MMP-9 cleaving cellular E-cadherin from epithelial cells to generate sE-cadherin. Najy et al. reported enhanced migration and invasion of breast cancer cells through sE-cadherin influence [40]. Finally, in squamous cell carcinoma cells, sE-cadherin supported carcinogenesis by activating the MAPK-PI3K-AKT-mTOR pathway and inhibiting apoptosis [41]. These findings establish an important role of sE-cadherin as a pro-oncogenic protein and indicate a potential therapeutic target.

Besides being an indicator of tumorigenesis due to tumor-associated proteolysis, overexpression of sE-cadherin has been linked to oxidative stress and production of proinflammatory cytokines [27,42,43]. Compared to controls, sE-cadherin's up-regulation has been reported in a variety of benign disorders, for example, in chronic obstructive pulmonary disease, HBV-related liver diseases (nonmalignant conditions + hepatocellular carcinoma), systemic lupus erythematosus, and acute pancreatitis [44–47]. This underscores a low specificity of sE-cadherin for a definite cancer type, thus precluding its use as a diagnostic or even clinically reasonable screening tool for the general population. Other problems are a lack of internal and external data validation, use of different measurement tools and, in particular, a considerable variation in sE-cadherin concentration reported in the literature for the healthy, making cut-off value generalization challenging [28,29,38,43]. Consequently, monitoring of treatment response and disease progression through sE-cadherin measurement by the same approach and in the same patient might be a necessary, viable option.

5. Conclusions

In conclusion, out of the cadherin family, the sE-cadherin level is up-regulated in the serum of patients with RCC and distinguishes them from healthy volunteers. It remains stably measurable in blood several days after tumor resection. Treatment of RCC cells with sE-cadherin induces a functional cellular switch from sessility to mobility. Further studies are warranted to investigate sE-cadherin's role

as a predictive and prognostic marker, as well as a therapeutic target in RCC.

Conflicts of interest

There are no conflicts of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.03.001.

References

[1] Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International variations and trends in renal cell carcinoma incidence and mortality. Eur Urol 2015;67(3):519–30.
[2] Mohammadian M, Pakzad R, Towhidi F, Makhsosi BR, Ahmadi A, Salehiniya H. Incidence and mortality of kidney cancer and its relationship with HDI (Human Development Index) in the world in 2012. Clujul Med 2017;90(3):286–93.
[3] Barrisford GW, Singer EA, Rosner IL, Linehan WM, Bratslavsky G. Familial renal cancer: molecular genetics and surgical management. Int J Surg Oncol 2011;2011:658767.
[4] Powles T, Albiges L, Staehler M, Bensalah K, Dabestani S, Giles RH, et al. Updated European Association of Urology Guidelines Recommendations for the treatment of first-line metastatic clear cell renal cancer. Eur Urol 2017;73(3):311–5.
[5] Motzer RJ, Jonasch E, Agarwal N, Bhayani S, Bro WP, Chang SS, et al. Kidney Cancer, Version 2.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2017;15(6):804–34.
[6] Farber NJ, Kim CJ, Modi PK, Hon JD, Sadimin ET, Singer EA. Renal cell carcinoma: the search for a reliable biomarker. Transl Cancer Res 2017;6(3):620–32.
[7] EAU Guideline on Renal Cell Carcinoma [Internet][cited 28.03.2018]. Available from: 2018. http://uroweb.org/wp-content/uploads/EAU-RCC-Guidelines-2018-large-text.pdf.
[8] Sim SH, Messenger MP, Gregory WM, Wind TC, Vasudev NS, Cartledge J, et al. Prognostic utility of pre-operative circulating osteopontin, carbonic anhydrase IX and CRP in renal cell carcinoma. Br J Cancer 2012;107(7):1131–7.
[9] Steffens S, Kohler A, Rudolph R, Eggers H, Seidel C, Janssen M, et al. Validation of CRP as prognostic marker for renal cell carcinoma in a large series of patients. BMC Cancer 2012;12:399.
[10] Pinho SS, Seruca R, Gartner F, Yamaguchi Y, Gu J, Taniguchi N, et al. Modulation of E-cadherin function and dysfunction by N-glycosylation. Cell Mol Life Sci 2011;68(6):1011–20.
[11] Stemmler MP. Cadherins in development and cancer. Mol Biosyst 2008;4(8):835–50.
[12] Berx G, van Roy F. Involvement of members of the cadherin superfamily in cancer. Cold Spring Harb Perspect Biol 2009;1(6):a003129.
[13] Sobin LH, Gospodariwicz M, Wittekind C. TNM classification of malignant tumors. UICC International Union Against Cancer. 7th ed. Wiley-Blackwell; 2011. Available from: http://www.uicc.org/tnm.
[14] Wittekind C, Compton C, Brierley JD, Sobin LH. TNM supplement, a commentary on uniform use. 4th ed. Wiley-Blackwell; 2012.Available from: http://eu.wiley.com/WileyCDA/WileyTitle/productCd-1444332430.html.
[15] Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982;6(7):655–63.
[16] Cho MC, Cho SY, Yoon CY, Lee SB, Kwak C, Kim HH, et al. EphA2 is a potential player of malignant cellular behavior in non-metastatic renal cell carcinoma cells but not in metastatic renal cell carcinoma cells. PLoS One 2015;10(7):e0130975.
[17] Heng DY, Xie W, Regan MM, Warren MA, Golshayan AR, Sahi C, et al. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. J Clin Oncol 2009;27(34):5794–9.
[18] Klatte T, Rossi SH, Stewart GD. Prognostic factors and prognostic models for renal cell carcinoma: a literature review. World J Urol 2018.
[19] Kattan MW, Reuter V, Motzer RJ, Katz J, Russo P. A postoperative prognostic nomogram for renal cell carcinoma. J Urol 2001;166(1):63–7.
[20] Zisman A, Pantuck AJ, Wieder J, Chao DH, Dorey F, Said JW, et al. Risk group assessment and clinical outcome algorithm to predict the natural history of patients with surgically resected renal cell carcinoma. J Clin Oncol 2002;20(23):4559–66.
[21] Repetto O, De Paoli P, De Re V, Canzonieri V, Cannizzaro R. Levels of soluble E-cadherin in breast, gastric, and colorectal cancers. Biomed Res Int 2014;2014:408047.
[22] Gheldof A, Berx G. Cadherins and epithelial-to-mesenchymal transition. Prog Mol Biol Transl Sci 2013;116:317–36.
[23] Figueiredo J, Soderberg O, Simoes-Correia J, Grannas K, Suriano G, Seruca R. The importance of E-cadherin binding partners to evaluate the pathogenicity of E-cadherin missense mutations associated to HDGC. Eur J Hum Genet 2013;21(3):301–9.
[24] Simoes-Correia J, Figueiredo J, Lopes R, Stricher F, Oliveira C, Serrano L, et al. E-cadherin destabilization accounts for the pathogenicity of missense mutations in hereditary diffuse gastric cancer. PLoS One 2012;7(3):e33783.
[25] Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, et al. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res 1994;54(14):3845–52.
[26] van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci 2008;65(23):3756–88.
[27] Grabowska MM, Day ML. Soluble E-cadherin: more than a symptom of disease. Front Biosci 2012;17:1948–64.
[28] Bodnar L, Stec R, Cierniak S, Synowiec A, Wcislo G, Jesiotr M, et al. Role of WNT/beta-Catenin Pathway as Potential Prognostic and Predictive Factors in Renal Cell Cancer Patients Treated With Everolimus in the Second and Subsequent Lines. Clin Genitourin Cancer 2018;16(4):257–65.
[29] Tsaur I, Thurn K, Juengel E, Gust KM, Borgmann H, Mager R, et al. sE-cadherin serves as a diagnostic and predictive parameter in prostate cancer patients. J Exp Clin Cancer Res 2015;34:43.
[30] Okugawa Y, Toiyama Y, Inoue Y, Iwata T, Fujikawa H, Saigusa S, et al. Clinical significance of serum soluble E-cadherin in colorectal carcinoma. J Surg Res 2012;175(2):e67–73.
[31] Weiss JV, Klein-Scory S, Kubler S, Reinacher-Schick A, Stricker I, Schmiegel W, et al. Soluble E-cadherin as a serum biomarker candidate: elevated levels in patients with late-stage colorectal carcinoma and FAP. Int J Cancer 2011;128(6):1384–92.
[32] Shariat SF, Matsumoto K, Casella R, Jian W, Lerner SP. Urinary levels of soluble e-cadherin in the detection of transitional cell carcinoma of the urinary bladder. Eur Urol 2005;48(1):69–76.
[33] Matsumoto K, Shariat SF, Casella R, Wheeler TM, Slawin KM, Lerner SP. Preoperative plasma soluble E-cadherin predicts metastases to lymph nodes and prognosis in patients undergoing radical cystectomy. J Urol 2003;170(6 Pt 1):2248–52.

[34] Chan AO, Lam SK, Chu KM, Lam CM, Kwok E, Leung SY, et al. Soluble E-cadherin is a valid prognostic marker in gastric carcinoma. Gut 2001;48(6):808–11.
[35] Soyama A, Eguchi S, Takatsuki M, Kawashita Y, Hidaka M, Tokai H, et al. Significance of the serum level of soluble E-cadherin in patients with HCC. Hepatogastroenterology 2008;55(85):1390–3.
[36] Gogali A, Charalabopoulos K, Zampira I, Konstantinidis AK, Tachmazoglou F, Daskalopoulos G, et al. Soluble adhesion molecules E-cadherin, intercellular adhesion molecule-1, and E-selectin as lung cancer biomarkers. Chest 2010;138(5):1173–9.
[37] Gofuku J, Shiozaki H, Doki Y, Inoue M, Hirao M, Fukuchi N, et al. Characterization of soluble E-cadherin as a disease marker in gastric cancer patients. Br J Cancer 1998;78(8):1095–101.
[38] Yang L, Fan T, Wei Q, Cui X, Bu S, Han P. Transient variations in the serum concentrations of cell adhesion molecules following retroperitoneal laparoscopic and open radical nephrectomy for localized renal-cell carcinoma. J Endourol 2012;26(10):1323–8.
[39] Patil PU, D'Ambrosio J, Inge LJ, Mason RW, Rajasekaran AK. Carcinoma cells induce lumen filling and EMT in epithelial cells through soluble E-cadherin-mediated activation of EGFR. J Cell Sci 2015;128 (23):4366–79.
[40] Najy AJ, Day KC, Day ML. The ectodomain shedding of E-cadherin by ADAM15 supports ErbB receptor activation. J Biol Chem 2008;283(26):18393–401.
[41] Brouxhon SM, Kyrkanides S, Teng X, Athar M, Ghazizadeh S, Simon M, et al. Soluble E-cadherin: a critical oncogene modulating receptor tyrosine kinases, MAPK and PI3K/Akt/mTOR signaling. Oncogene 2014;33(2):225–35.
[42] Parrish AR, Catania JM, Orozco J, Gandolfi AJ. Chemically induced oxidative stress disrupts the E-cadherin/catenin cell adhesion complex. Toxicol Sci 1999;51(1):80–6.
[43] Karayiannakis AJ, Syrigos KN, Savva A, Polychronidis A, Karatzas G, Simopoulos C. Serum E-cadherin concentrations and their response during laparoscopic and open cholecystectomy. Surg Endosc 2002;16(11):1551–4.
[44] Shirahata T, Nakamura H, Nakajima T, Nakamura M, Chubachi S, Yoshida S, et al. Plasma sE-cadherin and the plasma sE-cadherin/sVE-cadherin ratio are potential biomarkers for chronic obstructive pulmonary disease. Biomarkers 2018;23(5):414–21.
[45] Wang B, Liu J, Xiong Y, Yan Y, Sun B, Zhao Q, et al. Soluble E-cadherin as a serum biomarker in patients with HBV-related liver diseases. Clin Biochem 2016;49(16-17):1232–7.
[46] Jin T, Almehed K, Zhu Y, Carlsten H, Forsblad-d'Elia H. Soluble E-cadherin in systemic lupus erythematosus. J Rheumatol 2013;40 (10):1677–82.
[47] Sewpaul A, French JJ, Khoo TK, Kernohan M, Kirby JA, Charnley RM. Soluble E-cadherin: an early marker of severity in acute pancreatitis. HPB Surg 2009;2009:397375.