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Short Report

β_6-integrin serves as a novel serum tumor marker for colorectal carcinoma

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide and the need for novel biomarkers and therapeutic strategies to improve diagnosis and surveillance is obvious. This study aims to identify β_6-integrin (ITGB6) as a novel serum tumor marker for diagnosis, prognosis, and surveillance of CRC. ITGB6 serum levels were validated in retro- and prospective CRC patient cohorts. ITGB6 serum levels were analyzed by ELISA. Using an initial cohort of 60 CRC patients, we found that ITGB6 is present in the serum of CRC, but not in non-CRC control patients. A cut-off of ≥2 ng/mL ITGB6 reveals 100% specificity for the presence of metastatic CRC. In an enlarged study cohort of 269 CRC patients, ITGB6 predicted the onset of metastatic disease and was associated with poor prognosis. Those data were confirmed in an independent, prospective cohort

Key words: colorectal cancer, ITGB6, serum tumor marker, metastasis, surveillance, therapy response
Abbreviations: CEA: carcinoembryonic antigen; CRC: colorectal cancer; ITGB6: β_6-integrin; mCRC: metastatic CRC
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consisting of 40 CRC patients. To investigate whether ITGB6 can also be used for tumor surveillance, serum ITGB6-levels were assessed in 26 CRC patients, pre- and post-surgery, as well as during follow-up visits. After complete tumor resection, ITGB6 serum levels declined completely. During follow-up, a new rise in ITGB6 serum levels indicated tumor recurrence or the onset of new metastasis as confirmed by CT scan. ITGB6 was more accurate for prognosis of advanced CRC and for tumor surveillance as the established marker carcinoembryonic antigen (CEA). Our findings identify ITGB6 as a novel serum marker for diagnosis, prognosis, and surveillance of advanced CRC. This might essentially contribute to an optimized patient care.

What's new?

While serum biomarkers are ideal tools for colorectal cancer (CRC) detection, the identification of a marker with both high sensitivity and high specificity has proven challenging. Here, the authors investigated β_6-integrin (ITGB6), a molecule upregulated in association with epithelial-to-mesenchymal transition activities, such as wound healing and carcinogenesis, for its biomarker potential in CRC. In patients, ITGB6 serum levels were significantly associated with CRC diagnosis and prognosis. A cut-off of ≥2 ng/mL ITGB6 reliably predicted metastatic disease and poor CRC prognosis. The findings suggest that employing ITGB6 as a serum biomarker could greatly aid diagnosis and tumor surveillance in CRC patients.

Introduction

Colorectal cancer (CRC) is the third most common malignancy and one of the leading causes of cancer-related deaths worldwide. Every fourth patient with CRC exhibits metastases at time of diagnosis and every second will develop metastasis during disease course.[1] Easily accessible biomarkers, such as serum markers, are an important tool for optimizing patient care in patients with CRC. Such serum biomarkers should be ideally usable for diagnosis and surveillance of the disease. At present, carcinoembryonic antigen (CEA) is the clinical standard as CRC biomarker. However, it exhibits only a low sensitivity and specificity.

Previous studies suggested that β_6-integrin (ITGB6) may be involved in CRC pathogenesis.[2–6] ITGB6 is exclusively expressed on epithelial cells (EC) during embryogenesis, and upregulated in adult EC during processes involving epithelial-to-mesenchymal transition (EMT), such as wound healing, fibrosis, and carcinogenesis.[7,8] ITGB6 contributes to the activation of latent TGFβ and perpetuates TGFβ-mediated EMT,[9,10] which is a key process during CRC progression and metastasis.[11] In CRC cells, ITGB6 promotes its own expression in an autocrine manner and induces cell proliferation, chemo-resistance, cell migration, and invasiveness, but also inhibits apoptosis *via* its unique cytoplasmic tail, absent in other integrins.[2,12–16]

The purpose of the present study, using retro- and prospectively collected patient cohorts, was to investigate, whether ITGB6 in serum might serve as a novel tumor marker for CRC patients. We hypothesized that ITGB6 in patients' serum might represent a novel marker for diagnosis, prognosis, as well as tumor surveillance in CRC patients. The identification of such a novel serum marker would be a great benefit for daily patient care, and represent a crucial step forward towards a personalized medicine approach for CRC patients.

Material and Methods

Study population

Samples from CRC patients and healthy controls were recruited and collected at the University Hospital Zurich (Switzerland), the University Hospital Erlangen (Germany), the University Hospital Basel (Switzerland), the University of Oulu (Finland), and ProteoGenex Inc. (USA) between 06/2003 and 01/2017 (Supporting Information Methods and Supporting Information Tables S1, S3–S5).

Ethical consideration

The studies were approved by the Cantonal Ethics Committee of the Canton Zurich (Switzerland; Approval-No. EK-1755) and by the local ethics committees Erlangen (Germany; Approval-No. 3402; Ethikkommission der FAU) and Oulu (Finland), respectively. All participants were informed personally and provided written informed consent for this study. Patients' data were pseudonymized and all analyses were carried out in accordance with the Helsinki declaration.

Human ITGB6 and CEA detection

Serum ITGB6-levels were measured using human ITGB6 ELISA kit (E92099Hu, USCN Life Science Inc., Wuhan; China) according to manufacturer's instructions. CEA serum levels were analyzed at the Institute of Clinical Chemistry at the University Hospital Zurich (see supplemental methods for more details).

Statistical analyses

Data are reported according to the remark criteria.[17] Statistical analyses were performed using IBM SPSS statistics 22.0 and 23.0 (SPSS Inc., Chicago, IL, USA) (see Supporting Information Methods for more details).

Results

Proof-of-concept validation of Beta-6-integrin as a serum marker for colorectal cancer

To explore whether ITGB6 is present in the serum of CRC patients, we first investigated serum ITGB6-levels in an initial retrospective study cohort including 60 CRC patients including all four UICC tumor stages and 19 healthy volunteers (*Cohort 1*, Supporting Information Table S1). Here, we found that ITGB6 was present in the serum of CRC patients, within a range between 0 and 10.203 ng/mL. In contrast, serum ITGB6-levels of healthy volunteers were always 0 ng/mL (Fig. 1*a*). We then performed receiver-operating-characteristic (ROC) analysis and determined an area under the curve (AUC) of 0.858 ± 0.040 (95% CI 0.779–0.937; $p = 0.000003$; Fig. 1*b*). In the next step, we evaluated which cut-off value of ITGB6 serum level might be useful for daily clinical practice. We defined two cut-off values of either 0.1 and 2.0 ng/mL ITGB6 concentration in the serum that both revealed 100% specificity and a sensitivity of 69.8% and 27.8%, respectively, for the presence of CRC overall (Fig. 1*b*). Beyond, we validated those observations in an independent, prospectively collected cohort consisting of 40 CRC patients featuring all four UICC tumor stages (*Cohort 2*, Supporting Information Table S2). Using this cohort 2, we were able to confirm our previous findings obtained using cohort 1 ($p = 0.000002$; Fig. 1*c*) that ITGB6 revealed an equally good performance with an AUC of 0.847 ± 0.052 (95% CI 0.746–0.948; $p = 0.000026$; Fig. 1*d*).

In summary, these results suggest that ITGB6 is detectable in the serum of CRC patients and represents a feasible serum marker for the diagnosis of CRC.

Beta-6-integrin as a marker for diagnosis of advanced colorectal cancer

To understand whether serum ITGB6 concentrations also reflect tumor stages in CRC patients, an enlarged cohort of 269 CRC patients was studied (*Cohort 3*, Figs. 1*e* and 1*f*, Supporting Information Table S3). Increased ITGB6 serum levels in CRC patients from cohort 3 significantly correlated with features of advanced CRC, such as presence of lymph node (LN) and distant organ metastases at time of blood draw (Figs. 1*e* and 1*f*; Supporting Information Figs. S1A–S1C and S2A, Supporting Information Table S4). Considering our predefined cut-off levels, we found that every second patient with an ITGB6 serum level ≥0.1 ng/mL ITGB6 suffered from metastatic CRC (mCRC), however, all patients with a serum level ≥2.0 ng/mL ITGB6 exhibited metastatic disease at time of blood draw (Fig. 1*e*, Supporting Information Tables S4 and S5).

We then analyzed the association of serum ITGB6-levels with TNM-classification at time of CRC diagnosis. The percentage of patients with tumor positive lymph nodes (N-status) and metastasis (M-status) at diagnosis increased with higher serum ITGB6-levels (Supporting Information Figs. S1A and 1B and 2A, Supporting Information Tables S3 and S5). In contrast, tumor infiltration (T-status) and differentiation (G-status) were not associated with ITGB6 serum levels (Supporting Information Table S3). However, an elevated lymph node ratio (LNR), defined as the ratio of positive mesenteric LN to the total number of resected mesenteric LN during surgery, directly correlated with increased ITGB6 serum levels (overall $p = 1.46 \times 10^{-7}$; Fig. 1*f*, Supporting Information Tables S4 and S5).

To examine ITGB6 protein expression and the cell type expressing ITGB6 in primary and metastatic CRC tissue, we analyzed 39 primary CRC and 23 mCRC from patients of cohort 3. As previously reported,[18–20] we also found that ITGB6 was present in cells within the tumor, along the invasion front and in cells within the tumor stroma of primary CRC and metastasis (Supporting Information Fig. S3). ITGB6 staining intensity correlated with the ITGB6 serum levels in primary CRC, but not in mCRC (Supporting Information Fig. S3A). Notably, ITGB6 staining intensity decreased the further the distance from the primary CRC (Supporting Information Fig. S3B),[20] but was to some extent still present in microscopically normal appearing colon along the invasion front. CRC cells within liver, lung and LN metastasis revealed a strong staining intensity for ITGB6 independent from ITGB6 serum level (Supporting Information Figs. S3C–S3E).

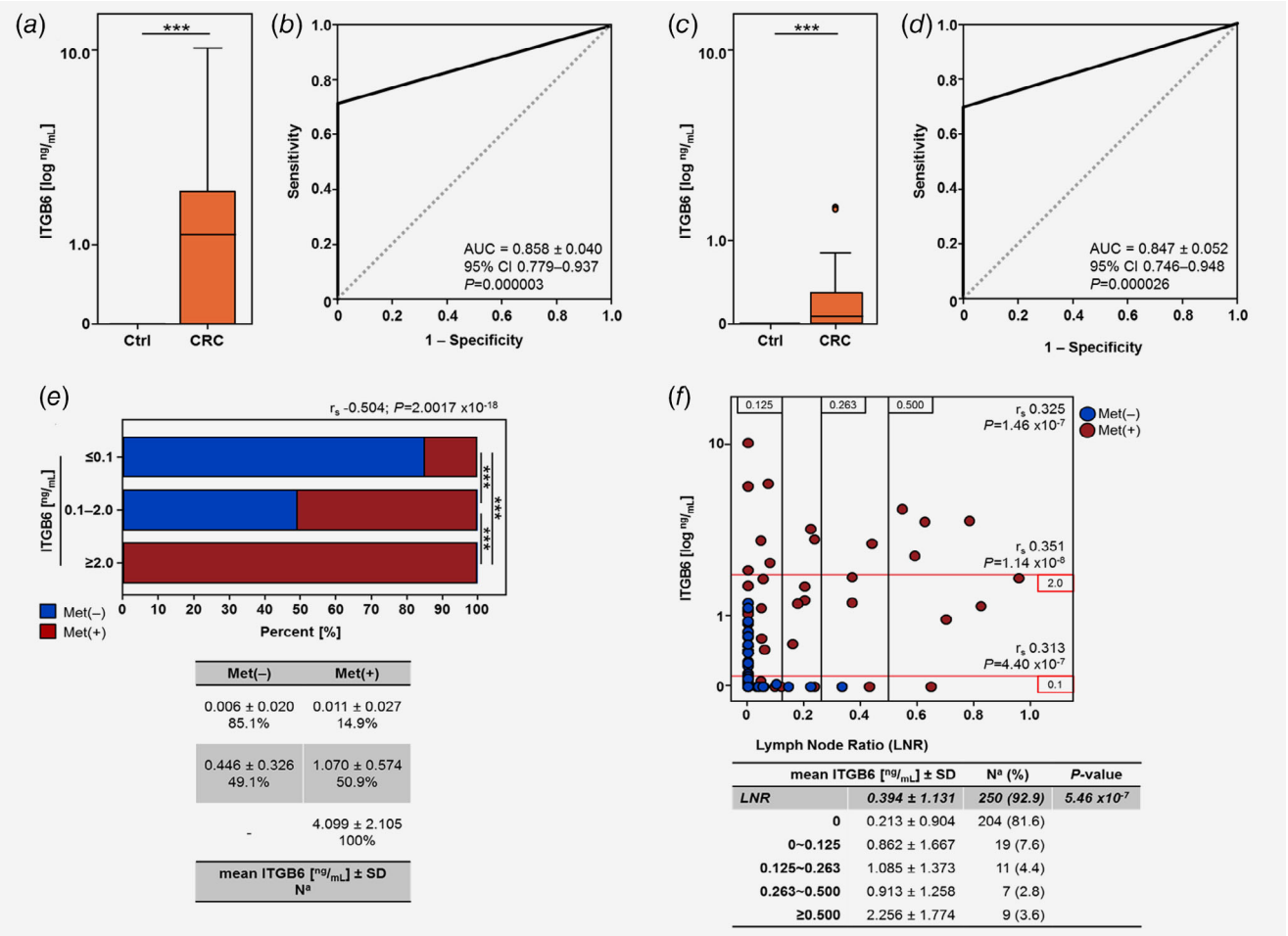
Interestingly, ITGB6 was not only present in the tumor cells of the primary CRC and mCRC as indicated by CDX2 co-staining (marker for intestinal epithelial cells), but also demonstrated a co-expression with either CD68 (marker for macrophage-lineage cells) or vimentin (VIM, marker for mesenchymal-like cells) within the tumor stroma (Supporting Information Fig. S4).

To further investigate molecular differences between colon cancer tissue from patients with high *vs.* low serum ITGB6 level, we used colon cancer tissue samples and serum samples from a prospectively collected cohort of colon cancer patients (*Cohort 4*, Supporting Information Table S6). We analyzed ITGB6 mRNA levels in colon cancer tissues from 352 patients representing all four UICC tumor stages. We found that mRNA levels did not differ between UICC tumor stages (Fig. 2*a*, Supporting Information Table S6). However, analysis of ITGB6 serum levels in 40 patients from cohort 2 that were selected according to UICC stages out of the cohort 4 patients (cohort 2 is a subgroup of patients from cohort 4), revealed that ITGB6 serum levels increased with UICC tumor stage despite no obvious change in mRNA levels (Figs. 2*a* and 2*b*, Supporting Information Table S6).

Taken together, our data confirm on the one hand that ITGB6 is present in the serum and tumor tissue of CRC patients, and even more importantly, that serum ITGB6 concentrations represent a marker for advanced CRC.

Serum levels of beta-6-integrin indicate prognosis in patients with colorectal cancer

We then examined the prognostic value of ITGB6 serum levels with respect to patient survival in cohort 3. We found



Met(–)	Met(+)
0.006 ± 0.020 85.1%	0.011 ± 0.027 14.9%
0.446 ± 0.326 49.1%	1.070 ± 0.574 50.9%
-	4.099 ± 2.105 100%
mean ITGB6 [ng/mL] ± SD N[a]	

	mean ITGB6 [ng/mL] ± SD	N[a] (%)	*P*-value
LNR	***0.394 ± 1.131***	***250 (92.9)***	***5.46 x10^-7***
0	0.213 ± 0.904	204 (81.6)	
0~0.125	0.862 ± 1.667	19 (7.6)	
0.125~0.263	1.085 ± 1.373	11 (4.4)	
0.263~0.500	0.913 ± 1.258	7 (2.8)	
≥0.500	2.256 ± 1.774	9 (3.6)	

Figure 1. Validation of ITGB6 as a possible serum marker for colorectal cancer (CRC). (*a*) Serum levels of ITGB6 was assessed in a retrospective study cohort, *cohort 1*, of 60 CRC patients ($n = 60$) and 19 healthy volunteers ($n = 19$). (*b*) ROC curve was applied to reveal a good accuracy of ITGB6 to predict the presence of CRC, and reveals two cut-off values at 0.1 and 2.0 ng/mL ITGB6. (*c*, *d*) In a prospective and independent serum cohort of 40 CRC patients ($n = 40$, *cohort 2*) and 19 healthy volunteers ($n = 19$), ITGB6 serum levels were analyzed, and the ROC-curve applied, confirming the good performance of ITGB6 to predict CRC in those patients. (*a*, *c*) Mann–Whitney-U Exact Sig. 2-tailed test was performed. AUC (area under the curve); CI (confidence interval); CRC (colorectal cancer patients); Ctrl (healthy volunteers); ROC curve (receiver-operating-characteristic curve). *** $p < 0.001$. In an enlarged serum cohort of 269 CRC patients ($n = 269$, *cohort 3*), serum levels of ITGB6 were assessed (*e* and *f*). The two cut-off values at 0.1 and 2.0 ng/mL ITGB6 compared to the disease severity and disease characteristics of these patients. (*e*) Classification of ITGB6 cut-off values according to the metastatic disease of CRC patients at time of blood draw ($n = 264$). (*f*) Two-dimensional scatterplot depicts serum levels of ITGB6 in relation to the lymph node ratio (LNR $n = 250$) of each patient, and according to their metastatic disease. In (*e*) Mann–Whitney-U Exact Sig. 2-tailed, and (*f*) Fisher's Exact Sig. 2-tailed test were performed. In (*e*) data are shown as means ITGB6 ± SD. (*f*) Red lines indicating ITGB6 cut-off values at 0.1 and 2.0 ng/mL; black lines indicating LNR at 0.125, 0.263 and 0.500. [a] (Missing data); LNR (lymph node ratio); Met(–) (patients without metastasis); Met(+) (patients with metastasis); N (Percentage according to total N within each group); r_s (Spearman's rho correlation); SD (standard deviation). ** $p < 0.01$; *** $p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

that ITGB6 serum concentrations are correlated with overall survival (r_s 0.522, $p = 8.43 \times 10^{-19}$; Fig. 2*c*) in all subgroups of CRC patients (stage I–IV patients; Supporting Information Figs. S2B–S2E). The 5-year survival rate was clearly lower in patients with serum concentrations ≥2.0 ng/mL ITGB6 (mean survival 2.6 ± 1.5 years; hazard ratio [HR] of 5.189, 95% CI 2.801–9.612; $p = 1.65 \times 10^{-7}$). In contrast, patients with lower ITGB6 serum levels, between 0.1 and 1.99 ng/mL ITGB6 displayed a HR of 2.524 (95% CI 1.463–4.355; $p = 0.001$) and a mean survival of 4.6 ± 2.4 years (Fig. 2*d*, Supporting Information Table S2). Kaplan–Meier analysis confirmed that elevated ITGB6 serum levels were significantly associated with poor prognosis and a reduced survival rate ($p = 1.23 \times 10^{-8}$; Fig. 2*d*). Those observations might be due to the fact that all patients with ≥2.0 ng/mL ITGB6 suffered from metastatic disease (Fig. 2*c*).

Subsequently, we performed a subgroup analysis of cohort 3 and investigated only CRC patients that underwent chemoradiotherapy before blood draw and categorized them according to their metastatic status and serum ITGB6-levels (Fig. 2*e*). Patients with no metastasis and low ITGB6 serum levels that

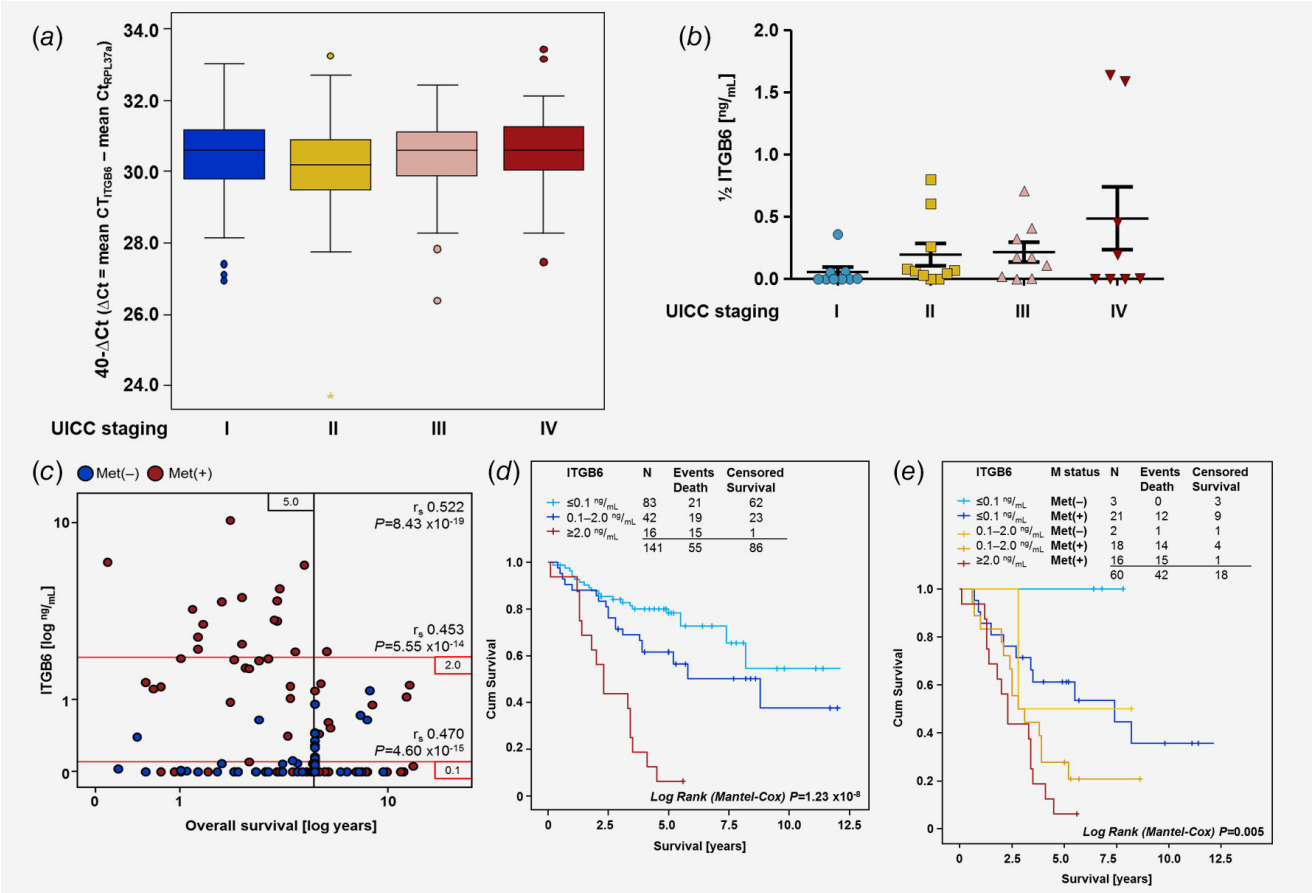


Figure 2. ITGB6 mRNA expression in colon cancer tissue and prognostic value of serum ITGB6-levels for patients with CRC. (*a*) mRNA analysis for ITGB6 was performed using primary colon cancer tissue from patients with UICC stage I–IV tumors (*n* = 352, *cohort 4*). (*b*) Serum levels of ITGB6 was assessed in a prospective study cohort of 40 CRC patients (*cohort 2*, a subgroup of *cohort 4*) and is depicted according to UICC tumor stages I–IV. (*c*) Two-dimensional scatterplots depict serum ITGB6-levels in relation to the overall survival (*n* = 249, *cohort 3*) from each CRC patient, and according to their metastatic disease. (*d*) Survival of CRC patients (*n* = 141, *cohort 3*) is plotted according to ITGB6. The HR was calculated with the Cox regression according to the ITGB6 cut-off values of 0.1 and 2.0 ng/mL (*n* = 141). (*e*) Survival rates of patients that underwent chemo and or radiotherapy before blood draw, were plotted according to the metastatic disease (n = 60, subgroup of *cohort 3*). (*c*) Spearman's rho correlation (r_s) Sig. 2-tailed test, and (*d* and *e*) Kaplan–Meier estimation was performed and plotted according to the ITGB6 cut-off ranges, ≤0.1, 0.1–2.0 and ≥2.0 ng/mL, respectively. CRC patients defined as an event dies during 12-years follow-up and survived CRC patients were defined as censored cases. (*c*) Red lines indicating ITGB6 cut-off values at 0.1 and 2.0 ng/mL; black line indicates 5-year survival. HR (hazard ratio); Met(–) (patients without metastasis); Met(+) (patients with metastasis); r_s (Spearman's rho correlation); SD (standard deviation). [Color figure can be viewed at wileyonlinelibrary.com]

underwent chemoradiotherapy treatment before blood draw revealed a significantly better survival ($p = 0.005$) compared to CRC patients with metastasis and high levels of ITGB6 (Fig. 2*e*). In general, these data indicate that serum levels of ITGB6 might predict the prognosis of CRC patients.

Beta-6-integrin improves the diagnosis of advanced CRC

Next, we compared serum ITGB6 levels with serum CEA levels (carcinoembryonic antigen; normal value <5.0 μg/L) in patients from cohort 3. We found a moderate correlation of serum levels of ITGB6 and CEA in CRC patients (r_s 0.510, $p = 0.003$; Fig. 3*a*). High serum CEA levels were associated with elevated ITGB6 serum levels. However, CEA serum levels were highly variable, ranging between 1.2 and 9,081.0 μg/L even in patients with mCRC, confirming that CEA is not optimal for reliable diagnosis of advanced CRC. In contrast, patients with ≥2.0 ng/mL ITGB6 displayed 100% mCRC, indicating a clearly higher accuracy for ITGB6 to delineate the presence of metastasis at time of blood draw than CEA (Fig. 3*a*, Supporting Information Table S4). A similar high accuracy for presence of metastasis was detected for ≥0.1 ng/mL ITGB6 serum level in parallel with ≥5.0 μg/L CEA serum level at time of blood draw. Our results indicate that ITGB6 serum concentrations together with CEA would refine the diagnosis of metastatic disease in CRC in clinical practice.

Beta-6-integrin in the serum represents a reliable marker for tumor surveillance in CRC patients

Next, we investigated whether ITGB6 serum levels can be used for tumor surveillance and monitoring of the disease course over time in CRC patients. To address this goal, we established

a prospective patient cohort consisting of 26 CRC patients undergoing surgery due to CRC (*Cohort 5*, Supporting Information Table S7). Here, we studied ITGB6 serum concentrations directly pre-surgery (day −1 to −3) and post-surgery (day 2–6), as well as during follow-up visits (weeks 3–20) (Fig. 3*b*). Additionally, we analyzed the clinical standard marker CEA as a reference serum marker (Fig. 3*c*), to compare the accuracy of ITGB6 with CEA for tumor surveillance.

Almost no detectable ITGB6 serum concentrations were observed in CRC patients from cohort 5 without lymph node- or solid organ metastasis at all-time points of blood draw (Fig. 3*b*, Supporting Information Table S7). Five patients with LN metastases, but without distant metastases, revealed ITGB6 serum concentrations of 0.00–1.94 ng/mL pre-surgery, which dropped to 0.00–0.07 ng/mL post-surgery, including the follow-up visits of those patients, representing complete tumor resection. Interestingly, ITGB6 serum concentration increased to 1.40 ng/mL in one patient, who was diagnosed with liver metastases at a second follow-up visit (80 weeks after surgery). Of note, CEA levels did not show any increase at this time point (Fig. 3*c*).

In six patients with liver metastases, pre-surgery ITGB6-levels were between 0.05–1.98 ng/mL (Figs. 3*b* and 3*e*, Supporting Information Table S8). Post-surgery, those patients also revealed lower levels of ITGB6 overall (0.06–1.21 ng/mL). One of those patients underwent resection of the primary tumor and the liver metastases and ITGB6-levels declined after surgery (Supporting Information Table S8). Three patients exhibited a clear decline of ITGB6 serum levels after primary tumor resection, whereas two patients remained stable during adjuvant chemotherapy for the remaining liver metastases. However, in one patient, a significant increase during follow-up visit was observed, and the patients

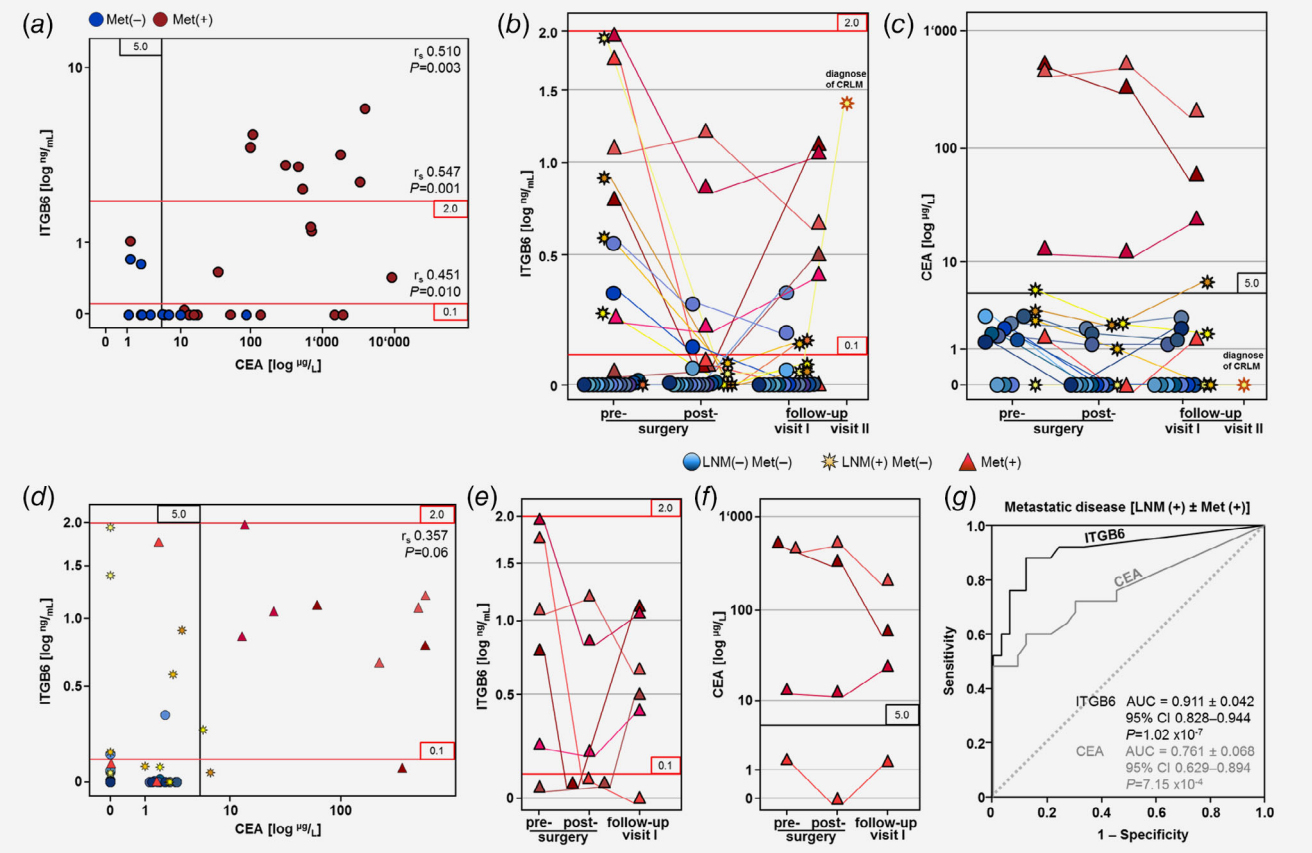


Figure 3. Serum ITGB6-levels represents an improved diagnostic performance compared to CEA. (*a* and *d*) Two-dimensional scatterplots depict ITGB6 serum levels in relation to serum levels of CEA from each CRC patient according to their metastatic disease, of (*a*) *cohort 3* (*n* = 32), and (*d*) the follow-up serum cohort (*n* = *19, cohort 5*). Time course of ITGB6 (*b, n = 26;* and *e, n = 6*) and CEA (*c*, *n = 19;* and *f*, *n = 4*) serum levels from each CRC patient from *cohort 5*, directly pre-surgery (day −1 to −3), post-surgery (day 2–6), as well as during follow-up visits (I: weeks 3–20; II: week 80), and according to their metastatic disease (LNM(–)Met(–), *n = 15*; LNM(–)Met(+), *n* = 5; LNM(+)Met(+), *n = 6*). (*g*) To compare the diagnostic performance of ITGB6 and CEA to detect the presence of metastatic CRC, ROC curve was applied for serum ITGB6- and CEA-levels (*n = 25*). (*a* and *d*) Spearman's rho correlation (r_s) Sig. 2-tailed test was applied. (*a* and *d*) Red lines indicating ITGB6 cut-off values at 0.1 and 2.0 ng/mL; black line indicates CEA cut-off value at 5.0 µg/L. AUC (area under the curve); CI (confidence interval); CEA (carcinoembryonic antigen); CRLM (colorectal cancer metastasis in the liver); LNM(–) (without lymph node metastasis); LNM(+) (with mesenteric lymph node metastasis); Met(–) (patients without metastasis); Met(+) (patients with metastasis); ROC curve (receiver-operating-characteristic curve); r_s (Spearman's rho correlation). [Color figure can be viewed at wileyonlinelibrary.com]

was then diagnosed with progression of the remaining liver metastases (Figs. 3*b* and 3*e*). While we detected a direct correlation between tumor resection resulting in a reduction of tumor load in the body and a decrease of ITGB6 serum levels, no such correlation was found for serum CEA levels in our patients (Figs. 3*c* and 3*f*). Moreover, while we found a moderate correlation between ITGB6 and CEA serum levels in the retrospective cohort, we found only a weak correlation in the follow-up cohort (r_s 0.357, $p = 0.006$; Fig. 3*d*), implying that there is no direct correlation between ITGB6 and CEA during disease course and after resection of the primary tumor. In particular, while ITGB6 serum levels indicated the presence of mCRC, CEA serum levels were elevated without signs of any metastatic disease in those CRC patients. This was further defined by ROC curve analysis for CEA serum levels that showed only a fair AUC of 0.761 ± 0.068 (95% CI 0.629–0.894; $p = 7.15 \times 10^{-4}$; Fig. 3*g*) to define metastatic disease. In comparison, serum levels of ITGB6 showed an excellent AUC of 0.911 ± 0.042 (95% CI 0.828–0.944; $p = 1.02 \times 10^{-7}$; Fig. 3*g*) to identify metastatic disease in the same patient cohort.

These observations suggest that ITGB6 might serve as a serum biomarker for diagnosis and prognosis of metastatic CRC patients, and indicates tumor surveillance, relapse, and treatment response to surgery.

Discussion

Our findings suggest that serum ITGB6 concentration can be used as a biomarker for diagnosis, prognosis, and surveillance of CRC, especially for patients with advanced CRC. We were able to define two cut-off values for ITGB6 with 100% specificity, and a diagnostic sensitivity of 69.8% (0.1 ng/mL) and 27.8% (2.0 ng/mL), respectively, for the detection of CRC. Those observations were confirmed by analyzing ITGB6 serum levels in a separate and independent prospective CRC patient cohort, namely cohort 2.

Our data also suggested that ITGB6 in the serum can be used as a reliable marker for tumor surveillance. Here, elevated serum levels of ITGB6 clearly declined after successful tumor therapy and remained at very low or even undetectable levels as long as no tumor recurrence was detected. However, tumor progression or onset of new metastasis resulted in a rise in serum ITGB6 levels and correlated with the observed tumor load in those patients. In contrast, CEA serum concentrations of those patients were not directly related to treatment response. These findings demonstrated that ITGB6 might be superior to CEA serum levels for tumor surveillance and delineated the importance of serum ITGB6-levels for routinely tumor surveillance.

A limitation of our study is the relatively small number of patients in the prospective tumor surveillance part of the study. However, establishing such a prospective cohort and the respective analyses and read-outs would require a large number of patients and was therefore beyond the scope of this present proof-of-principle study. Nevertheless, our data already demonstrated the potential relevance of serum ITGB6 levels for tumor surveillance in CRC patients. A further limitation is that our study utilizes different patient cohorts what might make the studied patient collective heterogeneous. We cannot exclude some biases arising due to this heterogeneity. In particular, the years of recruitment span from 2003 and 2017, four hospitals and one private company. On the other hand, this can also be regarded as a strength of the study, since the results obtained by analyzing those different cohorts are highly conserved and suggest that the observations made in this study are consistent and can be applied to the real life setting.

In summary, our findings provide evidence that ITGB6 can serve as a novel serum biomarker for diagnosis and prognosis of advanced CRC and as a marker for tumor surveillance, relapse, and treatment response. Herein, ITGB6 serum levels are clearly superior to ITGB6 tumor tissue levels.[18] Those findings provide a crucial step towards a personalized medicine approach for the diagnosis and surveillance of patients with CRC. Further, ITGB6 serum level can be used for the detection of advanced CRC as well as for tumor surveillance *via* an easy blood (serum) test that seems to be superior to the current clinical standard CEA. In conclusion, our findings suggest that measuring serum levels of ITGB6 may be a novel and promising diagnostic strategy in the treatment of CRC patients and a critical step towards a personalized medicine approach.

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