High TrkB expression levels are associated with poor prognosis and EMT induction in colorectal cancer cells

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Abstract

Background The neurotrophic receptor tropomyosin related kinase (TrkB) is associated with tumor progression in neuroblastoma and certain human malignancies. Recent reports indicate TrkB may participate in the epithelial–mesenchymal transition (EMT). This study investigates whether TrkB expression is associated with the clinical outcome of colorectal cancer (CRC) patients and whether TrkB induces EMT in CRC cells.

Methods TrkB and E-cadherin expression in surgical tissue samples and clinicopathological data from 102 CRC patients were analyzed by real-time polymerase chain reaction and immunohistochemistry. The biological role of TrkB in CRC was analyzed using RNA interference against TrkB in the CRC cell line SW480 to assess tumor progression and the correlation between TrkB and E-cadherin expression.

Results Patients with high TrkB mRNA expression in clinical samples had a significantly poorer prognosis relative to those with low TrkB levels ($p = 0.03$). TrkB was inversely correlated with E-cadherin at both the mRNA and protein levels. In vitro, cell proliferation ($p = 0.02$), migration ($p < 0.001$), and invasion ($p < 0.001$) were significantly inhibited by TrkB knockdown while the anoikis rate increased in TrkB siRNA-transfected cells compared to control siRNA. Interestingly, E-cadherin expression in TrkB siRNA-transfected cells was higher than in control cells and vimentin was lower conversely.

Conclusions High TrkB expression is associated with poor prognosis in CRC patients and enhanced malignant potential in terms of proliferation, migration, invasion, and anoikis inhibition in CRC cells. These results indicate TrkB could induce EMT and play an important role in CRC progression to metastasis.

Keywords TrkB · Colorectal cancer · E-cadherin · EMT

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Introduction

Colorectal cancer (CRC) is the most common malignant disease worldwide with an estimated 5-year survival rate ranging from 90% for stage 1 patients to 10% for metastatic cases [1]. Thus, distant metastases formation is the decisive and most lethal event during the disease course.

The neurotrophic receptor tropomyosin related kinase B (TrkB) is a neurotrophic tyrosine kinase receptor that has brain-derived neurotrophic factor (BDNF) as a primary ligand [2]. Recent studies indicated that TrkB is oncogenic in tumors of neurogenic origin, such as neuroblastoma, and is linked phenotypically to chemotherapeutic resistance, cellular motility, hypoxic response, and anoikis detachment-induced apoptosis inhibition [3–5], while also regulating neuronal cell proliferation, differentiation, and death. TrkB is also overexpressed by solid tumors of the lung, prostate, pancreatic, and ovarian cancer [6–9]. Furthermore, TrkB overexpression in colon cancer is implicated in apoptosis inhibition, proliferation and invasion promotion,

and facilitating tumor progression by lymphangiogenesis-associated metastasis [10]. Previously, we demonstrated that high TrkB expression is an independent prognostic marker in gastric cancer patients [11] and a chemoresistant marker for esophageal cancer [12].

Epithelial-to-mesenchymal transition (EMT) is a well-characterized embryological process that could play a vital role in tumor progression [12–17]. During EMT, epithelial cells actively downregulate cell–cell adhesion systems, lose polarity, and acquire a mesenchymal phenotype having reduced intercellular interactions and increased migratory capacity. The cell adhesion molecule E-cadherin, a transmembrane protein localized to the adherens junctions and basolateral plasma membrane of epithelial cells, is correlated with invasiveness and poor differentiation and malignant phenotype in CRC [18]. In addition, E-cadherin is the best-characterized molecular marker of EMT and loss of E-cadherin expression is an EMT hallmark [16]. Recent in vitro studies suggest that TrkB could regulate EMT [19, 20]. To our knowledge, neither the prognostic significance of TrkB expression in CRC patients nor the relationship between TrkB and EMT in CRC has been sufficiently evaluated. In this study, we investigated the association between TrkB expression and CRC clinical outcome and whether TrkB regulates EMT in a CRC cell line.

Materials and methods

Patients and tissue samples

A total of 102 patients treated at the Department of Gastrointestinal and Pediatric Surgery, Mie University Graduate Hospital, Japan, between January 2004 and January 2007 were included in this study. All patients underwent surgical resection without prior chemical or radiation therapy, and were classified according to Union for International Cancer Control (UICC)/TNM classification, the sixth edition. Tumor specimens were frozen in liquid nitrogen immediately after surgical resection, and were stored at −80°C until RNA extraction. The study design was approved by the institutional review board, and written informed consent was obtained from all patients.

Total RNA extraction and cDNA synthesis

Tumor specimens were homogenized and total RNA was isolated with RNeasy mini kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized from 5.0 mg total RNA using a random hexamer primer and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Quantitative real-time reverse transcription PCR

Quantitative real-time reverse transcription analysis (qRT-PCR) was performed with the StepOne™ Real Time PCR System instrument and software (Applied Biosystems, Foster City, CA, USA). Primers for TrkB, E-cadherin, and β-actin were designed by using Primer3 software (Biology Workbench version 3.2, San Diego Supercomputer Center, University of California, San Diego). The following sequences were used: TrkB: forward, 5′-CGTGTACAGCACTGACTACTACAGG-3′; reverse, 5′-GTACATGATGCTCTCTGGAGGC-3′; E-cadherin: forward, 5′-GCCGAGAGCTACACGTTCAC-3′; reverse, 5′-ACTTTGAATCGGGTGTCGAG-3′; β-actin: forward, 5′-ACAGAGCCTCGCCTTTGC-3′; reverse, 5′-GCGGCGATATCATCATCC-3′. PCR was performed with Power SYBR Green PCR Master Mix ($\times 2$) (Applied Biosystems). The following cycling conditions were used: 95°C, 10 min, 40 cycles at 95°C for 15 s and 60°C for 1 min.

Relative TrkB and E-cadherin expression levels

The relative gene expression levels of TrkB and E-cadherin were determined by the standard curve method. Standard curves and linear equations were generated using fivefold serial dilutions of qPCR Human Reference cDNA, random-primed (Takara Bio Inc., Clontech). Within the range analyzed, all standard curves were linear with an acceptable correlation coefficient (R^2). The extent of target gene expression was calculated from the standard curve, and the cDNA in each sample was quantitatively normalized with respect to the β-actin gene, which served as an internal control. Finally, the target gene mRNA levels were expressed as the respective ratio to β-actin mRNA levels. Real-time PCR assays were performed in duplicate for each sample, and the mean value used to calculate mRNA expression levels.

Immunohistochemistry

Formalin-fixed paraffin-embedded sections (2–3 μm thickness) from surgical CRC patients were used. After deparaffinization and dehydration, specimens were boiled in 10 mM sodium citrate buffer to unmask antigens. Specimens were then blocked and incubated with primary antibody overnight at 4°C. Antibody binding was detected by Envision reagents (Envision kit/HRP, Dako Cytomation, Denmark). All sections were counterstained with hematoxylin. Primary mouse monoclonal antibodies against TrkB (R&D Systems) and E-cadherin (BD Biosciences) were diluted 1:60 and 1:1000, respectively. Positive and negative controls were also run simultaneously.

Cell culture and treatment

Human colorectal adenocarcinoma cell lines (Caco2, DLD1, HT29, and SW480) were obtained from RIKEN Cell Bank (Ibaragi, Japan) and grown in RPMI1640 (Sigma-Aldrich, St Louis, MO, USA), supplemented with fetal bovine serum [FBS; 10% (v/v); Gibco BRL, Tokyo, Japan], glutamine (2 mM), penicillin (100 IU/mL), and streptomycin (100 μg/mL) at 37°C in 5% CO_2. Recombinant human TrkB Fc chimera (rhTrkB; R&D systems) was used to examine whether TrkB induced EMT morphologically. rhTrkB was exposed to cells at a concentration of 100 ng/mL at 24 h after plating. Cells were maintained in a humidified atmosphere and assays performed after a 24-h incubation.

TrkB RNA interference

Small interfering RNA (siRNA) was used for RNA interference. TrkB-specific siRNA (Silencer Validated siRNA; sense: 5′-GAAUUGACGAUGGUGCAAAtt; antisense: 5′-UUUGCACCAUCGUCAAUUCca, Ambion®, Applied Biosystems) and control siRNA (Silencer Select Negative Control #1 siRNA, Ambion®, Applied Biosystems) were purchased from Ambion (Austin, TX, USA). Reverse transfections were performed by mixing suspended cells with siRNA oligonucleotides (50 nM), Opti-MEM I (Invitrogen), and Lipofectamine RNAiMAX (Invitrogen) before plating. Cells were maintained in a humidified atmosphere and assays performed after a 24-h incubation.

Protein extraction and Western blot analysis

Cells were homogenized in lysis buffer for 5 min on ice. The supernatants were collected after centrifugation at 15000 rpm for 15 min at 4°C and frozen at −20°C until use. The protein concentration was measured by BCA protein assay (Pierce, Rockford, IL, USA). Lysates containing 20 μg total protein were mixed with appropriate quantities of Lammeli's loading buffer containing β-mercaptoethanol, and heated at 100°C for 5 min. Samples were separated by electrophoresis on 12.5% gradient polyacrylamide gels containing 0.1% SDS at 25 mA for 30 min, followed by semi-dry transfer to an Immun-Blot PVDF membrane (Bio-Rad, Hercules, CA, USA) at 12 V for 30 min. The membranes were blocked for 2 h at room temperature with 5% skim milk in TBS, pH 7.5, supplemented with 0.1% Tween 20 (TBS-T). The blots were then incubated overnight at 4°C with mouse monoclonal TrkB antibody (R&D Systems) diluted 1:250, mouse monoclonal E-cadherin antibody (BD Biosciences) diluted 1:2000, mouse monoclonal vimentin antibody (BD Biosciences) diluted 1:5000, and mouse monoclonal anti-actin (clone

C4) antibody (ICN Biomedicals, Aurora, OH, USA) diluted 1:400 in 5% skim milk in TBS-T. After three TBS-T washes, blots were incubated for 2 h at room temperature with alkaline phosphatase-conjugated goat anti-mouse IgG (Promega, Madison, WI, USA) diluted 1:200 in TBS-T with 5% skim milk. Following addition of enhanced chemiluminescence detection solution, chemiluminescent signals were visualized with a CS Analyzer and AE-6962 Light Capture (ATTO Corp., Tokyo, Japan).

Proliferation assay

Cell proliferation was evaluated with a WST-8 colorimetric assay kit (Cell Counting Kit-8, Dojindo Laboratories, Japan). Twenty-four hours after reverse transfection, TrkB siRNA- and control siRNA-transfected SW480 cells were seeded at 5×10^3 cells per well in 96-well flat-bottomed microtiter plates, in a final volume of 100 μL culture medium per well, and incubated in a humidified atmosphere. After 0- to 48-h culture, 10 μL Cell Counting Kit-8 reagent was added to each well, and the plates incubated for 1 h in a humidified atmosphere whereupon the absorbance of each well was measured by SoftMax Pro (Molecular Devices Corp., Sunnyvale, CA, USA) at a wavelength of 450 nm. Each independent experiment was performed three times.

Anoikis assay

Anoikis assays were performed in six-well Costar ultra low attachment microplates (Corning, NY). TrkB siRNA- or negative control siRNA-transfected SW480 cells were suspended in RPMI-1640 with 10% FBS at a concentration of 5×10^5 cells/mL. Cell suspensions (2 mL) were added to each well and incubated for 18 h in a humidified atmosphere. After anoikis induction, cells were washed and resuspended in 0.5 mL binding buffer, and annexin V:fluorescein isothiocyanate/propidium iodide labeling performed according to the manufacturer's protocol (Bio Vision, Mountain View, CA, USA). Analysis was performed with FACSCalibur (BD Biosciences). Each sample contained 5×10^5 cells. Data were analyzed by CellQuest Pro software (BD Biosciences).

Migration and invasion assay

Transfected cells (5×10^4 cells/well) were seeded in serum-free media (in triplicate) into 24-well (8 μm pore size) Falcon™ cell culture inserts (BD Biosciences) or 24-well (8 μm pore size) BD Matrigel™ Invasion Chambers (BD Biosciences) for migration and invasion assays, respectively. Inserts were placed into Falcon companion plates containing 10% FBS and incubated for 48 h. The

incubation media plus cells were then removed from the top chamber using cotton swabs and phosphate buffered saline and the number of cells migrating to or invading the membrane underside was determined. Membranes were fixed and stained with Diff-Quik stain™ (Sysmex, Kobe, Japan) and mounted on glass slides. The number of migrating or invading cells in 10 fields was counted with a light microscope at ×100 magnification.

Statistical methods

The results are presented as the mean ± SD, and were subjected to a nonparametric Mann–Whitney U test. To calculate cutoff values, nonparametric operating characteristics (ROC) were analyzed according to the most accurate value obtained using Medcalc 7.2 for Windows (Broekstraat 52, 9030, Mariakerke, Belgium). Survival probabilities were calculated by the Kaplan–Meier product

limit method, and intergroup differences were determined using the log-rank test. Prognostic factors were examined by univariate and multivariate analyses (Cox proportional hazards model). All statistical analyses were carried out using Stat View 5.0 for Windows (SAS Institute Inc., Cary, NC, USA). Two-sided p values of less than 0.05 were considered statistically significant.

Results

Association between TrkB and E-cadherin expression and CRC clinicopathological characteristics

Table 1 relates TrkB and E-cadherin mRNA expression to clinicopathological findings in CRC patients. TrkB expression correlated with disease progression factors, including lymph node involvement and occurrence of

Table 1 Relationship between TrkB and E-cadherin gene expression and pathological parameters in colorectal cancer patients

Variables	Number	TrkB/β-actin ($\times 10^{-2}$)	p value	E-cadherin	p value
Gender					
Male	58	0.35 (±0.45)		3.83 (±3.83)	
Female	44	0.61 (±1.30)	0.66	3.64 (±2.92)	0.87
Age (year)					
>65	55	0.37 (±0.57)		3.96 (±2.96)	
≤65	47	0.57 (±1.21)	0.88	3.51 (±3.98)	0.14
Tumor size (mm)					
≥45	51	0.49 (±1.17)		3.73 (±4.01)	
<45	51	0.43 (±0.58)	0.74	3.77 (±2.83)	0.29
Vessel involvement					
Absent	29	0.28 (±1.07)		3.43 (±2.75)	
Present	73	0.53 (±0.13)	0.43	3.88 (±3.71)	0.7
Lymphatic vessel involvement					
Absent	10	0.33 (±0.35)		5.08 (±3.70)	
Present	92	0.47 (±0.96)	0.98	3.61 (±3.42)	0.13
Histological grade					
Differentiated (well/mod)	92	0.43 (±0.92)		3.89 (±3.56)	
Undifferentiated (por/muc)	10	0.69 (±0.92)	0.2	2.42 (±1.97)	0.09
Pathological T category					
pT1	8	0.29 (±0.21)		5.73 (±3.32)	
pT2	13	0.30 (±0.34)		4.52 (±4.29)	
pT3	54	0.32 (±0.42)		3.54 (±3.63)	
pT4	27	0.86 (±1.63)	0.23	3.21 (±2.47)	0.07
Lymph node metastasis					
Absent	56	0.24 (±0.25)		4.11 (±3.89)	
Present	46	0.73 (±1.30)	<0.01	3.31 (±2.82)	0.25
Distant metastasis					
Absent	72	0.29 (±0.37)		4.10 (±3.74)	
Present	30	0.87 (±1.54)	<0.01	2.92 (±2.50)	0.09

distant metastasis. E-cadherin mRNA expression was lower in undifferentiated CRC (poorly and mucinous) than differentiated CRC (well and moderately), though statistical significance was not reached. On the other hand, TrkB expression was not related to histological grade.

Figure 1a illustrates the distribution of TrkB and E-cadherin mRNA expression subdivided by UICC stage. TrkB expression increased according to stage classification progression. Figure 1b shows that TrkB and E-cadherin mRNA expression was inversely correlated in CRC tissues. Immunohistochemistry (IHC) of CRC clinical tissue specimens revealed a correlation between TrkB and E-cadherin expression at the protein level (Fig. 1c).

To examine the predictive value of TrkB expression for prognosis, we defined the cutoff value by ROC analysis. Figure 1d shows CRC patient survival curves subdivided according to TrkB mRNA expression (cutoff value 0.001) and E-cadherin mRNA expression (cutoff value 1.036). They indicated that high TrkB expression and low E-cadherin expression were associated with poor prognosis in CRC patients. On the basis of Cox univariate proportional hazards analysis, age ($p = 0.05$), pathological T category ($p < 0.01$), lymph node metastasis ($p < 0.01$), E-cadherin ($p < 0.01$), and TrkB ($p = 0.04$) were significant prognostic factors for 5-year survival (data not shown). However, pathological T category ($p < 0.01$) was the only independent risk factor to predict poor prognosis by multivariate analysis (data not shown).

TrkB knockdown affects CRC cell proliferation

To investigate whether TrkB alters the biological characteristics of CRC cells, TrkB gene silencing was performed using siRNA. TrkB mRNA expression was assessed by real-time RT-PCR in CRC cell lines Caco2, DLD1, HT29, and SW480. SW480 cells were chosen for siRNA transfections because they showed the most intense TrkB gene expression levels (data not shown). TrkB mRNA expression was considerably reduced in TrkB siRNA (siTrkB)-transfected cells (Fig. 2a). Cell proliferation was also impaired after TrkB knockdown relative to cells transfected with negative control siRNA (siControl) as measured by a WST-8 proliferation assay (Fig. 2b).

TrkB knockdown affects CRC migration and invasion

To test whether TrkB silencing inhibited cell migration and invasion, cell motility and invasiveness were assessed using in vitro migration and invasion assays. The number of migratory cells treated with siTrkB was markedly decreased compared to siControl-treated cells (Fig. 3a, b). The number of invading cells treated with siTrkB was also significantly reduced relative to control cells (Fig. 3c, d).

Anoikis is enhanced in TrkB siRNA-transfected cells

Anoikis is apoptosis induced by lost or inappropriate cell adhesion [21]. Since TrkB was recently identified as a potent anoikis suppressor [3], we performed an anoikis assay in the TrkB knockdown model. After anoikis induction, an annexin V-FITC assay using flow cytometry revealed that a greater percentage of siTrkB cells were apoptotic (16.9%) compared to siControl cells (11.4%) (Fig. 4).

E-cadherin and vimentin expression change upon TrkB knockdown, and morphology changes upon rhTrkB treatment

To assess the malignant potential of TrkB in CRC further, E-cadherin repression, a hallmark of EMT, was investigated by Western blotting. In addition, vimentin expression and morphologic changes were investigated. Upon TrkB protein knockdown, expression of E-cadherin was higher and that of vimentin was lower in siTrkB cells relative to siControl cells (Fig. 5a). In siTrkB cells, TrkB and vimentin expression decreased by 48.9 and 64.8% respectively while E-cadherin expression increased by 53.5% relative to that of siControl cells (Fig. 5b). Furthermore, we investigated morphologic change by SW480 cells treated with rhTrkB. rhTrkB-treated cells developed morphologic changes and mesenchymal-like cells (spindle-shaped cells) slightly increased by rhTrkB treatment (Fig. 5c). However, we could not find a statistically significant difference between control cells and rhTrkB-treated cells in terms of the number of spindle cells (data not shown).

Discussion

Here we show the biological and clinical significance of TrkB expression in CRC. To our knowledge, no previous studies have investigated the correlation between CRC tissue TrkB expression and clinicopathological variables, including prognosis. We found that TrkB mRNA expression was increased according to stage classification progression, and high TrkB expression in CRC was associated with poor prognosis by survival curve analysis. Previous studies demonstrated that TrkB overexpression could be associated with poor prognosis in neuroblastoma [22, 23] and ovarian cancer [24], and is an independent prognostic factor for gastric cancer [11], suggesting that TrkB may indeed be an independent prognostic factor for CRC as well, but perhaps was not in this study due to sample size.

Since we found high TrkB mRNA expression was associated with poor prognosis, and because TrkB can enhance malignant potential (e.g., proliferation, migration,

Fig. 1 TrkB and E-cadherin expression in clinical tissue samples. **a** Boxplot of TrkB mRNA expression in 102 CRC patients subdivided by TMN staging. **b** Inverse correlation between TrkB and E-cadherin mRNA expression in CRC samples. **c** Representative immunohistochemical analysis of TrkB and E-cadherin expression ($\times 100$, respectively). Patient A was representative of patients having high TrkB and low E-cadherin mRNA expression in CRC tissue, whereas patient B represented patients with low TrkB and high E-cadherin. Serial sections were stained with anti-TrkB monoclonal antibody and anti-E-cadherin at a dilution of 1:60 and 1:1000, respectively. For patient A, TrkB (*A-1*) had strong cytoplasmic expression, whereas E-cadherin (*A-2*) was weakly expressed at the cytoplasmic membrane. For patient B, TrkB (*B-1*) was weakly expressed, whereas E-cadherin (*B-2*) was strongly expressed. **d** CRC patient survival curves according to TrkB and E-cadherin mRNA expression status
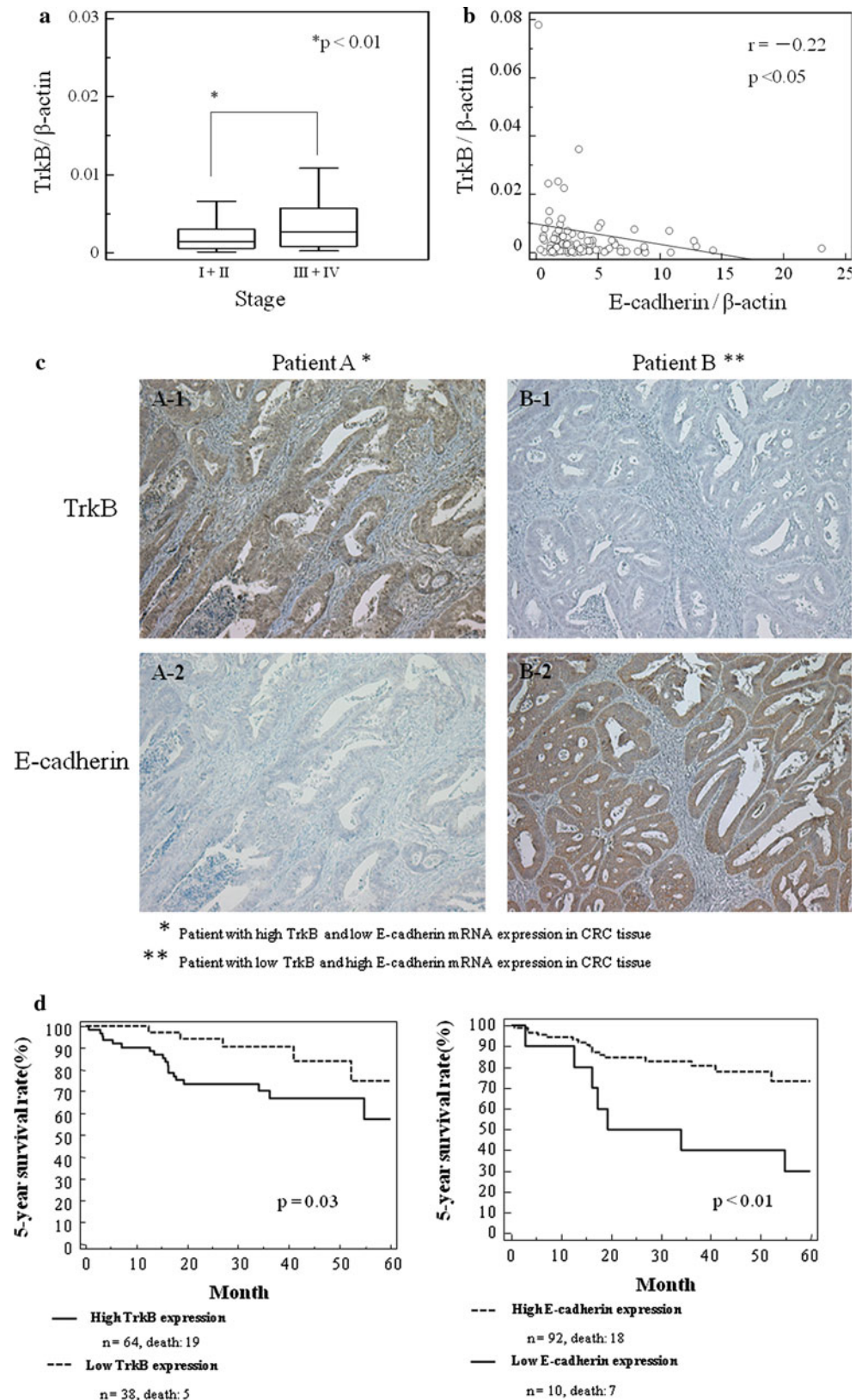
invasion) in several malignancies [7, 9, 24, 25], we investigated the malignant features of TrkB in the CRC cell line SW480 using siRNA transfection. Recently, TrkB overexpression in colon cancer was reported to play possible roles in inhibiting apoptosis, promoting proliferation and invasion, and facilitating tumor progression by

Fig. 2 TrkB expression knockdown in SW480 cells. SW480 cells were transfected with either TrkB-siRNA (*siTrkB*) or negative control-siRNA (*siControl*). Control is positive control without transfection. **a** mRNA expression ratio of siTrkB to siControl were measured 24 h after transfection by qRT-PCR. **b** Proliferation assay. The proliferation rate was impaired after TrkB knockdown compared to siControl cells
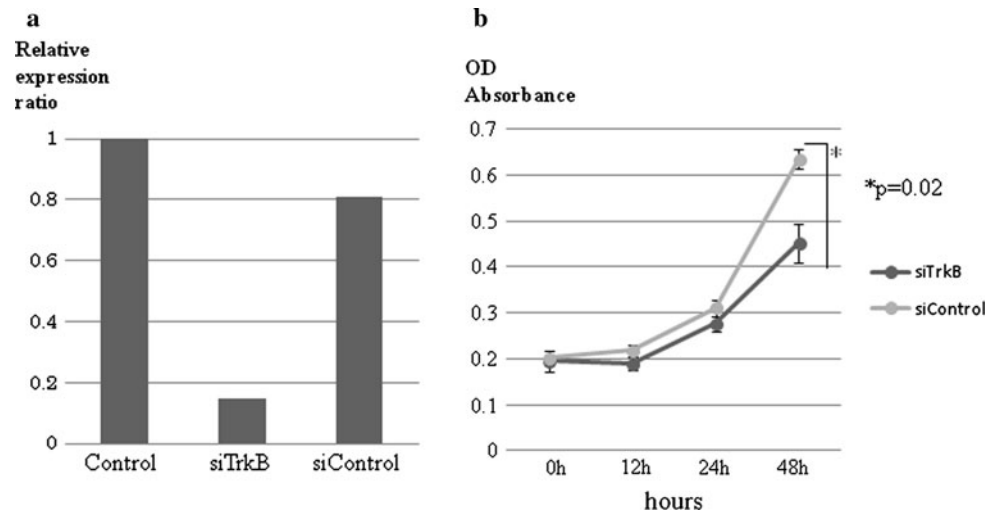

Fig. 3 Reduction of TrkB expression enhances cancer cell migration and invasion. **a**, **c** The transwell migration system demonstrates enhanced migrative and invasive capacity after TrkB knockdown. Images of migrating (**a**) and invading (**c**) cells were taken by phase contrast microscopy at ×100. **b**, **d** Quantitative transwell migration and invasion assay. *Y*-axis represents the number of migrating (**b**) and invading (**d**) cells
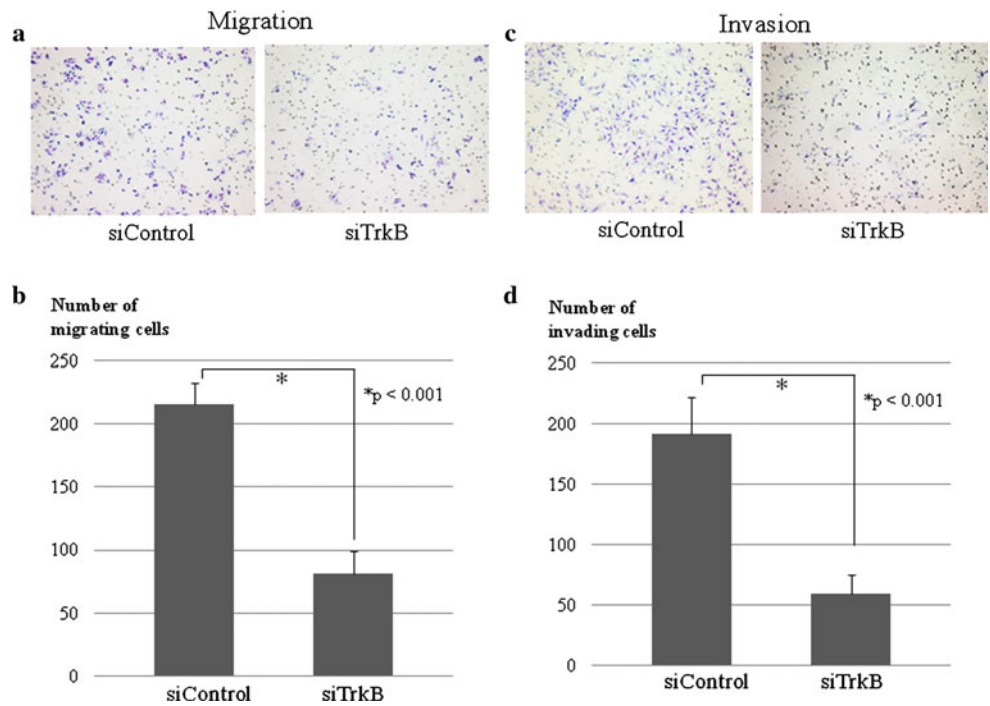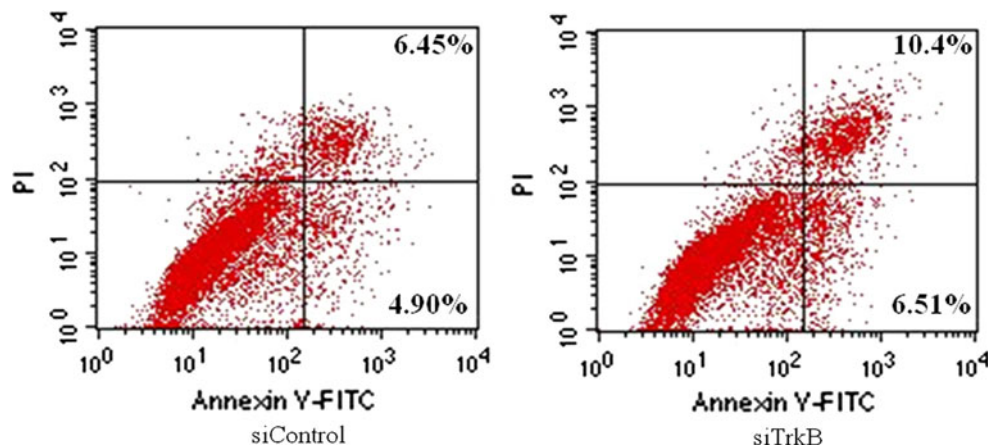

Fig. 4 Anoikis assay. Apoptosis rates were measured by flow cytometry using annexin V and propidium iodide staining 24 h after transfection. Apoptotic cell percentage is the sum of the *upper* and *lower right panels*
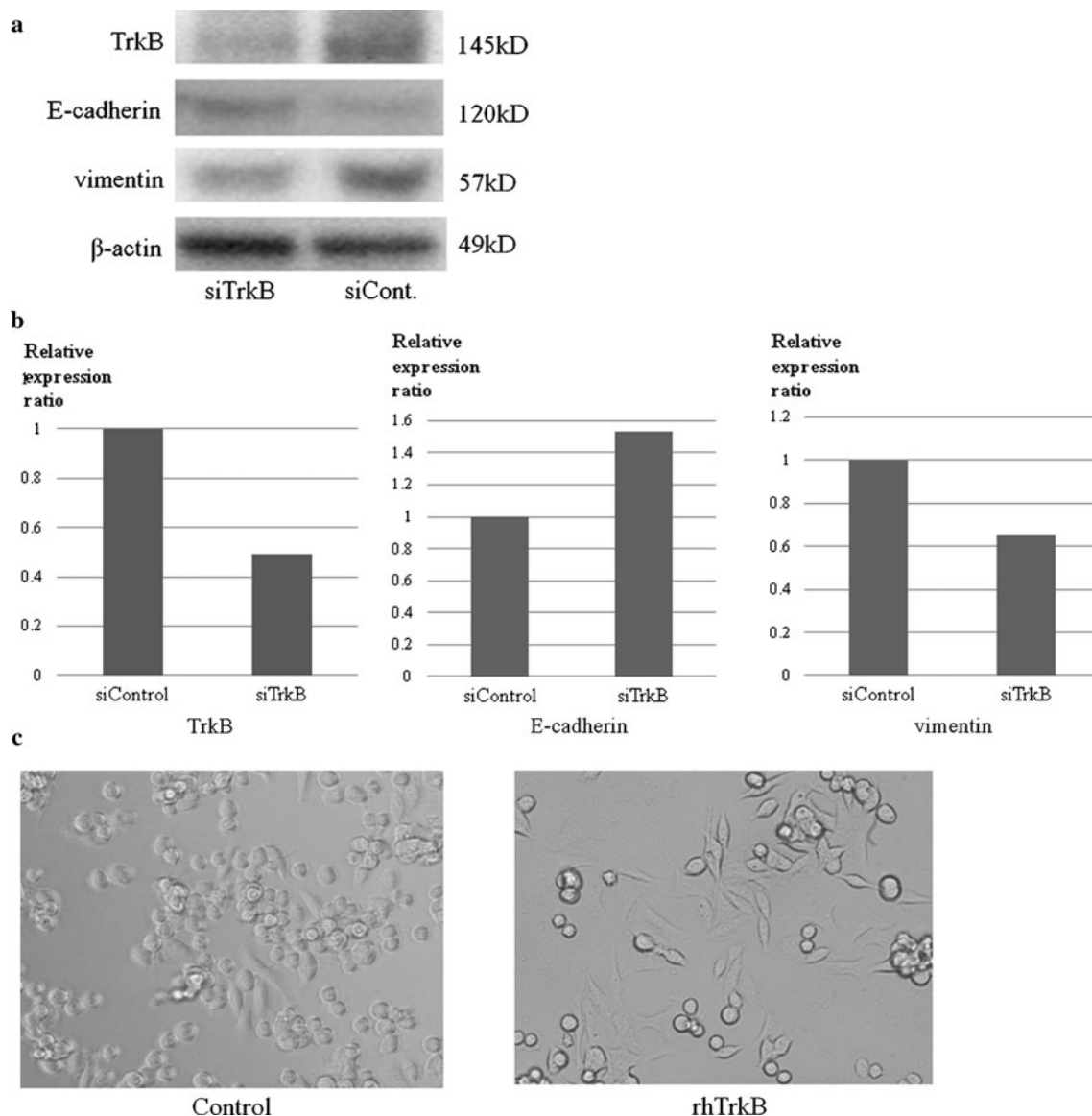
Fig. 5 High TrkB expression decreases E-cadherin levels and increases vimentin levels, and rhTrkB treatment induces morphologic change. **a** Western blotting. **b** The ratio of β-actin to optical density. Upon TrkB inhibition, expression of E-cadherin was higher and that of vimentin was lower in siTrkB cells relative to siControl cells. **c** Representative morphologic changes after rhTrkB treatment were visualized with a light microscope at ×200

lymphangiogenesis-associated metastasis [10]. We found that high TrkB expression promoted not only cellular proliferation and invasion but also migration. Together, these results suggest that TrkB has metastatic potential in CRC cells.

Anoikis is apoptosis resulting from loss of cell–matrix interactions that represent a physiological barrier to metastasis [3]. In fact, TrkB is increased in anoikis-surviving cells, and TrkB overexpression could play a role in anoikis suppression via the PI3K–AKT pathway [3, 9]. We showed that the CRC cell anoikis rate was suppressed by TrkB knockdown, suggesting that TrkB is important for anoikis regulation in CRC cells. To our knowledge, this is the first report demonstrating TrkB-dependent anoikis induction in CRC cells.

Furthermore, a recent study showed that tumor cells lacking E-cadherin expression are anoikis-resistant [26]. Loss of E-cadherin expression is a defining characteristic of EMT, which may be a fundamental cellular strategy to evade the anoikis barrier [27]. Recently, TrkB has been reported to promote EMT in head and neck squamous cell carcinoma cells and a rat kidney cell line [19, 20]. Smit et al. [20] showed that TrkB activation induces expression of Twist and Snail transcription factors. These transcriptional factors may induce EMT by suppressing E-cadherin expression and are correlated with poor prognosis and CRC

chemoresistance [28–30]. We thus evaluated the relationship between TrkB and the EMT signature adhesion molecule E-cadherin. We found a significant inverse correlation between the expression of TrkB and E-cadherin mRNA in colorectal tumors, and IHC provided supporting evidence that TrkB expression is inversely correlated with E-cadherin in representative cases. TrkB mRNA expression has not been significantly associated with histological grade in this study though it has been reported that loss of E-cadherin is associated with poor differentiation [18]. On the other hand, E-cadherin levels were increased and vimentin levels were decreased in siTrkB-treated SW480 cells compared to siControl cells. These results show that TrkB could induce EMT in CRC cells. Yu et al. [10] first reported that TrkB played important roles in facilitating metastasis CRC. However, the important and new finding of our report is that TrkB is associated with malignant potential including EMT in the studies using CRC clinical samples as well as CRC cell lines.

In conclusion, TrkB promotes proliferation, migration, and invasion of a CRC cell line and furthermore inhibited anoikis and E-cadherin expression, indicating that TrkB elicits an important effect on an EMT mediator. High TrkB expression in CRC tissue was also associated with poor prognosis in CRC patients. These results suggest that TrkB plays an important role in CRC progression to metastasis.

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