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TMEM52B-derived peptides inhibit generation of soluble E-cadherin and EGFR activity to suppress colon cancer growth and early metastasis

Yunhee Lee[1], Dongjoon Ko[1,2], Junghwa Yoon[1] and Semi Kim[1,2*]

Abstract

Background Transmembrane protein 52B (TMEM52B) is a novel gene expressed widely in various normal human tissues; however, the biological function of TMEM52B in cancer remains largely unknown. Previously, we demonstrated that TMEM52B is a novel modulator of E-cadherin and EGFR activity, and that it has tumor suppressor-like activity using both experimental and clinical analyses. Here, we hypothesized that the extracellular domain (ECD) of TMEM52B may exert tumor-suppressing activity.

Methods We designed and evaluated the therapeutic potential of TMEM52B ECD-derived peptides in vitro and in vivo. The molecular mechanisms underlying the anti-cancer activity of the peptides were explored.

Results TMEM52B ECD-derived peptides reduced cancer cell survival, invasion, and anchorage-independent growth, which was accompanied by decreased phosphorylation of ERK1/2 and AKT. The peptides maintained intact E-cadherin at organized cell–cell junctions, leading to reduced β-catenin activity. They also inhibited generation of soluble E-cadherin and activation of EGFR by binding directly to the E-cadherin ECD and interfering with the interaction between soluble E-cadherin and EGFR. Peptides fused to the Fc domain of human IgG1 efficiently inhibited tumor growth in a colon cancer xenograft model and reduced survival of circulating tumor cells in an early metastasis model.

Conclusions These results strongly suggest that TMEM52B ECD-derived peptides could provide a platform for the development of novel anti-cancer therapeutics and furnish a useful tool for exploring the function of TMEM52B in modulating the interplay between E-cadherin and EGFR.

Keywords TMEM52B, Peptide, Therapeutics, E-Cadherin, EGFR, β-Catenin

*Correspondence:
Semi Kim
semikim@kribb.re.kr
[1] Korea Research Institute of Bioscience and Biotechnology, Microbiome Convergence Research Center, 125 Gwahak-ro, Yuseong-gu, Daejon 34141, Korea
[2] Department of Functional Genomics, Korea University of Science and Technology, Daejon 34113, Korea

Background

Cancer metastasis, the leading cause of cancer-related mortality, is a multistep process comprising several steps: local invasion, intravasation, cancer cell survival in the circulation, extravasation, micrometastasis, and metastatic colonization [1]. Epithelial-mesenchymal transition (EMT) plays a critical role in local invasion, and in subsequent steps such as intravasation, survival of cancer cells in the circulation, and micrometastasis.

EMT is a reversible process during which organized epithelial cells undergo morphological and molecular changes to acquire a more mesenchymal phenotype [2–5]. Reduced expression or functional loss of E-cadherin, a well-known metastasis suppressor, is a key event of EMT [5]. The extracellular domain of E-cadherin mediates cell–cell adhesion through Ca^{2+}-dependent homophilic interactions, thereby inhibiting cell migration and dissemination. Furthermore, the intracellular domain of E-cadherin anchors β-catenin to the cell membrane, which prevents β-catenin from entering the nucleus and inducing expression of EMT-related genes, thereby suppressing EMT and cancer metastasis [5]. Usually, reduced expression of E-cadherin is mediated at the transcriptional level by E-cadherin transcriptional repressors/EMT-inducing transcription factors, as well as at the post-translational level [5, 6]. Furthermore, a soluble fragment of E-cadherin (sE-cad; an 80 kDa soluble form), formed by shedding of the E-cadherin ectodomain, contributes to tumor progression by promoting invasion and metastasis [6, 7]. Clinically, sE-cad serum levels are a prognostic marker for multiple types of cancers, including gastric, colorectal, breast, and ovarian cancers [8, 9].

Transmembrane protein 52B (TMEM52B, C12orf59) is a novel gene first cloned in 2002 [10]. TMEM52B mRNA isoform 1 (NM_153022; 2789 bp) encodes a 163 amino acid protein, and isoform 2 (NM_001079815; 2563 bp), which differs with respect to the 5′ UTR and contains an alternate exon in the 5′ coding region, meaning that it initiates translation from an alternate upstream start codon, encodes a distinct N-terminal, 183 amino acid protein. Both isoforms are predicted to encode transmembrane proteins. Xie et al. (2016) showed that TMEM52B is broadly expressed in normal human tissues, with high expression in the kidney; by contrast, expression in a panel of cancer cell lines was not detectable. Decreased expression of TMEM52B correlates with a poor prognosis and von Hippel-Lindau mutations in renal cell carcinoma (RCC), suggesting that TMEM52B is a tumor suppressor in RCC [11]; however, to date, the expression pattern and biological functions of TMEM52B in cancer remain largely unknown.

Previously, we reported that suppressing TMEM52B mediates shedding of extracellular E-cadherin and contributes to activation and internalization of the epidermal growth factor receptor (EGFR), resulting in increased activation of downstream signals and subsequent cancer cell survival and invasion. These data imply that TMEM52B has tumor/metastasis-suppressing activity. Clinically, high expression of TMEM52B correlates positively with increased survival of patients with breast, lung, kidney, or rectal cancers, suggesting that a reduction in TMEM52B expression may contribute to tumor progression.

These previous results led us to hypothesize that TMEM52B may be exploited as part of a novel cancer treatment strategy. We hypothesized that the extracellular domain (ECD) of TMEM52B may exert tumor/metastasis-suppressing activity because both TMEM52B isoform 1 and isoform 2 localize at the plasma membrane and interact with E-cadherin to reduce its shedding. Here, we report that TMEM52B ECD-derived peptides suppress cancer cell survival and invasion; inhibit production of soluble E-cadherin and the activation of EGFR by interfering with the interaction between soluble E-cadherin and EGFR. We generated peptide-Fc fusion proteins and evaluated their therapeutic potential in colorectal cancer xenograft models. We found that recombinant peptide-Fc fusion proteins reduced tumor growth and early metastasis in vivo. Taken together, these results strongly suggest that the peptides could be further developed to provide a novel anti-cancer agent.

Materials and methods

Cell lines

SW480, HCT-15, HCT-116, HT-29 (colon cancer), FHC (normal colon epithelial), and CCD-18Co (normal colon tissue) cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The SW480sub cell line is a subclone isolated from SW480 cells in-house [12]; these cells display higher matrix adhesion capacity than SW480 cells. SW480, HCT-15, HCT-116, and HT-29 cells were maintained at 37 °C/5% CO_2 in RPMI1640 supplemented with 10% FBS. Stable cells (TMEM52B-suppressed *vs.* control cells) established from the HCT-15 cell lines were described previously [12]. FHC cells were maintained in DMEM/F12 supplemented with 10% FBS, 10 mM HEPES, 5 μg/mL insulin, 100 ng/mL hydrocortisone, 5 μg/mL transferrin, 10 ng/mL human recombinant EGF. CCD-18Co cells were maintained in Eagle's MEM with 10% FBS. C8161 cells (melanoma) were a kind gift from Dr. C-R Jung (KRIBB, Korea) [13]. Cells were checked for mycoplasma and their identities were confirmed using STR-PCR analysis.

Synthesis of TMEM52B-derived peptides and production of Fc-fused proteins

Peptides corresponding to the extracellular domain (ECD) of TMEM52B isoform 1 (SWRPQPCCISSCCLTTDWVH) and isoform 2 (EENCGNPEHCLTTDWVH) were synthesized by Peptron (Daejon, Korea). To produce recombinant TMEM52B ECD-Fc fusion proteins (the ECD fused to the Fc of human IgG1), cDNA fragments encoding the ECD of TMEM52B isoform 1 and isoform 2 were subcloned into the pCMV-Fc-myc

vector [12]. The resulting recombinant TMEM52B ECD-Fc fusion proteins were produced using the Expi293 Expression system (Thermo fisher Scientific, Waltham, MA, USA) and purified by protein A affinity chromatography, as previously described [14]. Recombinant soluble E-cadherin-Fc (sE-cad-Fc) fusion protein and mock Fc protein were produced as previously described [12].

Transfection of cells with expression vectors and small interfering RNA (siRNA)

Isoform 1- and isoform 2-expressing constructs within the pcDNA3.1-myc vector were described previously [12]. cDNA encoding the truncated form of isoform 2 lacking the cytoplasmic domain was amplified by PCR using primer set (5′- AACATCTCGAGGCCGCCA TGGGAGTCCGAGTTCAT-3′ and 5′- AATTCAAGC TTGCGGAAGCACAGGGAC-3′) and then subcloned into the pcDNA3.1-myc vector to generate pcDNA3.1-isoform 2ΔC. Prior to analysis, cells were transfected for 48 h with siRNA specific for EGFR (5′-CGCAAAGUG UGUAACGGAAUATT-3′) [12] using Lipofectamine 2000.

Immunoblot analysis

Whole-cell lysates were prepared using RIPA buffer, followed by immunoblotting as described previously [12]. Protein bands were detected using the following primary antibodies: anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-β-catenin, anti-E-cadherin, and anti-integrin α5 (BD Biosciences, San Jose, CA, USA); anti-α smooth muscle actin (αSMA) and anti-vimentin (Sigma, St Louis, MO, USA); anti-phospho-extracellular signal-regulated kinase 1/2 (ERK1/2), anti-ERK1/2, anti-phospho-AKT(S473), anti-AKT, anti-survivin, anti-phospho-c-Jun(S63), anti-c-Jun, anti-survivin, anti-phospho-EGFR(Y1068), anti-EGFR, anti-phospho-β-catenin(S33, 37/T41), anti-phospho-β-catenin(S675), and anti-phospho-β-catenin(S552) (Cell Signaling, Danvers, MA, USA); anti-E-cadherin (R&D Systems, Minneapolis, MN, USA); and rabbit anti-TMEM52B (produced in-house) [12]. To detect the soluble E-cadherin fragment, conditioned medium from cells was collected for 48 h.

Invasion assay

Cells in serum-free medium were plated on Transwell inserts (Corning, NY) coated with 25 μg of Matrigel in the presence of the peptides. The underside of the insert was pre-coated with 2 μg of collagen type I (Sigma). After incubation for 48 h at 37 °C/5% CO_2, the inserts were fixed with 10% formalin and stained with 2% crystal violet. Invasiveness was determined by calculating the cell-stained area relative to the total area using ImageJ software.

Cell survival analysis and anoikis assay

Cell survival under suspension culture conditions was assessed. Briefly, cells were seeded ($1.5–2\times10^4$ cells/well) into 96-well plates with an Ultra-Low Attachment Surface (Corning) and incubated for 3 days in the presence of the peptides. Cells were then incubated with WST reagent (Ez-Cytox; Dogenbio, Seoul, Korea; one-tenth of the medium volume) and the amount of formazan dye formed was determined by measuring absorbance at 450 nm in a spectrophotometric microplate reader (BMG LABTECH GmbH, Ortenber, Germany). For HT-29 cells, cell viability was analyzed using the CellTiter-Glo 3D Cell Viability Assay kit (Promega, Southampton, UK). To induce anoikis, cells were seeded into 6-well plates with an Ultra-Low Attachment Surface for 48 h. Cells (1×10^5) were then stained with annexin V (5 μL) and propidium iodide (5 μL) for 15 min at 25 °C in the dark, and the percentage of apoptotic cells was determined by flow cytometry.

Anchorage-independent soft agar assay

Cells in 0.4% agar (Sigma) were seeded over a 0.6% agar feed layer at a density of 1×10^3 cells/well in 6-well tissue culture plates. Cells were allowed to grow at 37 °C in 5% CO_2 for 13 days in the presence of the peptides, and the number of resulting colonies with diameter > 0.3 mm was counted per well.

Tumorsphere formation assay

To induce tumorsphere formation, cells were dissociated into single cells and seeded at a density of 200 cells/well into 96-well plates with an Ultra-Low Attachment Surface to prevent the cells from attaching to the surface. Cells were incubated in the presence of the peptides for 10 days at 37 °C/5% CO_2 in serum-free DMEM/F12 containing 20 ng/mL EGF (Peprotech, Seoul, Korea), 10 ng/mL bFGF (Peprotech), and 2% B27 supplement (Thermo fisher Scientific). The number of spheroids with a diameter > 50 or 100 μm was counted.

Promoter reporter assay

Cells were seeded into 6-well plates at a density of 2×10^5 cells/well and incubated for 24 h, followed by transfection with 4 μg of reporter plasmid DNA using Lipofectamine 2000 and treatment with the peptides. At 48 h post-transfection, firefly luciferase activity was measured using a Dual-luciferase reporter assay system (Promega). Transfection efficiency was normalized by measuring Renilla luciferase activity, encoded by the co-transfected Renilla luciferase vector (pRL-TK). The AP-1 cis-element

reporter (AP-1 reporter) and cyclic AMP response element reporter (CRE reporter) plasmids were purchased from Stratagene (La Jolla, CA). β-Catenin-responsive firefly luciferase reporter plasmid TOPFlash, and the negative control FOPFlash, were purchased from Millipore.

Co-immunoprecipitation

TMEM52B-suppressed HCT-15 stable cells were incubated for 48 h with or without 100 μg/mL peptide and then lysed in co-immunoprecipitation buffer (10 mM Tris pH 7.4, 150 mM NaCl, 0.56 mM EGTA, 1% Triton X-100, 0.5% NP-40) supplemented with a protease inhibitor (Complete, Roche). Lysates (1 mg, equivalent to 5×10^6 cells, per condition) were centrifuged for 20 min at 10 000 g and the resulting supernatant was precleared by incubation with protein A/G–agarose for 2 h at 4 °C. The precleared supernatant was immunoprecipitated at 4 °C for 16 h using an anti-EGFR (sc-120; Santa Cruz Biotechnology) antibody. The protein complexes were then collected by incubation with protein A/G–agarose for 2 h at 4 °C and washed four times with co-immunoprecipitation buffer. The protein complexes were eluted by boiling in sodium dodecyl sulfate sample buffer and analyzed by immunoblotting with anti-EGFR (#4267; Cell Signaling) and anti-E-cadherin (MAB1838) antibodies.

Immunofluorescence staining

Cells were plated on coverslips and treated with peptide (100 μg/mL) for 48 h. Cells were then fixed for 15 min in 10% formalin and permeabilized for 5 min with 0.3% Triton X-100. After blocking in 10% normal goat serum, cells were stained using an anti-E-cadherin antibody (R&D Systems; 1:50), followed by an Alexa546-conjugated secondary antibody. Cells were counterstained with 4,6-diamidino-2-phenylindole (DAPI; Sigma) to visualize nuclei. Mounted samples were visualized under a confocal microscope (LSM 510 META; Carl Zeiss, Jena, Germany). The intensity of E-cadherin staining was quantitated by ImageJ software.

Enzyme-linked immunosorbent assay (ELISA)

Ninety-six-well Immunoplates (eBiosciences, San Diego, CA, USA) were coated with recombinant human E-cadherin ECD protein (R&D systems; 100 ng/well) diluted in 50 mM sodium carbonate buffer (pH 9.6) at 4 °C overnight, and then blocked with 2% BSA in PBS. The plates were washed three times with PBS containing 0.05% Tween 20 between each step. The peptide-Fc fusion proteins (amounts ranging from 0 to 270 ng per well) were added to each well, and then horseradish peroxidase (HRP)-conjugated anti-human Fc was added. Mock Fc protein was used as a negative control. All incubations were carried out at 37 °C for 1 to 2 h. Color was developed with 3,3′,5,5′-tetramethylbenzidine substrate solution, and the absorbance was measured at 450 nm using a microplate reader (BMG LABTECH GmbH).

Mouse xenograft model and circulating tumor cell (CTC) survival analysis

All animal procedures were performed in accordance with the guidelines of the Animal Care Committee at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), and with approval of the bioethics committee of the KRIBB (KRIBB-AEC-22107). Nude mice (BALB/c-nude, 5-week-old females) were purchased from Nara Biotech (Seoul, Korea). TMEM52B-suppressed HCT-15 stable cells mixed with Matrigel (1:1) were injected subcutaneously into the right flank of each mouse (5×10^6 cells per mouse). After 21 days, when the tumor volume reached approximately 80 mm^3, the tumor-bearing mice were randomly assigned to groups ($n=6$ per group). Normal IgG (negative control), peptide-Fc ($_{pep}$Iso2-Fc and $_{pep}$Iso1-Fc), or cetuximab (300 μg/mouse) was injected intraperitoneally at 2- or 3-day intervals (total eight times). Body weight and tumor volume were measured before injection of protein. Tumor volume was calculated using the formula $width^2\times length/2$. On Day 39, mice were sacrificed, and dissected tumor masses were lysed as previously described [14] prior to immunoblot analysis.

For CTC survival analysis (early metastasis model), mice were injected intraperitoneally with two peptide-Fc ($_{pep}$Iso2-Fc and $_{pep}$Iso1-Fc) or normal IgG (300 μg/mouse; n = 5–6 per group). Six hours later, TMEM52B-suppressed stable HCT-15 cells (5×10^6) were injected into nude mice via the tail vein. Twenty-four hours later, mice were sacrificed and total DNA was isolated from the lungs, as previously reported [12, 15]. To quantify survival and early seeding/arrest of circulating tumor cells, human tumor cells present in mouse lungs were assessed by real-time qPCR analysis of human prostaglandin E receptor 2 (PTGER2) genomic DNA, as previously reported [12, 16]. A standard curve was generated by qPCR using genomic DNA extracted from HCT-15 cells and nude mouse lungs. The amounts of human genomic DNA present initially in the qPCR reaction tube were estimated from the standard curve.

mRNA sequencing analysis

Total RNA was extracted from HCT-15 (TMEM52B-suppressed and control) cells, followed by mRNA sequencing analysis by a commercial service (Macrogen; Seoul, Korea).

Statistical analysis

Statistical analyses were performed using Student's *t*-test and one-way ANOVA with Dunnett's or Tukey's multiple comparison post-tests (SPSS software; IBM, NY). GSEA of TCGA mRNA data was performed using R program. $P < 0.05$ was considered statistically significant.

Results

Truncated form of TMEM52B and TMEM52B extracellular domain-derived peptides suppress cancer cell invasion, survival, and growth

Previously, we reported that TMEM52B suppression promotes cancer cell survival and invasion, potentially by increasing phosphorylation of EGFR, MAPKs, and AKT [12]. Transcription factor activating protein-1 (AP-1) activity has also been reported to be involved in the tumor-suppressing activity of TMEM52B [12]. We performed mRNA sequencing analysis to determine the effect of TMEM52B on gene expression in TMEM52B-suppressed HCT-15 cells and control cells. Analysis of KEGG pathways revealed that TMEM52B suppression correlated significantly with "Pathways in cancer", "PI3K-Akt signaling pathway", "MAPK signaling pathway", and "Ras signaling pathway" (Figure S1a), confirming the tumor suppressor-like activity of TMEM52B and its modulation of MAPK and AKT pathways. Previously, we observed that overexpression of TMEM52B suppresses invasion and survival of cancer cells [12]. In addition, we observed that the truncated form of isoform 2 (lacking the cytoplasmic domain) also suppressed invasion and the activity of AP-1 cis-element reporter plasmid (AP-1 reporter) in SW480 cells as efficiently, or more efficiently, than intact isoform 2 (Figure S1b, c). Therefore, we explored whether peptides derived from the extracellular domain (ECD) of isoform 1 and isoform 2 (Fig. 1a) inhibit cancer cell invasion and survival. Treatment with the peptides reduced invasion of SW480, SW480sub, and HCT-15 cells significantly, and in a dose-dependent manner (Fig. 1b). Cell survival under suspension conditions was also reduced significantly by both peptides (Fig. 1c). Flow cytometry analysis to determine the percentage of apoptotic cells revealed that the peptides increased the sensitivity of SW480 and SW480sub cells to anoikis (Figure S2a). The peptides decreased anchorage-independent growth of stable HCT-15 cells (TMEM52B-suppressed cells and control cells) significantly, and in a dose-dependent manner (Fig. 1d), although cell proliferation was not affected substantially (data not shown), a finding consistent with our previous report [12]. In addition, the peptides reduced significantly tumorsphere formation by SW480, SW480sub, and HCT-15 cells (Figure S2b), further supporting the anti-cancer activity of the peptides. Immunoblot analysis showed that both peptides decreased phosphorylation of ERK1/2, AKT, and c-Jun, as well as expression of mesenchymal markers, including vimentin and α smooth muscle actin (αSMA), and the anti-apoptotic factor survivin, largely in a dose-dependent manner (Fig. 1e), indicating that the peptides suppress EMT events, leading to suppression of cancer cell invasion and survival. Consistent with this, a reporter assay showed that both peptides decreased the activity of AP-1 cis-element reporter plasmid (AP-1 reporter) significantly in HCT-15 cells (Fig. 1f, upper), indicating that the peptides suppress the transcriptional activity of AP-1. On the other hand, the activity of another AP-1 binding site, the cyclic AMP response element (CRE), was decreased only moderately in the presence of the isoform 1 peptide (Fig. 1f, lower), suggesting that the peptides cause more substantial inhibition of certain components of AP-1. Overall, the inhibitory activities of the isoform 1-derived peptide tended to be greater than those of the isoform 2-derived peptide.

To further confirm the inhibitory activity of the peptides, the effects of the peptides on additional colon cancer cell lines (HCT-116 and HT-29) were examined. Similar to the previously tested cell lines, the peptides reduced drastically the invasion and survival of HCT-116 cells in a dose-dependent manner (Figure S2c, d). The peptides also reduced significantly the survival of HT-29 cells in a dose-dependent manner (Figure S2d).

(See figure on next page.)

Fig. 1 TMEM52B ECD-derived peptides suppress cancer cell invasion, survival, and anchorage-independent growth. **a** Peptide sequences derived from the ECD of isoform 1 and isoform 2. **b** Cells were allowed to invade Matrigel for 48 h in the presence of the peptides. Cell invasion was determined by calculating the cell-stained area relative to the total area using ImageJ software. **c** To induce anoikis, cells were seeded into 96-well plates with an Ultra-Low Attachment surface and then grown for 72 h in the presence of the peptides. Cell viability was determined in a colorimetric WST assay. **d** Anchorage-independent growth soft agar assay in the presence of peptides. The total number of colonies > 0.3 mm in diameter in each well was counted. Scale bar, 100 μm. **e** Cells were treated with the peptides for 48 h prior to lysis and immunoblot analysis. Densitometric quantification was performed using GAPDH as a loading control; phosphorylated proteins were normalized against the amount of the corresponding total protein. **f** Cells were transfected with AP-1 or CRE reporter plasmid and then treated with the peptides for 48 h. AP-1 activity was determined in reporter assays. All determinations were performed in three independent experiments. Values represent mean ± standard deviation (SD). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

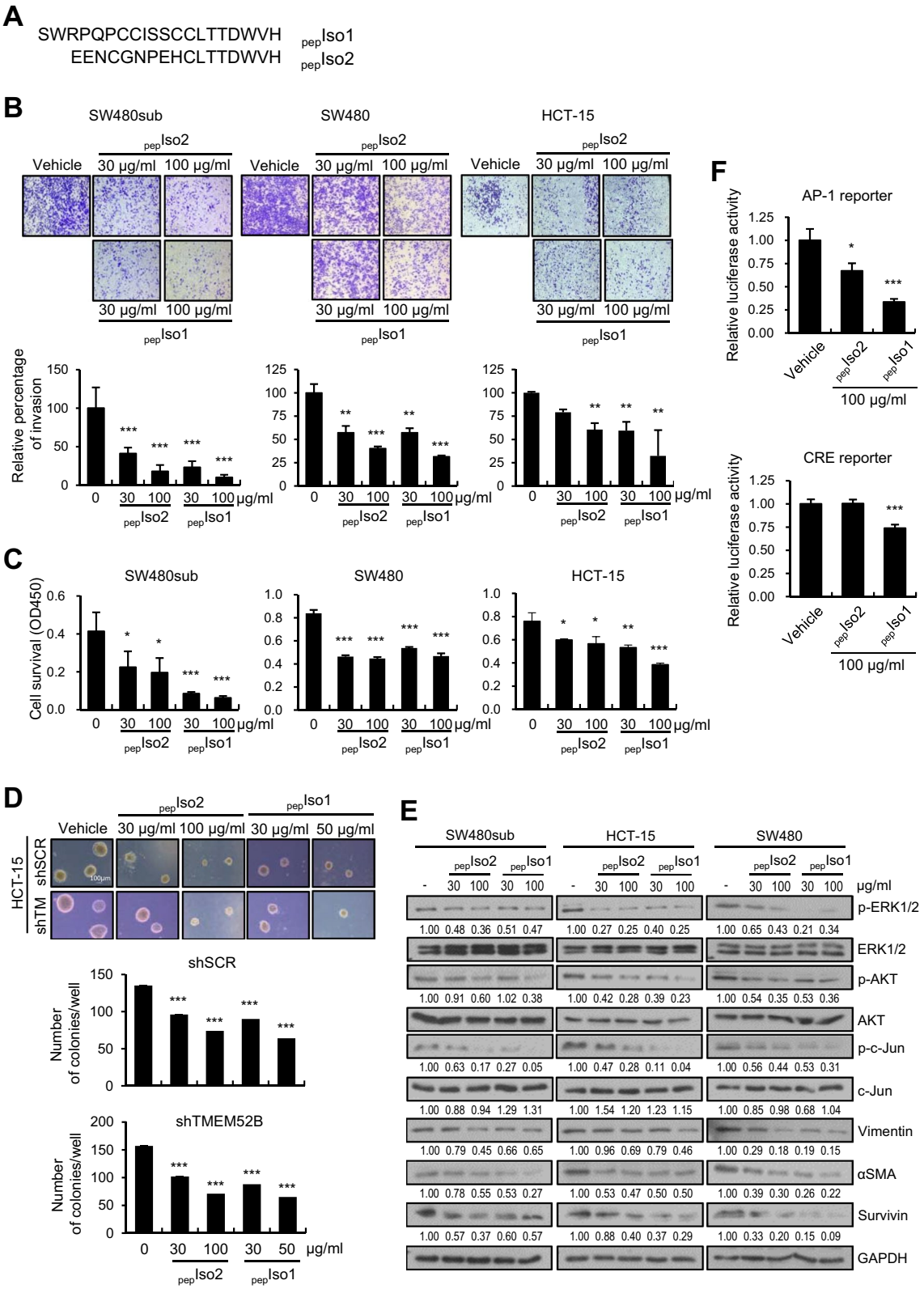

Fig. 1 (See legend on previous page.)

TMEM52B ECD-derived peptides suppress generation of the soluble E-cadherin fragment and the activation of EGFR

Previously, we observed that suppressing TMEM52B reduces transcription of E-cadherin in a context-dependent manner [12]. Consistent with this, gene set enrichment analysis (GSEA) of cancer patient datasets from The Cancer Genome Atlas (TCGA) showed that TMEM52B expression correlated positively with KEGG pathway gene sets related to "Cell adhesion molecules_CAMS" in the TCGA-COAD, TCGA-KIRP, and TCGA-READ datasets (Figure S1d). Furthermore, we observed previously that suppressing TMEM52B reduces E-cadherin stability to enhance β-catenin activity, and that it promotes generation of a soluble E-cadherin fragment that contributes to EGFR activation [12]. Therefore, we examined whether the peptides affect the stability of the E-cadherin protein. Treatment with the peptides led to a moderate increase in expression of intact E-cadherin, and a reduction in expression of soluble E-cadherin, in SW480 cells and TMEM52B-suppressed HCT-15 cells (Fig. 2a). Notably, the effect of the peptides on HCT-15 cells was weaker than that on SW480 cells. Immunofluorescence analysis showed that HCT-15 control cells maintained E-cadherin expression, whereas TMEM52B-suppressed HCT-15 cells displayed significantly less E-cadherin than the control cells at organized cell–cell junctions. Treatment of TMEM52B-suppressed HCT-15 cells with the peptides restored E-cadherin expression at organized cell–cell junctions to levels that were comparable to those of the control cells (Fig. 2b). In addition, the peptides reduced phosphorylation of β-catenin at Ser552 and Ser675, but enhanced phosphorylation at Ser33/37/Thr41 (Fig. 2a). Reporter assays showed that the peptides reduced the transcriptional activity of β-catenin in SW480 cells and TMEM52B-suppressed HCT-15 cells (Fig. 2c). Both peptides also reduced phosphorylation of EGFR in both cell lines, which may be, at least in part, due to reduced levels of soluble E-cadherin (Fig. 2a). Of note, and consistent with our previous observations [12], intact E-cadherin levels were lower and soluble E-cadherin levels were higher in TMEM52B-suppressed HCT-15 cells than in control cells. Phosphorylation of β-catenin (at S552 and S675) and EGFR was also increased by suppression of TMEM52B (Fig. 2a, right).

In HCT-116 and HT-29 colon cancer cells, immunoblot analysis showed that the peptides decreased phosphorylation of EGFR, ERK1/2, and AKT (Figure S2e). The peptides moderately increased the expression of intact E-cadherin and reduced the expression of soluble E-cadherin. In addition, the peptides reduced phosphorylation of β-catenin at Ser552 and Ser675, but increased phosphorylation at Ser33/37/Thr41 (Figure S2e). A Reporter assay showed that the peptides significantly reduced the transcriptional activity of β-catenin in HCT-116 cells (Figure S2f).

We also explored whether the peptides affect activation of EGFR mediated by soluble E-cadherin. Immunoblot analysis showed that phosphorylation of EGFR, ERK1/2, and AKT was higher in HCT-15 cells treated with purified recombinant soluble E-cadherin-Fc (sE-cad-Fc) fusion protein than in cells exposed to mock Fc protein or no treatment at all. This increase in phosphorylation decreased substantially after treatment with the peptides (Fig. 3a).

Soluble E-cadherin, an extracellular proteolytic fragment of E-cadherin, can activate EGFR family members by binding to them [6]. Co-immunoprecipitation experiments using whole-cell extracts from TMEM52B-suppressed HCT-15 cells, which express EGFR and produce soluble E-cadherin, revealed that EGFR co-precipitated with soluble E-cadherin (Fig. 3b), a finding consistent with reports from other groups [17, 18]. This co-precipitation was reduced after treatment of cells with the peptides (Fig. 3c), indicating that the peptides may inhibit EGFR activation by interfering with its interaction with soluble E-cadherin. We next explored whether the peptides bind directly to soluble E-cadherin. ELISA showed that the both peptides fused to Fc protein (see below), but not mock Fc protein, bound significantly to recombinant E-cadherin ECD protein, while isoform 1-derived peptide bound more strongly than isoform 2-derived peptide (Fig. 3d), suggesting that the peptides bind directly to E-cadherin, interfering with the interaction between soluble E-cadherin and EGFR.

Taken together, these results suggest that the peptides suppress production of the soluble E-cadherin fragment, as well as activation of β-catenin and soluble E-cadherin-mediated activation of EGFR.

TMEM52B ECD-derived peptides suppress invasion in an EGFR-dependent manner

Next, we examined the effect of the peptides on EGFR in the presence of EGF. Exogenous EGF-induced phosphorylation of EGFR, and subsequent phosphorylation of ERK1/2 and AKT, in SW480 and HCT-15 cells was suppressed by the peptides (although phosphorylation of AKT was suppressed moderately by the isoform 2 peptide in HCT-15 cells) (Fig. 4a). Invasion by SW480 and HCT-15 cells, induced by exogenous EGF, was reduced by both the isoform 1 peptide (more potently) and the isoform 2 peptide (Fig. 4b). Consistent with the results shown in Fig. 1b, basal cell invasion was inhibited by both peptides (Fig. 4b). EGFR expression and activity are increased in melanoma cells and are associated with poor prognosis of malignant melanoma patients [19, 20]. Both

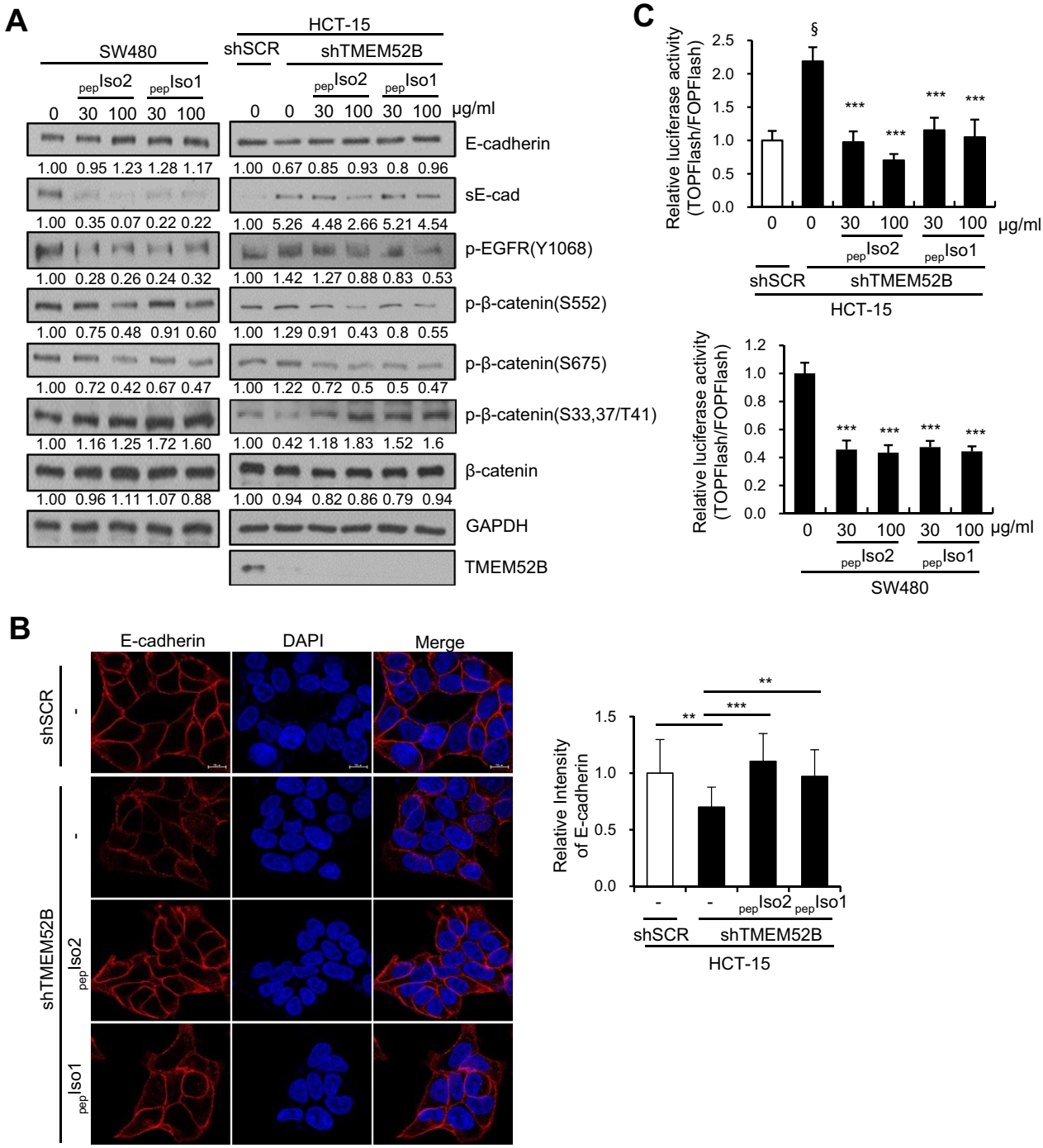

Fig. 2 TMEM52B ECD-derived peptides stabilize intact E-cadherin and reduce β-catenin activity. **a** Cells were treated with the peptides for 48 h and cell conditioned medium was collected over an additional 48 h. Lysates from cells and conditioned medium (containing soluble E-cadherin; denoted as sE-cad) were analyzed by immunoblotting. Densitometric quantification was performed using GAPDH as a loading control. **b** Immunofluorescence analysis of TMEM52B-suppressed HCT-15 cells following treatment with peptides; E-cadherin (red) and nuclei (blue). Scale bar, 10 μm. ImageJ software was used to calculate the level of E-cadherin staining based on the ratio of the E-cadherin-positive area to the total observed area. At least 100 cells per condition were analyzed. Values represent mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$. **c** The transcriptional activity of β-catenin was analyzed using the TOP/FOP reporter system. Cells were transfected with reporter plasmids and incubated with the peptides for 48 h. Firefly luciferase activity was normalized against the Renilla luciferase activity, and fold increases in TOPFlash activity compared with FOPFlash activity were calculated. All determinations were performed in three independent experiments. Values represent mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, compared with vehicle; $^{§}P < 0.05$ compared with shSCR (C upper)

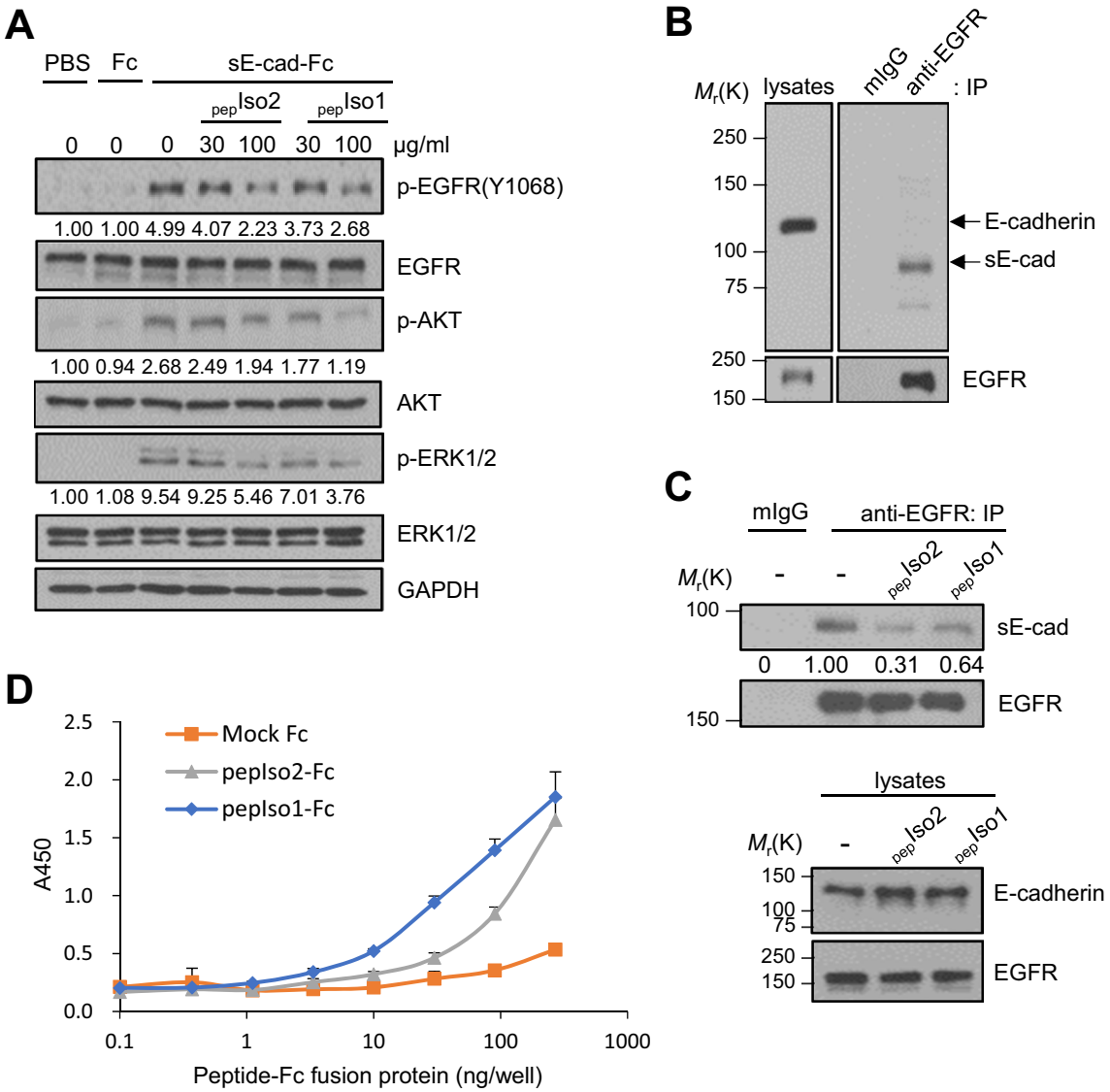

Fig. 3 TMEM52B ECD-derived peptides suppress EGFR activation by interfering with the interaction between soluble E-cadherin and EGFR. **a** Cells were treated for 48 h with recombinant extracellular E-cadherin protein fused to Fc (sE-cad-Fc; 25 μg/mL) and with the peptides. Recombinant Fc protein (25 μg/mL) was used as a negative control. Cells were lysed prior to immunoblot analysis. Densitometric quantification was performed using GAPDH as a loading control; phosphorylated proteins were normalized against the corresponding total protein. **b** Co-immunoprecipitation analysis was performed to examine the interaction between EGFR and E-cadherin in HCT-15 cells. **c** Co-immunoprecipitation was performed using lysates from HCT-15 cells treated with the peptides (100 μg/mL) for 48 h. **d** E-cadherin-binding activities of the peptides were determined by ELISA. Immunoplate wells were coated with the recombinant human E-cadherin ECD protein (100 ng/well), and then incubated with varying amounts of peptide-Fc fusion proteins. Bound peptide-Fc fusion proteins were detected by using HRP-conjugated anti-human Fc antibodies. Mock Fc protein was used as a negative control

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Fig. 4 TMEM52B ECD-derived peptides suppress cancer cell invasion in an EGFR-dependent manner. **a** Cells were treated with EGF (10 ng/mL) and the peptides for 48 h prior to lysis and immunoblotting. Densitometric quantification was performed. Phosphorylated proteins were normalized against the amount of corresponding total protein. **b** Cells were allowed to invade Matrigel for 48 h in the absence or presence of EGF (10 ng/mL) and the peptides. Cell invasion was determined by calculating the cell-stained area relative to the total area using ImageJ software. **c** HCT-15 cells were transfected with siRNA specific to EGFR for 48 h. Transfected cells were subjected to invasion assay and lysis prior to immunoblot analysis. Values represent mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared with siControl + Vehicle (C). n.s., non-significant

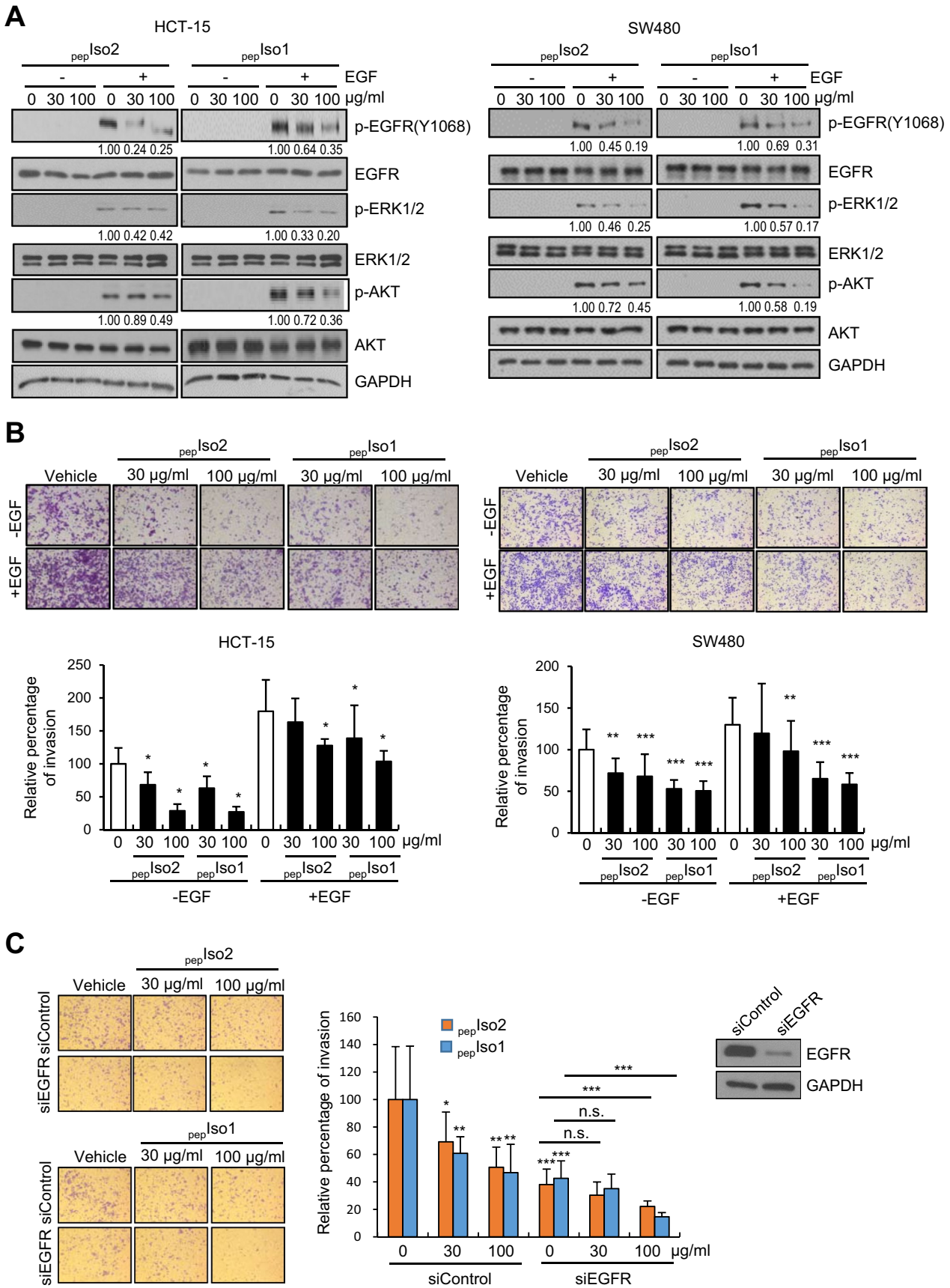

Fig. 4 (See legend on previous page.)

peptides suppressed basal and EGF-induced invasion and survival of C8161 melanoma cells (Figure S3a, b), and reduced phosphorylation of EGFR, AKT, and ERK1/2 (albeit moderately by the isoform 2-derived peptide; Figure S3c). The anti-cancer effects of the peptides may not be limited to colon cancer cells and may extend to other cancer cell types. This will require further investigation using various cell lines and in vivo models.

In addition, EGFR knockdown reduced the invasiveness of HCT-15 (Fig. 4c) and C8161 (Figure S3d) cells; however, the peptides at 30 μg/mL did not inhibit substantially the invasion of HCT-15 and C8161 cells expressing reduced levels of the EGFR. Of note, the peptides at 100 μg/mL had a suppressive effect on HCT-15 cells (Fig. 4c) and isoform 1 peptide at 100 μg/mL only had a modest, non-significant suppressive effect on C8161 cells (Figure S3d). These observations are consistent with our previous observation that suppressing TMEM52B promotes invasion and cell survival in an EGFR-dependent manner [12]. Taken together, the data indicate that the peptides suppress cancer cell invasion, at least in part, in an EGFR-dependent manner.

TMEM52B ECD-derived peptide-Fc fusion proteins inhibit tumor growth and early metastasis in vivo

Next, to evaluate the in vivo antitumor efficacy of the peptides, we generated peptide-Fc fusion proteins, because biologically active peptides or small proteins are often fused to human serum albumin or the constant fragment (Fc) domain of a human immunoglobulin (Ig) G to extend their plasma half-life [21]. First, peptides fused to the Fc domain of human IgG1 were expressed in Expi293 cells and then purified by protein A affinity chromatography (Figure S4a). Next, we confirmed the activity of the purified peptide-Fc fusion proteins. The peptide-Fc fusion proteins decreased cancer cell survival in a manner similar to the native peptides (Figure S4b).

Next, we investigated the antitumor efficacy of the peptide-Fc fusion proteins in nude mouse models bearing tumor xenografts. TMEM52B-suppressed HCT-15 cells were injected subcutaneously into the flanks of nude mice. As shown in Fig. 5a, intraperitoneal administration of the peptide-Fc fusion proteins (300 μg/mouse/dose; three times per week; eight times in total) inhibited tumor growth by 60% without affecting body weight. The antitumor activity of the peptide-Fc fusion proteins was comparable with that of cetuximab, an EGFR-blocking therapeutic antibody used to treat metastatic colorectal cancer with wild-type KRAS, which inhibited tumor growth by 67%. Immunoblot analysis of xenograft tumor tissues revealed that peptide-Fc fusion reduced phosphorylation of EGFR and ERK1/2 in a manner similar to cetuximab (of note, phosphorylation of ERK1/2 was not substantially reduced by cetuximab in our system), although there was no apparent effect on phosphorylation of AKT (Fig. 5b). Phosphorylation of β-catenin at S552 and S675 was also reduced by the peptide-Fc fusions (Fig. 5b). It is possible that the anti-cancer activity of the peptides is increased when they are used in combination with anti-EGFR antibody and/or chemotherapy. However, this will require further investigation.

Finally, we examined the effect of the peptide-Fc fusion on survival and subsequent seeding of circulating tumor cells in vivo using an early metastasis model. TMEM52B-suppressed HCT-15 cells were injected intravenously into nude mice pretreated with the peptide-Fc fusion (300 μg/mouse, intraperitoneal injection). The mice were then sacrificed 24 h after tumor cell injection, and the lungs were removed immediately for total DNA extraction and to determine their tumor cell content. Real-time qPCR analysis revealed that the amount of human genomic DNA in the lungs of mice injected with the peptide-Fc fusion was significantly lower than that in the lungs of mice injected with control Fc protein (Fig. 5c), indicating that peptide-Fc fusion effectively reduces early metastasis.

Discussion

TMEM52B is a novel gene, and to date little is known about its biological functions or expression patterns in cancer. Previously, we demonstrated that TMEM52B exhibits tumor/metastasis suppressor-like activity and is

(See figure on next page.)

Fig. 5 TMEM52B ECD-derived peptide-Fc fusion proteins reduce tumor growth and early metastasis in vivo. (**a**, **b**) TMEM52B-suppressed HCT-15 cells (5×10^6) were mixed with Matrigel and injected subcutaneously into the backs of mice. Mice were then injected intraperitoneally with the peptides and cetuximab (300 μg/mouse; n = 6 per group) at 2 or 3 day intervals (total of eight injections). **a** Left: Tumor volume (length × width^2/2). Right upper: Photos of tumor masses dissected on Day 39. Right lower: Body weight of injected mice. **b** Immunoblot analysis of tumor extracts. Phosphorylated proteins were normalized against the corresponding total protein except that phosphorylated β-catenin was normalized using GAPDH as a loading control. **c** TMEM52B-suppressed HCT-15 cells (5×10^6) were injected intravenously into nude mice (n = 5–6 per group) that had been pre-injected with the peptides. Twenty-four hours after cell injection, the lungs were removed and total DNA extracted. Real-time qPCR analysis was then performed to detect human *PTGER2*. The amount of human genomic DNA present initially in a qPCR reaction tube was estimated (left) from the standard curve (right). Statistical analyses were performed using one-way ANOVA with Tukey's post-test. Values represent mean ± SD. *P*-values are shown on the graph

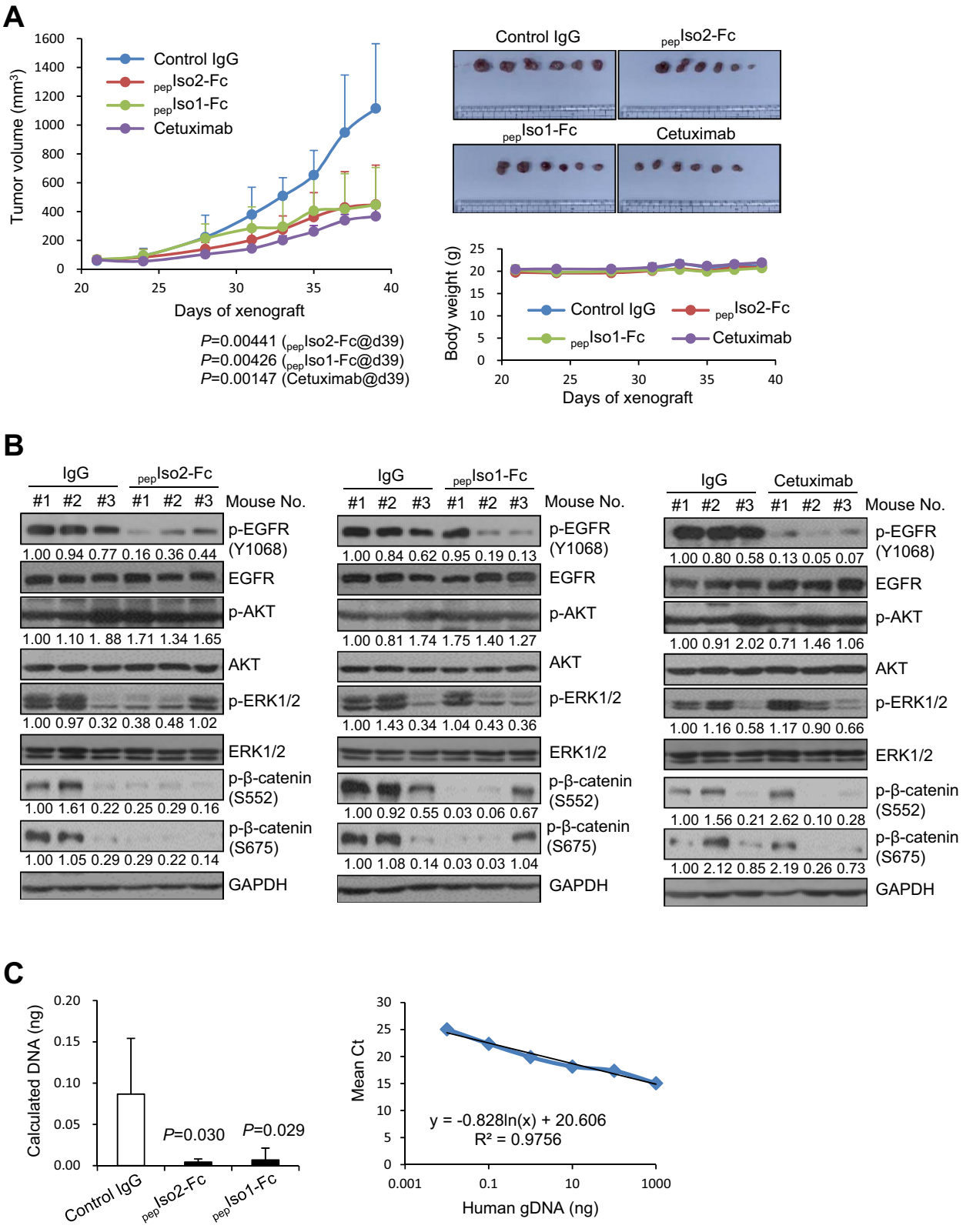

Fig. 5 (See legend on previous page.)

a novel modulator of the interplay between E-cadherin and EGFR. Analysis of clinical datasets shows that high expression of TMEM52B correlates with increased survival of patients with different types of cancer. Consistent with our previous results, Li and colleagues reported that downregulation of TMEM52B correlates with poor survival of RCC patients [11]. By contrast, the same group reported recently that TMEM52B promotes progression of esophageal squamous cell carcinoma through Yes-associated protein (YAP)-mediated EMT [22]. It is possible therefore that TMEM52B exerts positive or negative effects according to cancer type, or in a context-dependent manner. TMEM52B function and the precise molecular mechanisms that drive tumor development and progression remain to be explored.

Previously, we suggested that tumor suppressor-like TMEM52B may be exploited as part of a novel strategy for cancer treatment. For example, partial fragments of TMEM52B may have utility as anti-cancer agents through therapeutic administration of recombinant suppressor proteins or peptide-mimetics. Indeed, restoration of the function of tumor/metastasis suppressors through gene transfer, epigenetic re-induction of the gene, or administration of suppressor proteins is considered to be a novel therapeutic strategy [23, 24]. For example, direct therapeutic administration of bone morphogenetic protein (BMP) 4, a member of the transforming growth factor-beta family, drives differentiation of glioma cancer stem-like cells and thereby sensitizes human glioma to chemotherapy and radiation therapy [25]. Here, we demonstrate that TMEM52B ECD-derived peptides reduce production of soluble E-cadherin and activation of EGFR to suppress cancer cell survival and invasion. Fc-fused peptides efficiently reduced tumor growth and early metastasis in vivo (Fig. 6). This study indicates that these peptides could serve as a platform for development of cancer treatments. Briefly, this will involve determining the anti-cancer activity spectrum of the peptides using various in vitro and in vivo models, exploring the molecular mechanisms, and identifying surrogate biomarker(s) of the activity of the peptides. The pharmacophore of the peptides will also need to be identified and may correspond to the shared amino acid sequence (CLTTDWVH) between the two peptides. The specific domain of E-cadherin ECD that interacts with the peptides will also require further study.

The EGFR is a target for an expanding class of anti-cancer therapies. EGFR-inhibiting agents such as

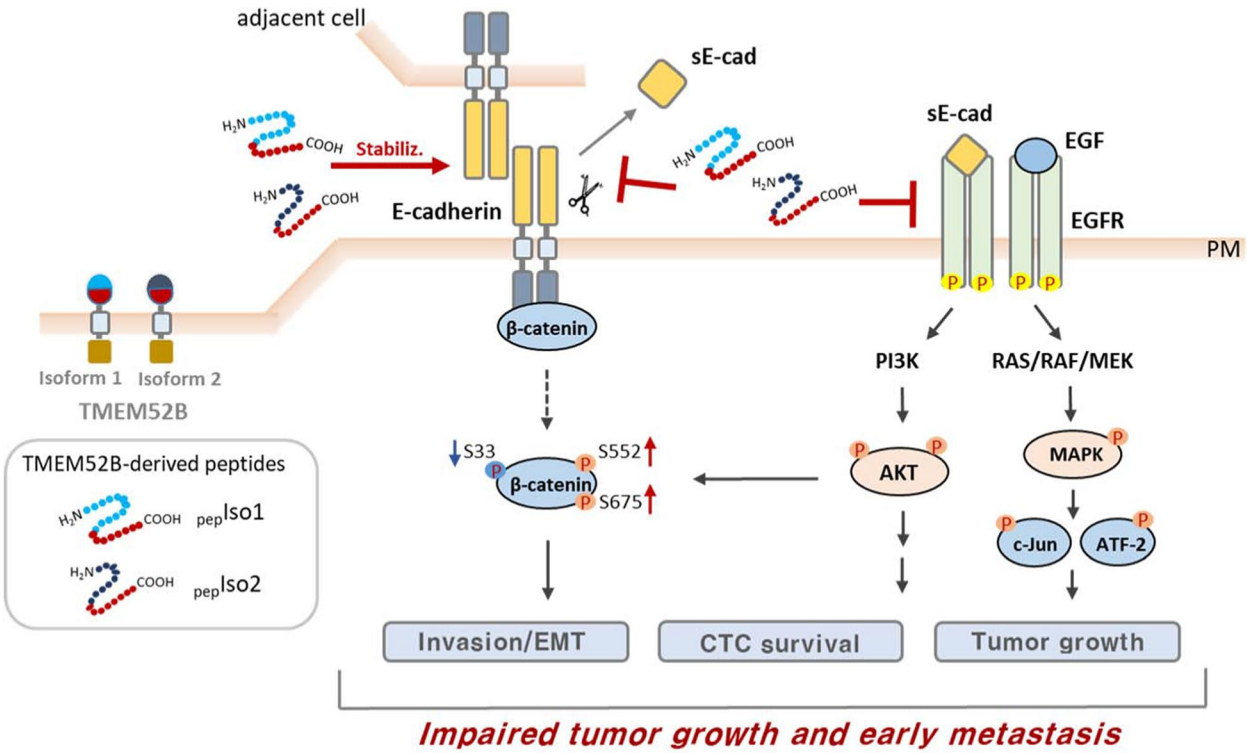

Fig. 6 A schematic representation illustrating the anti-cancer activity of TMEM52B ECD-derived peptides. TMEM52B ECD-derived peptides stabilized intact E-cadherin at cell–cell junctions, leading to reduced β-catenin transcriptional activity. They also inhibited generation of soluble E-cadherin and the activation of EGFR by interfering with the interaction between soluble E-cadherin and EGFR. These activities may reduce tumor growth and early metastasis. The nucleus was not distinguished. The dashed arrow indicates translocation

EGFR-blocking antibodies and EGFR tyrosine kinase inhibitors have been approved for a variety of cancers, including colorectal, non-small-cell lung, pancreatic, and breast cancers. For example, EGFR-blocking antibodies (for example, cetuximab or panitumumab) combined with chemotherapy are used clinically to treat metastatic colorectal cancer patients with wild-type KRAS; however, only about a third of the colon cancer patients respond to EGFR inhibition, and patients with KRAS mutations are resistant to anti-EGFR antibodies [26]. Innate or acquired resistance to these EGFR inhibitors means that there remains a need to identify potential molecular targets/pathways to enable development of novel effective therapeutics. Our results show that TMEM52B-derived peptides inhibit the survival, growth, and invasion of colorectal cancer cells harboring an activating KRAS mutation (HCT-15, KRAS G13D; SW480, KRAS G12V; HCT-116, KRAS G13D). These results support the possibility that using TMEM52B-derived peptides as TMEM52B functional-mimetics can increase the therapeutic benefit of EGFR-inhibiting agents, although this requires further investigation.

Most cancers (> 90%) derived from the epithelial cells are classified as carcinoma. These cells display unique morphologic features such as cell polarity and cell–cell adhesion. Loss of intercellular adhesion mediated by E-cadherin is an essential change that occurs during invasion; therefore, E-cadherin is categorized as a tumor/metastasis suppressor [5, 27]. By contrast, several reports suggest that E-cadherin promotes tumor progression. For example, some aggressive carcinomas retain E-cadherin expression [28], and E-cadherin is required for metastasis in multiple models of breast cancer [29]. Abnormal expression of E-cadherin is associated with a more aggressive brain tumor phenotype and a poor prognosis [30]. In addition, proteolysis of intact E-cadherin generates soluble extracellular fragments that promote tumor growth, survival, and invasion by activating EGFR family members and insulin-like growth factor receptor 1 (IGF-1R) in cancer cells [6, 7]. Soluble E-cadherin localizes to exosome surfaces and promotes angiogenesis in ovarian cancer [31]. Thus, cleavage of E-cadherin converts a tumor suppressor into an oncogenic factor. This soluble E-cadherin is shed constitutively at low levels by normal epithelial cells, but shedding is elevated significantly at primary tumor sites and metastatic foci. Correlations between decreased survival and elevated soluble E-cadherin levels in serum, urine, and tumors have been reported [8, 32]. In colorectal cancer, soluble E-cadherin is associated with a poor prognosis [32] and is a potential diagnostic marker [33]. A soluble E-cadherin-Fc chimera protein increases cancer stem-like properties, including chemoresistance, in colon cancer cells [34]. The opposing roles of E-cadherin may be attributed to the presence of interacting partner(s) for intact E-cadherin on cell surfaces, as well as modulation of soluble E-cadherin production. Our previous and present studies suggest that TMEM52B may be an important modulator of functional E-cadherin in cancer, and that the peptides may be useful for exploring the dual functions of E-cadherin.

The intestinal epithelial barrier protects the underlying tissues from bacteria within the gut lumen. Within this layer, epithelial cells are connected by intercellular junctions, called adherens junctions, which mediate cell–cell adhesion mainly through E-cadherin; therefore, E-cadherin is essential for maintenance of intestinal epithelial homeostasis [35]. The physiological significance of E-cadherin is well known; targeted E-cadherin defects in adult mice are associated with epithelial shedding, leading to bloody diarrhea and death [36]. Inflammatory cytokine-mediated signaling leads to cleavage or internalization of intercellular junction proteins, including E-cadherin, which disassembles adherens junctions and destabilizes the cell contacts. This facilitates invasion (translocation) of pathogens to trigger further inflammation [35, 37]. In addition, cleavage of E-cadherin during pathogenic microbial invasion is mediated by proteases (sheddases) derived from the pathogens themselves, which results in release of soluble E-cadherin, which can trigger inflammatory and pro-oncogenic programs [38]. Therefore, it is possible that TMEM52B may play a role in maintenance of intestinal epithelial homeostasis, and that TMEM52B-derived peptides may modulate/reduce intestinal inflammation by maintaining or promoting E-cadherin stability on epithelial cells. In our previous and present studies using cancer cells, TMEM52B suppression reduced the stability of E-cadherin, whereas TMEM52B-derived peptides inhibited production of soluble E-cadherin and stabilized intact E-cadherin on the plasma membrane. Therefore, it would be worth exploring whether TMEM52B and these peptides promote intestinal integrity and modulate inflammation by blocking cleavage of E-cadherin mediated directly or indirectly by pathogens in the gut. In preliminary experiments, we observed that TMEM52B is expressed in FHC normal colonic epithelial cells and CCD-18Co normal colon tissue cells (Figure S5a). The peptides did not reduce the viability of FHC and CCD-18Co cells, indicating that they lacked substantial toxicity toward normal intestinal cells (Figure S5b). Peptide treatment did not substantially increase the intact E-cadherin levels of FHC and CCD-18Co cells, in which the basal E-cadherin level was undetectable using immunoblot analysis, under 2D culture conditions (Figure S5c). However, further investigations using ex vivo intestinal organoids and in vivo mouse models are warranted.

Conclusions

In summary, based on our previous study that demonstrated that TMEM52B is a novel modulator of E-cadherin and EGFR activity, and that TMEM52B has tumor suppressor-like activity, we designed and evaluated the therapeutic potential of TMEM52B ECD-derived peptides in vitro and in vivo. Both peptides derived from the ECD of isoform 1 (more potently) and isoform 2 inhibited invasion, growth, and survival of colon cancer cells, which occurred concomitantly with a reduction in phosphorylation of EGFR, ERK1/2, and AKT. The peptides stabilized intact E-cadherin at cell–cell junctions, leading to reduced transcriptional activity of β-catenin. In addition, the peptides inhibited production of soluble E-cadherin and activation of EGFR by interfering with the interaction between soluble E-cadherin and EGFR. Fc-fused peptides efficiently suppressed tumor growth and early metastasis in colon cancer xenograft models. Thus, these peptides could serve as a platform for development of novel anti-cancer therapeutics, be a useful tool for investigating the function of TMEM52B in cancer and health, and help to explore the interplay between E-cadherin and EGFR.

Abbreviations

AP-1	Activating protein-1
CTC	Circulating tumor cell
ECD	Extracellular domain
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
ERK1/2	Extracellular signal-regulated kinase 1/2
GSEA	Gene set enrichment analysis
PTGER2	Prostaglandin E receptor 2
qPCR	Quantitative polymerase chain reaction
siRNA	Small interfering RNA
TCGA	The Cancer Genome Atlas

Supplementary Information

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Additional file 1.

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Author contributions

SK conceptualized and supervised the study. YL designed and performed the in vitro experiments, analyzed results, and prepared figures. DK and JY performed the in vivo experiments and analyzed results. SK and YL analyzed clinical datasets and wrote the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The data supporting the conclusions of this article are included within the article and its supplementary data files. The RNA sequencing data used and extended materials and methods in this study are available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

The animal study was performed in accordance with the guidelines of the Animal Care Committee at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), and with approval of the bioethics committee of the KRIBB (KRIBB-AEC-22107).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science. 2011;331(6024):1559–64.
2. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell. 2008;14(6):818–29.
3. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871–90.
4. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006;7(2):131–42.
5. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15(3):178–96.
6. Hu QP, Kuang JY, Yang QK, Bian XW, Yu SC. Beyond a tumor suppressor: soluble E-cadherin promotes the progression of cancer. Int J Cancer. 2016;138(12):2804–12.
7. David JM, Rajasekaran AK. Dishonorable discharge: the oncogenic roles of cleaved E-cadherin fragments. Cancer Res. 2012;72(12):2917–23.
8. Grabowska MM, Day ML. Soluble E-cadherin: more than a symptom of disease. Front Biosci (Landmark Ed). 2012;17:1948–64.
9. 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.
10. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, et al. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA. 2002;99(26):16899–903.
11. Xie J, Zhu C, Wu J, Li C, Luo L, Xia L, et al. Down-regulation of C12orf59 is associated with a poor prognosis and VHL mutations in renal cell carcinoma. Oncotarget. 2016;7(6):6824–34.
12. Lee Y, Ko D, Yoon J, Lee Y, Kim S. TMEM52B suppression promotes cancer cell survival and invasion through modulating E-cadherin stability and EGFR activity. J Exp Clin Cancer Res. 2021;40(1):58.
13. Lee Y, Ko D, Min HJ, Kim SB, Ahn HM, Lee Y, et al. TMPRSS4 induces invasion and proliferation of prostate cancer cells through induction of Slug and cyclin D1. Oncotarget. 2016;7(31):50315–32.
14. Ko D, Kim E, Shin EA, Nam SH, Yoon J, Lee JS, et al. Therapeutic effects of TM4SF5-targeting chimeric and humanized monoclonal antibodies in hepatocellular and colon cancer models. Mol Ther Oncolytics. 2022;24:452–66.
15. Bourcy M, Suarez-Carmona M, Lambert J, Francart ME, Schroeder H, Delierneux C, et al. Tissue factor induced by epithelial-mesenchymal transition triggers a procoagulant state that drives metastasis of circulating tumor cells. Cancer Res. 2016;76(14):4270–82.

16. Alcoser SY, Kimmel DJ, Borgel SD, Carter JP, Dougherty KM, Hollingshead MG. Real-time PCR-based assay to quantify the relative amount of human and mouse tissue present in tumor xenografts. BMC Biotechnol. 2011;11:124.
17. 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.
18. 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.
19. Pastwinska J, Karas K, Karwaciak I, Ratajewski M. Targeting EGFR in melanoma—the sea of possibilities to overcome drug resistance. Biochim Biophys Acta Rev Cancer. 2022;1877(4): 188754.
20. Rakosy Z, Vizkeleti L, Ecsedi S, Voko Z, Begany A, Barok M, et al. EGFR gene copy number alterations in primary cutaneous malignant melanomas are associated with poor prognosis. Int J Cancer. 2007;121(8):1729–37.
21. Strohl WR. Fusion proteins for half-life extension of biologics as a strategy to make biobetters. BioDrugs. 2015;29(4):215–39.
22. Xu C, Lin S, Lu Y, Mao L, Li S, Li Z. C12orf59 promotes esophageal squamous cell carcinoma progression via YAP-mediated epithelial-mesenchymal transition. Front Oncol. 2022;12: 927249.
23. Smith SC, Theodorescu D. Learning therapeutic lessons from metastasis suppressor proteins. Nat Rev Cancer. 2009;9(4):253–64.
24. Mukherjee AG, Wanjari UR, Gopalakrishnan AV, Bradu P, Biswas A, Ganesan R, et al. Evolving strategies and application of proteins and peptide therapeutics in cancer treatment. Biomed Pharmacother. 2023;163: 114832.
25. Xi G, Best B, Mania-Farnell B, James CD, Tomita T. Therapeutic potential for bone morphogenetic protein 4 in human malignant glioma. Neoplasia. 2017;19(4):261–70.
26. Asghar U, Hawkes E, Cunningham D. Predictive and prognostic biomarkers for targeted therapy in metastatic colorectal cancer. Clin Colorectal Cancer. 2010;9(5):274–81.
27. Bruner HC, Derksen PWB. Loss of E-Cadherin-Dependent Cell-Cell Adhesion and the Development and Progression of Cancer. Cold Spring Harb Perspect Biol. 2018;10(3): a029330.
28. Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ. E-cadherin's dark side: possible role in tumor progression. Biochim Biophys Acta. 2012;1826(1):23–31.
29. Padmanaban V, Krol I, Suhail Y, Szczerba BM, Aceto N, Bader JS, et al. E-cadherin is required for metastasis in multiple models of breast cancer. Nature. 2019;573(7774):439–44.
30. Lewis-Tuffin LJ, Rodriguez F, Giannini C, Scheithauer B, Necela BM, Sarkaria JN, et al. Misregulated E-cadherin expression associated with an aggressive brain tumor phenotype. PLoS ONE. 2010;5(10): e13665.
31. Tang MKS, Yue PYK, Ip PP, Huang RL, Lai HC, Cheung ANY, et al. Soluble E-cadherin promotes tumor angiogenesis and localizes to exosome surface. Nat Commun. 2018;9(1):2270.
32. 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.
33. Zhu S, Zhao G, Zhao X, Zhan X, Cai M, Geng C, et al. Elevated soluble E-cadherin during the epithelial-mesenchymal transition process and as a diagnostic marker in colorectal cancer. Gene. 2020;754: 144899.
34. Qian Y, Wu X, Yokoyama Y, Okuzaki D, Taguchi M, Hirose H, et al. E-cadherin-Fc chimera protein matrix enhances cancer stem-like properties and induces mesenchymal features in colon cancer cells. Cancer Sci. 2019;110(11):3520–32.
35. Schnoor M. E-cadherin is important for the maintenance of intestinal epithelial homeostasis under basal and inflammatory conditions. Dig Dis Sci. 2015;60(4):816–8.
36. Schneider MR, Dahlhoff M, Horst D, Hirschi B, Trulzsch K, Muller-Hocker J, et al. A key role for E-cadherin in intestinal homeostasis and Paneth cell maturation. PLoS ONE. 2010;5(12): e14325.
37. Citalan-Madrid AF, Garcia-Ponce A, Vargas-Robles H, Betanzos A, Schnoor M. Small GTPases of the Ras superfamily regulate intestinal epithelial homeostasis and barrier function via common and unique mechanisms. Tissue Barriers. 2013;1(5): e26938.
38. Devaux CA, Mezouar S, Mege JL. The E-cadherin cleavage associated to pathogenic bacteria infections can favor bacterial invasion and transmigration, dysregulation of the immune response and cancer induction in humans. Front Microbiol. 2019;10:2598.

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