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Presence of spontaneous epithelial-mesenchymal plasticity in esophageal cancer

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ABSTRACT

Epithelial-mesenchymal plasticity (EMP) refers to the reversible cellular transition between epithelial and mesenchymal status. Spontaneous EMP is also reported in breast and prostate cancer, leading to the acquisition of stem-cell properties and chemoresistance. However, the presence of spontaneous EMP is still not reported in esophageal cancer. We screened 11 esophageal squamous cancer cell (ESCC) cell lines by CD44 isoform expression. KYSE520 was found to comprise heterogenous populations consisting of $CD44v^+$ and $CD44v^-$ subpopulations. $CD44v^+$ and $CD44v^-$ cells showed the expression of epithelial and mesenchymal markers, respectively. Single-cell sorting of $CD44v^+$ and $CD44v^-$ cells revealed both cells gave rise to cell populations consisting of $CD44v^+$ and $CD44v^-$ cells, indicating $CD44v^+$ epithelial-like and $CD44v^-$ mesenchymal-like cells can generate counterparts, respectively. The ablation of Epithelial splicing regulatory protein 1 (ESRP1), a major regulator of CD44 mRNA splicing, resulted in the shift from CD44v+ to CD44v– cells in KYSE520. However, the expression of epithelial-mesenchymal transition (EMT)-related markers or transcriptional factors were almost not affected, suggesting ESRP1 functions downstream of EMP. Our results revealed the presence of spontaneous EMP in esophageal cancer and KYSE520 is useful model to understand spontaneous EMP.

1. Introduction

Esophageal cancer is the sixth leading cause of cancer-related mortality in the world [1]. It is classified histologically into squamous cell carcinoma (ESCC) and adenocarcinoma, with the incidence of these histological subtypes differing among geographic regions. ESCC is thus dominant in East Asia, eastern and southern Africa, and southern Europe [2].

Epithelial-mesenchymal transition (EMT) is an indispensable cellular program for embryogenesis and wound healing and has recently been shown to contribute to tumorigenesis by promoting tumor initiation, cell motility, and the resistance of cancer cells to chemotherapy [3,4]. Epithelial-mesenchymal plasticity (EMP) refers to a reversible process whereby cells alternate between EMT and mesenchymal-epithelial transition (MET) [5]. EMP is triggered by extrinsic factors and induced by the core EMT regulators. On the other hand, spontaneous EMP is also reported in breast and prostate cancer [6,7]. Spontaneous EMP leads to the acquisition of stem-cell properties and chemoresistance. However, the presence of spontaneous EMP is not well understood in esophageal cancer.

CD44 is thought to be associated with features of cancer stem cells such as interaction with stem-cell niche, the potential for cell migration and homing, the capacity for defense against reactive oxidative stress and resistance to cell death [8]. CD44 is a single-pass transmembrane

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protein that exists in numerous isoforms through alternative splicing of CD44 precursor mRNA [9]. Epithelial splicing regulatory protein 1 (ESRP1) and ESRP2 are a RNA binding proteins and play a key role in the inclusion of variant exons in mature forms of CD44 mRNA [10]. Whereas the standard isoform of CD44 (CD44s) is expressed predominantly in hematopoietic and mesenchymal cell types, CD44 variant (CD44v) isoforms, which contain additional sequences in the membrane-proximal extracellular region of the protein, are highly expressed in epithelial cell types [11]. Furthermore, CD44s, but not CD44v, has been implicated in the acquisition of mesenchymal properties, including enhanced extracellular matrix interaction and transforming growth factor-β signaling [8]. EMT has been shown to induce the alterative splicing of CD44 mRNA, resulting in a switch from CD44v to CD44s accompanied with the downregulation of ESRP1 [12,13]. However, the functional relevance of ESRP1 to EMP-associated CD44 variant isoform expression in esophageal cancer remains unclear.

EMT-related transcription factors (TFs) including Grainyhead-like–2 (GRHL2) have been shown to regulate the expression of ESRP1 [14]. On the other hand, forced expression of ESRP1 was found to attenuate the expression of EMT-related TFs including Zeb1 and Zeb2 and to up-regulate that of the epithelial marker E-cadherin in ovarian cancer cells [15]. The functional relevance of ESRP1 to EMT and CD44 isoform status in ESCC has been largely unknown, however.

We here investigated the expression of CD44 isoforms in 11 ESCC cell lines. KYSE520 comprised heterogenous populations consisting of $CD44v^+$ epithelial-like and $CD44v^-$ mesenchymal-like subpopulations. We examined the plasticity between $CD44v^+$ and $CD44v^-$ subpopulation. Further, we investigated the role of ESRP1 on CD44v expression and EMP in ESCC.

2. Materials and methods

2.1. Cell culture

KYSE520 cells (JCRB1439) were obtained from JCRB Cell Bank (Ibaraki-Osaka, Japan) [13]. TE1 (RBRC-RCB1894), TE4 (RBRC-RCB2097), TE5 (RBRC-RCB1949), TE6 (RBRC-RCB1950), TE8 (RBRC-RCB2098), TE9 (RBRC-RCB1988), TE10 (RBRC-RCB2099), TE11 (RBRC-RCB2100), TE14 (RBRC-RCB2101), and TE15 (RBRC-RCB1951) cell lines were obtained from RIKEN BRC Cell Bank (Tsukuba, Ibaraki, Japan). All cells were cultured under 5% CO_2 at 37 °C in RPMI-1640 medium (Sigma, Tokyo, Japan) supplemented with 10% fetal bovine serum.

2.2. Antibodies and reagents

Antibodies for flow cytometry included allophycocyanin-conjugated antibodies to human EpCAM (324207; Biolegend, Inc., San Diego, CA, USA), phycoerythrin-conjugated antibodies to human CD44 (103008, BioLegend), and antibodies to human CD44v9 [16]. Antibodies for immunoblot analysis included those to ESRP1 (HPA023719; Merck, Darmstadt, Germany), those to β-actin (sc-4778; Santa Cruz Biotechnology Inc. Dallas, TX, USA), and those to the cytoplasmic domain of human CD44 (CD44cyto) [17]. Recombinant human epidermal growth factor (EGF) and transforming growth factor–β (TGF-β) were obtained from PeproTech (Rocky Hill, NJ, USA).

2.3. Flow cytometric analysis

Cells were dissociated with the use of a cell dissociation buffer (Thermo Fisher Scientific, Tokyo, Japan), and the resulting single-cell suspensions were incubated with primary antibodies for 30 min at 4 °C. The cells were then washed with phosphate-buffered saline (PBS), and those that had been exposed to nonlabeled primary antibodies to CD44v9, pan-CD44 or EpCAM and were incubated with fluorescently labeled secondary antibodies for 20 min at 4 °C and then washed again with PBS. Apoptotic cells were excluded during flow cytometric analysis by elimination of cells positive for staining with propidium iodide. Flow cytometry was performed as previously described [18].

2.4. FACS sorting for establishment of single-cell clones and isolation of cell subpopulations

To obtain single cell–derived clones and cell subpopulations from the TE8 and KYSE520 cell lines, we stained the cells with antibodies to human EpCAM and to human CD44v and sorted them by fluorescence-activated cell sorting (FACS) with a FACSAria instrument (BD Biosciences). The resulting subpopulations or single cell–derived clones were cultured for >1 month to expand them for experiments.

2.5. Quantitative and semiquantitative RT-PCR analysis

Total RNA was extracted from cultured cells with the use of an RNeasy Mini Kit (Qiagen, Tokyo, Japan) and was subjected to reverse transcription (RT) with a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Tokyo, Japan). Semiquantitative polymerase chain reaction (PCR) analysis was performed with the use of Takara Taq DNA polymerase (Takara Bio, Shiga, Japan), and the PCR products were fractionated by agarose gel electrophoresis. Quantitative PCR analysis was performed with the use of a Thermal Cycler Dice Real Time System (Takara Bio). The amplification protocol comprised an initial incubation at 95 °C for 2 min and 40 cycles of incubation at 95 °C for 30 s and 60 °C for 30s, and was followed by dissociation-curve analysis to confirm specificity. Human primer sets for RT-PCR and semiquantitative RT-PCR were shown in Table S1. Data were normalized by the abundance of β-actin mRNA in RT-PCR.

2.6. Immunoblot analysis

Immunoblot analysis was performed as previously described [18]. Briefly, cells were washed with PBS and lysed in radioimmunoprecipitation (RIPA) buffer supplemented with phosphatase and protease inhibitors (PhosSTOP and cOmplete, mini, EDTA-free; Roche Diagnostics) and equal amounts of lysate protein were subjected to SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred to a PVDF membrane and then exposed to primary antibodies. Immune complexes were detected with horseradish peroxidase–conjugated secondary antibodies and Chemiluminescence Reagent Plus (PerkinElmer Japan). The protein bands were quantified by ImageJ software. β-actin was used as a loading control.

2.7. RNA interference

The sequences of a small interfering RNA (siRNA) (chimeric RNA-DNA duplex; Japan Bioservice, Saitama, Japan) were 5′-CACAAUGACAGAGUAUUUAAATT-3′ and 5′- UUUAAAUACUCUGUCAUUGUGTT-3′ (ESRP1), 5′-AGAUGAUGAAUGCGAGUCGTT-3′ and, 5′-UGAUCAGCCUCAAUCUGCATT-3′ (ZEB1), and 5′-CGUACGCGGAAUACUUCGATT-3′ and 5′-UCGAAGUAUUCCGCGUACGTT-3′ (Control). Cells were transfected with the annealed ESRP1 or control siRNAs for 48–72 h with the use of Lipofectamine RNAiMAX (Thermo Fisher Scientific). Vectors encoding control or ESRP1 short hairpin RNAs (shRNAs) were obtained from Horizon Discovery (Tokyo, Japan). Vectors were transfected into Lenti-X 293T (Takara Bio) packaging cells using FugeneHD (Roche Diagnostics). Viral supernatants were used for infection of target cells and selection of infected target cells was performed by puromycin (Nacalai).

2.8. Statistical analysis

Data are presented as means ± SD and were analyzed with the unpaired Student's *t*-test. A P value of <0.05 was considered statistically significant.

3. Results

3.1. CD44v isoform expression and epithelial-mesenchymal status in human ESCC cells

To study EMP in esophageal cancer, we first investigated the cell surface expression of CD44v isoforms in 11 human ESCC cell lines. Flow cytometry revealed that all of the ESCC cell lines were positive for pan-CD44 (Fig. 1A), whereas KYSE520 and TE8 consisted of both CD44v$^+$ (which predominantly expressed CD44v) and CD44v$^-$ (which expressed only CD44s) subpopulations. To investigate the characteristics of these CD44v$^+$ and CD44v$^-$ cells, we sorted them from KYSE520 and TE8 cells

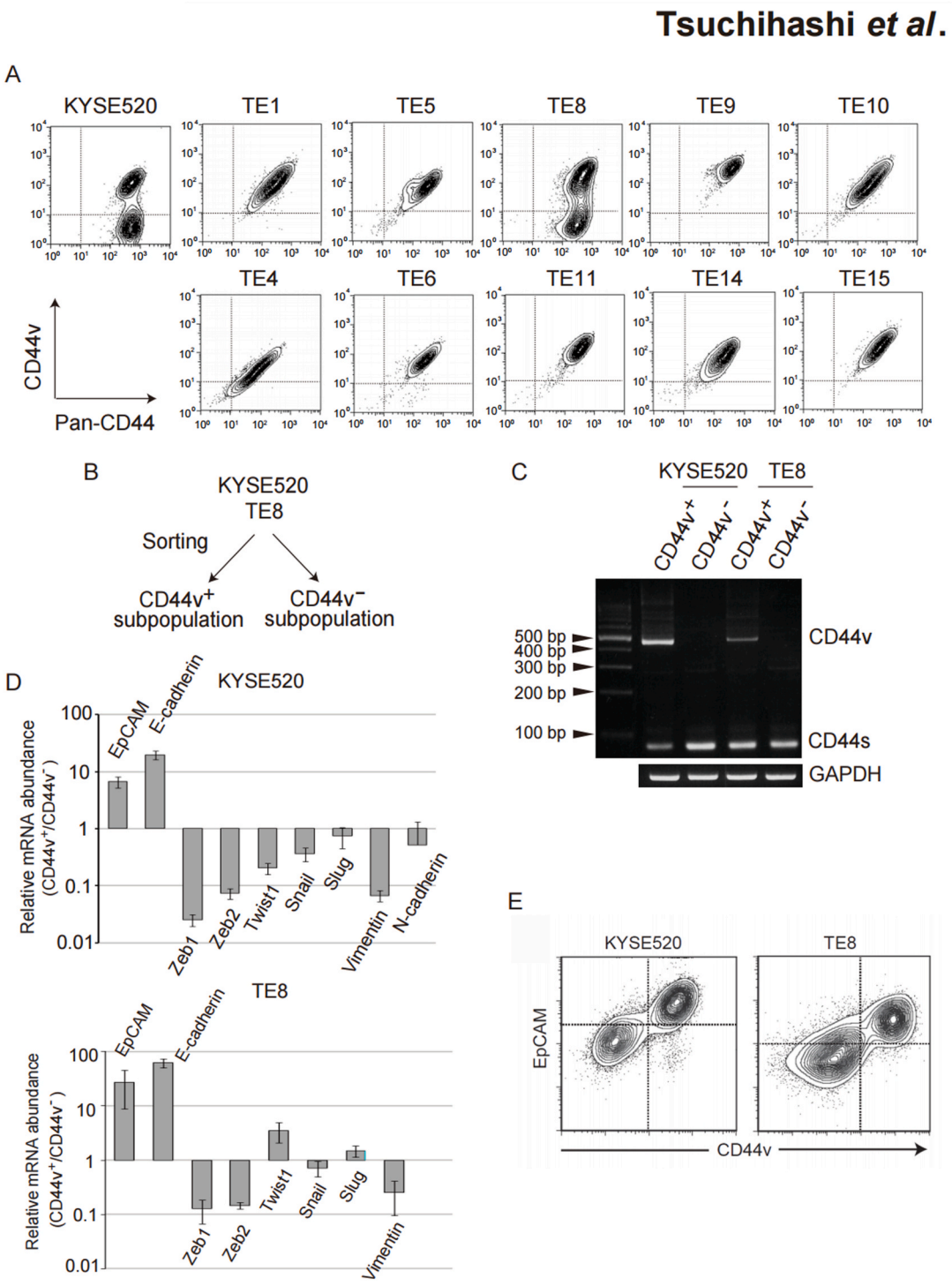

Fig. 1. KYSE520 and TE8 cell lines are heterogeneous and comprise CD44v$^+$ epithelial-like and CD44v$^-$ mesenchymal-like subpopulations. (A) Flow cytometric analysis of CD44v and pan-CD44 expression in 11 human ESCC cell lines. (B) Experimental protocol for sorting of KYSE520 and TE8 cells into CD44v$^+$ and CD44v$^-$ subpopulations by FACS using the monoclonal antibody to CD44v9. (C) Semiquantitative RT-PCR analysis of CD44v and CD44s isoform mRNAs in CD44v$^+$ and CD44v$^-$ subpopulations of KYSE520 and TE8 cells. GAPDH mRNA was examined as an internal control. The leftmost lane contains molecular size markers. (D) Quantitative RT-PCR analysis of mRNA abundance for EMT markers and TFs in CD44v$^+$ versus CD44v$^-$ subpopulations of KYSE520 and TE8 cells. Data are means ± SD from four independent experiments. (E) Flow cytometric analysis of the expression of CD44v and the epithelial cell marker EpCAM in KYSE520 and TE8 cells.

by FACS (Fig. 1B). Variant-specific semiquantitative RT-PCR analysis [19] showed that, for both KYSE520 and TE8 cell lines, the FACS-sorted CD44v$^+$ cells harbored both CD44v and CD44s mRNAs, whereas the CD44v$^-$ cells contained only CD44s mRNA (Fig. 1C). We next compared the expression of EMT-related markers and TFs between CD44v$^+$ cells and CD44v$^-$ cells. Quantitative RT-PCR analysis revealed that the abundance of mRNAs for epithelial markers including epithelial cell adhesion molecule (EpCAM) and E-cadherin was higher in CD44v$^+$ cells of both KYSE520 and TE8 cell lines, whereas that of mRNAs for mesenchymal markers and TFs such as Zeb1, Zeb2, Twist1, Snail, Slug, vimentin, and N-cadherin tended to be higher in CD44v$^-$ cells (Fig. 1D), with this pattern being more consistent in KYSE520 cells than in TE8

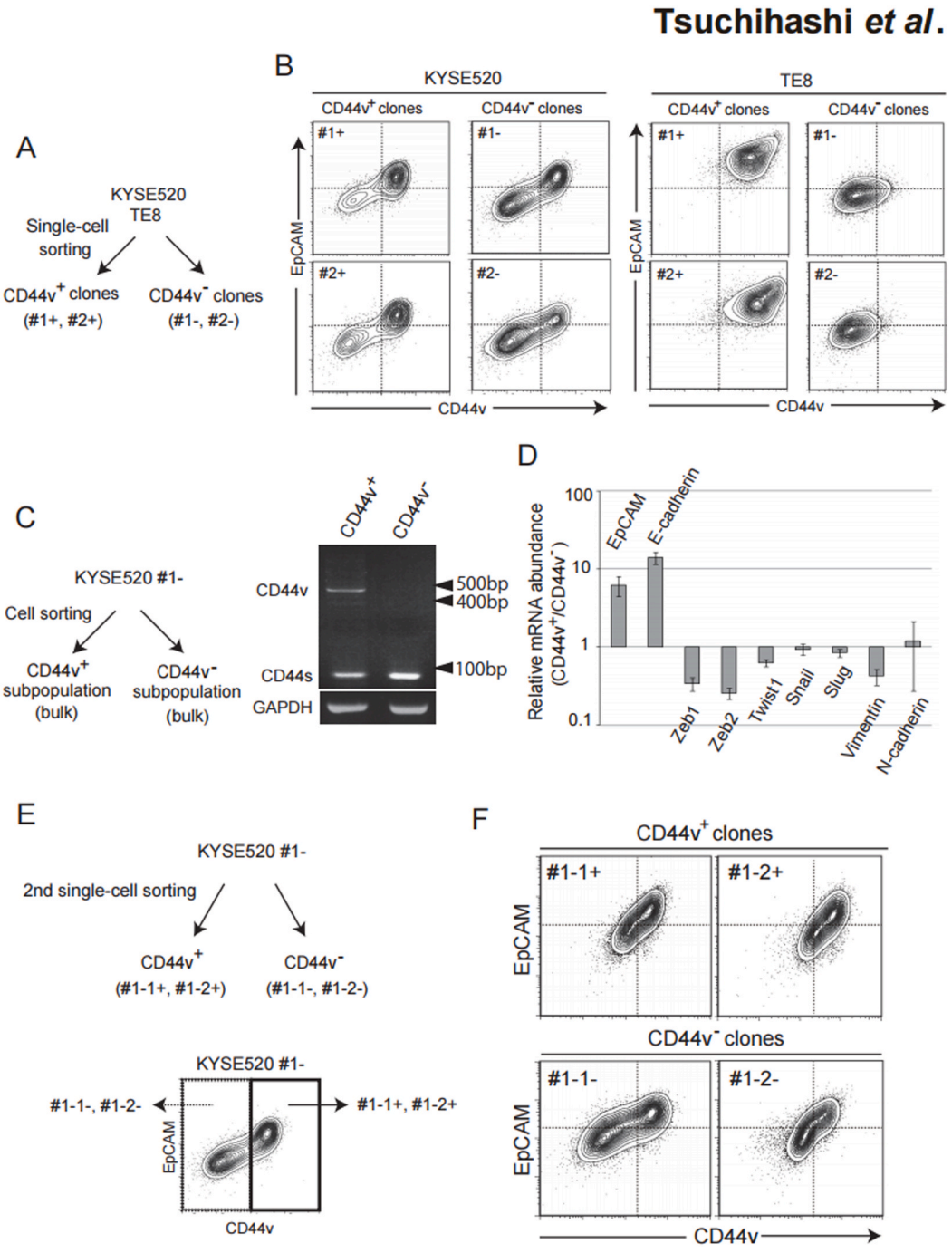

Fig. 2. Individual KYSE520 cells, but not TE8 cells, possess the ability to reconstitute a heterogeneous cell population consisting of CD44v$^+$ and CD44v$^-$ cells. (A) Experimental protocol for single-cell sorting using the monoclonal antibody to CD44v9. and expansion of CD44v$^+$ (#1+, #2+) and CD44v$^-$ (#1-, #2-) clones from KYSE520 and TE8 cell lines. (B) Flow cytometric analysis of EpCAM and CD44v expression in cell populations derived from single CD44v$^+$ (#1+, #2+) or CD44v$^-$ (#1-, #2-) KYSE520 and TE8 cells. (C) Experimental protocol for the sorting of CD44v$^+$ and CD44v$^-$ subpopulations derived from the #1- clone of KYSE520 cells using the monoclonal antibody to CD44v9 is shown on the left. Semiquantitative RT-PCR analysis of CD44 isoform mRNAs in these subpopulations is shown on the right. (D) Quantitative RT-PCR analysis of EMT-related markers and TFs in the CD44v$^+$ subpopulation relative to the CD44v$^-$ subpopulation isolated as in (C). Data are means ± SD from four independent experiments. (E) Experimental protocol for a second single-cell sorting and expansion of CD44v$^+$ (#1-1+, #1–2+) or CD44v$^-$ (#1-1-, #1-2-) clones derived from KYSE520 #1- cells. (F) Flow cytometric analysis of EpCAM and CD44v in the secondary CD44v$^+$ (#1-1+, #1–2+) or CD44v$^-$ (#1-1-, #1-2-) clones in (E).

cells. Flow cytometric analysis of both KYSE520 and TE8 cell lines also showed that CD44v$^+$ cells, but not CD44v$^-$ cells, expressed the epithelial cell marker EpCAM at the cell surface (Fig. 1E). These results suggested that KYSE520 and TE8 are heterogenous ESCC cell lines consisting of CD44v$^+$ cells that possess epithelial-like characteristics and CD44v$^-$ cells that manifest mesenchymal-like characteristics.

3.2. *Differential frequency of plasticity between epithelial-like CD44v$^+$ and mesenchymal-like CD44v$^-$ subpopulations of ESCC cells*

To study the dynamics of plasticity between CD44v$^+$ and CD44v$^-$ subpopulations, we next performed single-cell sorting of KYSE520 and TE8 cells with antibodies to CD44v (Fig. 2A). Single CD44v$^+$ and CD44v$^-$ cells of the KYSE520 cell line gave rise to heterogeneous cell populations consisting of epithelial-like CD44v$^+$ cells and mesenchymal-like CD44v$^-$ cells. In contrast, single CD44v$^+$ and CD44v$^-$ TE8 cells maintained their CD44v expression status after cell expansion (Fig. 2B). These results thus suggested that phenomenon of spontaneous EMP is dependent on kinds of cell lines. To investigate further the epithelial-mesenchymal status of CD44v$^+$ and CD44v$^-$ KYSE520 cells, we sorted cells derived from the CD44v$^-$ clone #1- into CD44v$^+$ and CD44v$^-$ subpopulations (Fig. 2C). Quantitative RT-PCR analysis revealed that the abundance of mRNAs for EpCAM and E-cadherin was higher in the CD44v$^+$ cells than in the CD44v$^-$ cells, whereas that of mRNAs for Zeb1, Zeb2, Twist1, Snail, Slug, and vimentin was higher in the CD44$^-$ cells (Fig. 2D). These results further suggested that CD44v isoform status is associated with epithelial-mesenchymal status in KYSE520 cells.

To examine further whether KYSE520 cells manifest EMP at the single-cell level, we performed single-cell sorting of cells from CD44v$^-$ clone #1- and thereby obtained cell clones derived from individual CD44v$^+$ cells (#1-1+, #1–2+) or from individual CD44v$^-$ cells (#1-1-, #1-2-) (Fig. 2E). Expansion of each of these four cell clones gave rise to heterogeneous populations consisting of both CD44v$^+$ and CD44v$^-$ cells (Fig. 2F). These data showed that KYSE520 cells possess the ability to shift their cellular status between a CD44v$^+$ (epithelial-like) and CD44v$^-$ (mesenchymal-like) phenotype in a cell-autonomous manner.

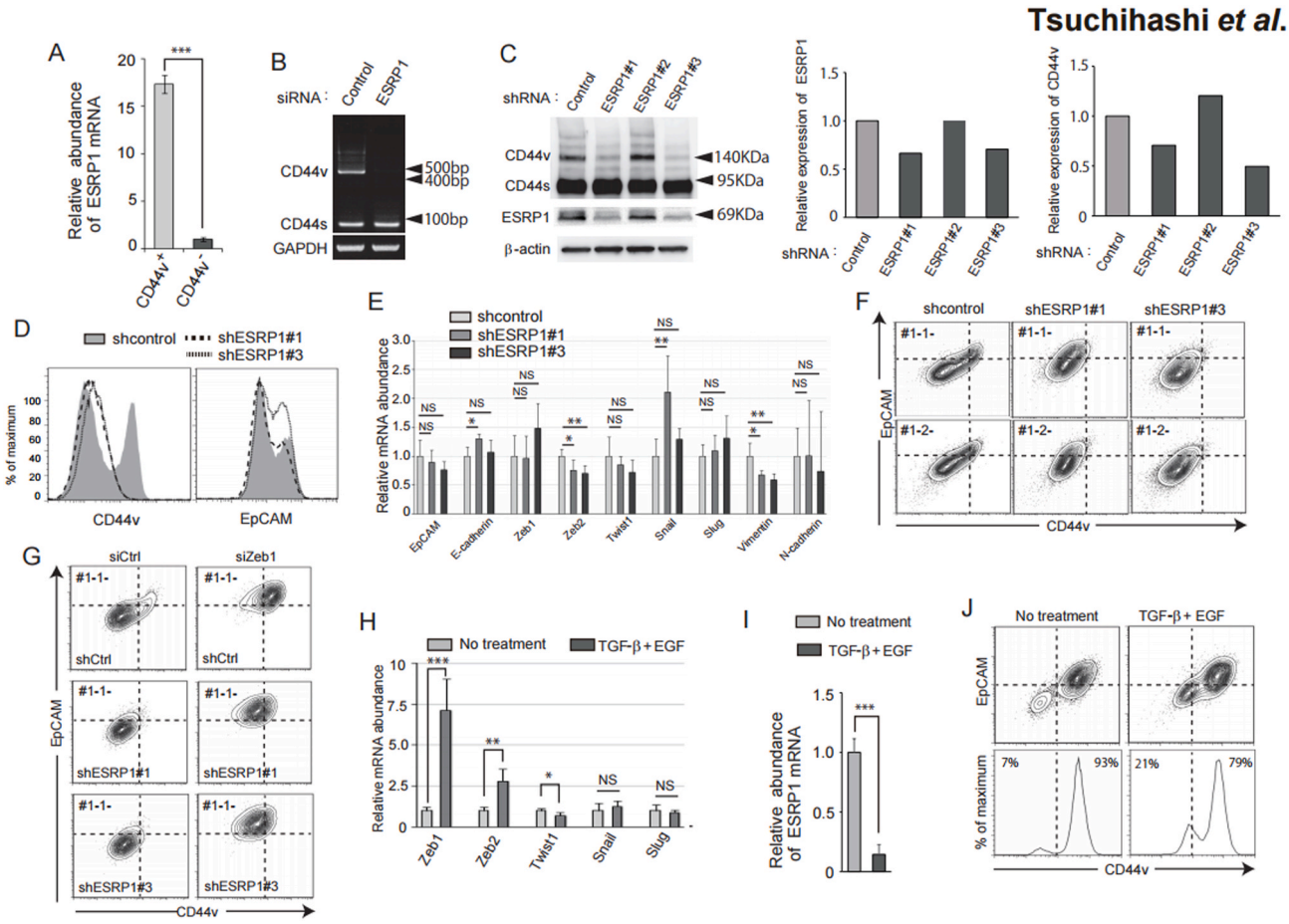

Fig. 3. ESRP1 regulates CD44 pre-mRNA splicing without affecting EMT status. (A) Quantitative RT-PCR analysis of ESRP1 mRNA abundance in CD44v$^+$ and CD44v$^-$ subpopulations of KYSE520 cells. Data are expressed relative to the corresponding normalized value for CD44v$^-$ cells and are means ± SD from four independent experiments. ***$P < 0.001$ (Student's *t*-test). (B) Semiquantitative RT-PCR analysis for CD44 isoform mRNAs in CD44v$^+$ KYSE520 cells transfected with control or ESRP1 siRNAs. (C) Immunoblot analysis of CD44 and ESRP1 in KYSE520 clone #1- cells stably transfected with vectors for Control or ESRP1 (#1, # 2, #3) shRNAs. ESRP1 shRNA#2 failed to suppress ESRP1 expression. β-actin was examined as a loading control. (D) Flow cytometric analysis of CD44v and EpCAM expression in KYSE520 clone #1- cells stably expressing ESRP1 (#1, #3) or control shRNAs. (E) Quantitative RT-PCR analysis of EMT-related marker and TF mRNA abundance in KYSE520 clone #1- cells stably expressing ESRP1 (#1, #3) or control shRNAs. Data are expressed relative to the corresponding normalized value for cells expressing the control shRNA and are means + SD from four independent experiments. NS (not significant), *$P < 0.05$, **$P < 0.01$ (Student's *t*-test). (F) Flow cytometric analysis of CD44v and EpCAM expression in KYSE520 clones (#1-1- and #1-2-) stably expressing ESRP1 (#1, #3) or control shRNAs. (G) Flow cytometric analysis of KYSE520 clone (#1-1-) stably expressing ESRP1 (#1, #3) or control shRNAs knocked down by control or Zeb1 siRNA. (H) Quantitative RT-PCR analysis of EMT-related marker and TF mRNA abundance in KYSE520 cells incubated with or without TGF-β (10 ng/ml) and EGF (25 ng/ml) for 72 h. Data are expressed relative to the corresponding normalized value for nontreated cells and are means + SD from four independent experiments. NS, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's *t*-test). (I) Quantitative RT-PCR analysis of ESRP1 expression in KYSE520 cells treated as in (H). (J) Flow cytometric analysis of CD44v and EpCAM expression in KYSE520 cells treated as in (G).

3.3. Spontaneous EMP and ESRP1-regulated CD44v expression in ESCC

ESRP1 is an RNA binding protein that regulates alternative splicing of CD44 pre-mRNA. Semiquantitative RT-PCR analysis showed that the abundance of ESRP1 mRNA was markedly greater in CD44v$^+$ KYSE520 cells than in their CD44v$^-$ counterparts (Fig. 3A). To examine the functional relevance of ESRP1 to CD44v expression status in ESCC cells, we first depleted ESRP1 mRNA by RNA interference with a specific siRNA. Knockdown of ESRP1 in CD44v$^+$ KYSE520 cells resulted in a decrease in the amount of CD44v mRNA (Fig. 3B), confirming a role for ESRP1 in the alternative splicing of CD44 pre-mRNA in this cell line. To study EMP in ESRP1-depleted cells, we generated CD44v$^+$ KYSE520 clone #1- cells that stably express shRNAs for ESRP1 (Fig. 3C and D). Quantitative RT-PCR analysis revealed that stable ESRP1 ablation had only small effects on the expression of EMT markers and TFs at the mRNA level (Fig. 3E), suggesting that ESRP1 is not an upstream regulator of the EMT program. On the other hand, depletion of ESRP1 in CD44v$^-$ clones derived from single KYSE520 cells prevented the generation of CD44v$^+$ cells (Fig. 3F). We next performed the ablation of the EMT-related TFs Zeb1 to study the impact of MET on the generation of CD44v+ subpopulation from CD44v– clones. Knockdown of Zeb1 in the control shRNA-expressing CD44v$^-$ cells were able to increase the EpCAM+/CD44v+ subpopulation (Fig. 3G), implying that the ablation of Zeb1 promoted MET in KYSE520 cells. On the other hand, the expression of CD44v in the EpCAM+ population generated from CD44v$^-$ clone was markedly suppressed in the ESRP1 shRNA-expressing CD44v$^-$ cells. Taken together, these results suggested that the ESRP1-regulated CD44 mRNA splicing occurs the downstream of cell-autonomous EMP in KYSE520 cells.

Given that EGF and TGF-β have been shown to induce EMT in a synergistic manner [20,21], we examined whether these factors might affect CD44 expression status in ESCC cells. Combined treatment with TGF-β and EGF resulted in the up-regulation of mRNAs for the EMT-associated TFs Zeb1 and Zeb2 (Fig. 3H), whereas it reduced the expression of ESRP1 and CD44v$^+$ subpopulation (Fig. 3I and J) in KYSE520 cells. These results suggested that EMP and its effect on CD44 isoform expression in ESCC cells might also be regulated by the EMT-inducing external stimulation including that with EGF and TGF-β.

4. Discussion

EMP is a feature of cancer cells that allows them to rapidly adapt to a changing tumor microenvironment during tumor progression [5,22]. On the other hand, spontaneous EMP is observed in breast and prostate cancer [6,7]. Spontaneous EMP also contributes to the acquisition of malignant properties of cancer cells. However, the presence of spontaneous EMP in ESCC is still not well understood. We report here the presence of spontaneous EMP in ESCC. KYSE520 is a useful model of spontaneous EMP.

CD44 exists in various isoforms generated through alternative splicing of CD44 mRNA. In the present study, KYSE520 and TE8 were heterogeneous in that they were composed of both CD44v$^+$ and CD44v$^-$ subpopulations. We previously reported murine breast cancer cell line 4T1 also comprises heterogenous CD44v$^+$ and CD44v$^-$ subpopulations [23]. CD44v$^+$ and CD44v$^-$ cells in KYSE520 manifested high expression of epithelial marker genes (EpCAM and E-cadherin) and mesenchymal marker genes (Zeb1, Zeb2, Twist1, Snail Slug, vimentin, and N-cadherin), respectively. This relationship between CD44 isoform expression and epithelial-mesenchymal status in ESCC is same as in other cancer [8,24]. Flow cytometric analysis of single cell–derived clones of CD44v$^+$ and CD44v$^-$ cells revealed that KYSE520 cells possess spontaneous plasticity between CD44v$^+$ epithelial-like and CD44v$^-$ mesenchymal-like cells. In contrast, TE8 cells did not show apparent plasticity. Given that not only cellular plasticity, but also expansion of different clones has been previously reported to impact the EMT status in breast and prostate cancer [6,7], there might be a possibility that CD44 variant expression status in TE-8 cell line might be regulated by the differential clone of CD44v+ and CD44v-cells rather than cellular plasticity. These observations together indicate that CD44v$^+$ epithelial-like and CD44v$^-$ mesenchymal-like cells in KYSE520 have spontaneous plasticity each other.

ESRP1 regulates alternative splicing of CD44 mRNA and its expression leads to inclusion of CD44 variant exons, shifting CD44 standard to CD44 variant isoform. However, the role of ESRP1 on CD44 isoform expression is still not investigated in esophageal cancer. Our result showed that the inhibition of ESRP1 expression shifted CD44v$^+$ to CD44v$^-$, indicating that ESRP1 plays a role in CD44 isoform expression in esophageal cancer. Further, ESRP1 has been shown to suppress the expression of EMT-related TFs in ovarian cancer cells, implicating ESRP1 in MET induction [15]. On the other hand, in our ESCC model, ESRP1 depletion did not affect the expression of EMT-related markers or TFs. Furthermore, treatment with the EMT-inducing growth factors EGF and TGF-β induced a splicing shift from CD44v+ to CD44v– in KYSE520 ESCC cells. Together, these findings suggest that regulation of alternative splicing of CD44 pre-mRNA by ESRP1 might occur downstream of EMT induction in ESCC cells.

Our data indicate that EMP associated with a shift in CD44v status in KYSE520 cells is not only regulated in a cell-autonomous manner but also promoted by growth factors in the ESCC tumor microenvironment. KYSE520 cells might thus be a useful to study the mechanisms underlying EMP.

Declaration of competing interest

The authors declare no conflict of interest related to this work.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2022.101246.

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