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RESEARCH ARTICLE

MECHANOSENSITIVE TRPV4 TRAFFICKING DRIVES TGF-B-MEDIATED MESENCHYMAL TRANSITION IN COLORECTAL CANCER

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ABSTRACT

Background: Epithelial-to-mesenchymal transition (EMT) enhances the invasive potential of cancers, significantly affecting survival rates in metastatic disease. TGF-β, a potent EMT regulator enriched in colon cancer (CRC), is influenced by bioelectric and biophysical forces. While some ion channels and mechanical forces are linked, TGF-β-coupled mechanosensing mechanisms in CRC remain poorly understood. This study investigates the mechanosensitive ion channel TRPV4 and its role in TGF-β-induced EMT, focusing on channel trafficking and its functional implications in CRC.
Methods: We analyzed mechanosensitive ion channels mRNA expressions in CRC stages and evaluated their association with survival through Kaplan-Meier analysis. Correlations were analyzed with mesenchymal gene sets, soluble factors, and TGF-β signaling. Immunofluorescence was used to visualize TRPV4 localization in untreated and 10 ng/mL TGF-β1-treated colon cell lines. Functional studies involved co-stimulation with TGF-β1 and TRPV4 modulators (GSK101 and HC-067047) to assess EMT-related changes.
Results: TRPV4 mRNA is elevated in CRC, with TRPV4-001 as the predominant isoform. High expression correlated with poor survival, EMT signatures, and TGF-β1 signaling. TGF-β1 induced out-of-nucleus TRPV4 translocation. TRPV4 inhibition reduced TGF-β-induced N-cadherin expression, mitigating EMT.
Conclusion: TRPV4 regulates TGF-β-induced EMT through trafficking mechanisms. Its inhibition presents anti-metastatic potential, identifying TRPV4 as a therapeutic target in CRC.

Keywords: TRPV4, TGF-β, EMT, Translocation, Ion channel, Mechanosensitive

INTRODUCTION

Colorectal cancer is the most common cancer in men and the second most common in women under the age of 50. Furthermore, its diagnosis in younger populations has been steadily increasing since 1991 (1). While the five-year survival rates for localized and regional stages are relatively high (91.3% and 71.7%, respectively), survival drops sharply to 15.7% in advanced cases with distant metastases (SEER). Epithelial-to-mesenchymal transition (EMT) is a well-known process that enhances the migratory and invasive capabilities of these malignancies (2). Moreover, the mesenchymal phenotype is associated with drug resistance and disease relapse (3,4). Thus, controlling the mesenchymal phenotype of tumors remains a critical objective in cancer treatment.

TGF-β is a well-known cytokine closely linked to metastatic colon cancer. This infamous cytokine promotes mesenchymal phenotype and increases cancer cell stemness. TGF-β receptor inhibitors and antibodies that neutralize soluble TGF-β ligands, offering potential combination therapies to overcome

metastatic disease and drug resistance (5–7). The TGF-β response is related to multiple signaling pathways, including TGF-β/SMAD, WNT/β-catenin, Notch, and receptor tyrosine kinase, can be induced by physical stimuli from the tumor microenvironment. Transcriptional factors such as snail, slug, ERK, and STAT3 are activated in response to the downstream, and signal for degradation of epithelial markers whereas increase the synthesis of mesenchymal markers such like N-cadherin and vimentin (8).

Communication through bioelectric signaling among cells is essential and widely studied for developmental biology. Tumors exhibit a higher resting membrane potential than healthy cells, ranging from −30 to −20 mV, similar to proliferative cells (9). Studies in the last decade indicate that altered bioelectricity is also very important for cancer progression and metastasis (10). Also, gap junctional coupling results in a multi-cellular network in bioelectrical state which is related to tissue level invasive behavior (11). Oncogene expression depolarizes cells that form tumor-like structures, but is unable to form tumors if this depolarization is artificially prevented by misexpression of hyperpolarizing ion channels (12). Recent studies investigate dysfunction of ion channelopathies and their role as both oncogenes and tumor suppressors (13,14).

Ca^{2+} influx acts as a modulator of TGF-β signaling affecting cellular plasticity and metastasis (15–17). Dysregulation of calcium and potassium channels enhance the invasive potential of cancer cells and may contribute to therapeutic resistance (18–20). TGF-β signaling also regulates ion channel expression and translocation in some diseases however the influence on translocation of ion channels in cancer is poorly understood (21,22).

TGF-β signaling is regulated by mechanical forces, such as shear stress and substrate stiffness (23–25). For example, cells sense the stiffness of their extracellular matrix, with changes in substrate elasticity influencing TGF-β signaling dynamics, such as during the fibroblast-to-myofibroblast transition (26). Moreover, physiologic range shear stress also induces endothelial generation of biologically active TGF-β1 coupled with K+ channel currents (27).

As tumor mass increases and tumor microenvironment undergoes alterations during cancer progression, the mechanical forces within the tissue become imbalanced, creating a high-pressure environment (28–30). Recent studies have shown that elevated

pressure in the cancer microenvironment impacts tumorigenesis, cancer progression, drug resistance, and metastasis (31–33). Mechanosensitive ion channels, which act as receptors sensing pathophysiological pressure, respond to mechanical forces such as tension, compression, and shear stress. These responses promote critical processes such as cell division, survival, invasion, and migration (30,34). Notably, PIEZO and TRPV4 channels are linked to increased cell proliferation and the acquisition of mesenchymal traits in various cancers, including colon cancer (35–37).

While studies have highlighted the importance of bioelectric signaling and physical forces in modulating TGF-β signaling, this process remains poorly understood. Furthermore, although TGF-β is known to regulate ion channels in various diseases, the mechanistic significance of these processes in colon cancer progression is unclear. This study establishes a mechanistic link between mechanosensitive ion channels and TGF-β-induced EMT in colon cancer, focusing on TRPV4 trafficking and function. Through a combination of transcriptomic analysis, functional assays, and immunofluorescence-based localization studies, we identify TRPV4 as a key mediator in TGF-β signaling. Additionally, we explore how TGF-β-induced TRPV4 translocation contributes to mesenchymal transition, providing new insights into the biophysical regulation of TRPV4 and its therapeutic potential in metastatic colon cancer.

MATERIALS AND METHODS

Dataset Selection and Normalization

The TCGA-COAD (The Cancer Genome Atlas-Colorectal Adenocarcinoma) RNA sequencing data were used as the primary patient cohort. Samples classified as ‘Normal’, ‘Primary Solid Tumor’, and cancer stages (stages 1–4) were selected from the available patient samples. Duplicate samples with the same patient ID were excluded. The data were downloaded using the TCGAbiolinks package. The downloaded data were prepared for analysis using the TCGAanalyze_Preprocessing function. A symmetric Spearman correlation matrix was computed (cor.cut=0.6) to identify low-correlation outlier samples among the dataset. Normalization of the counts within and across groups was performed using the TCGAanalyze_normalization function with the default parameters of the EDASeq R package.

Differential Gene Experission Analysis

Genes of mechanosensitive ion channels expressed and their isoforms in homo sapiens were retrieved based on the gene ontology (GO) molecular function term "mechanosensitive ion channel activity" [GO:8381]. The data used in this study were obtained from the colorectal cancer dataset of The Cancer Genome Atlas (TCGA) via the Genomics Data Commons (GDC) cancer data portal, which provides open access. Differential gene expression analyses were performed using the edgeR method. Genes with statistically significant expression changes were identified. An adjusted p-value of <0.05 was considered significant, and a fold-change threshold of |log2FC| ≥ 1 was applied. Significant expression changes were calculated while comparing gene expression levels between the selected groups.

Geneset Scoring and Correlation

Geneset scoring and correlation analyses were performed using a non-logarithmic scale for calculation, while a log-scale axis was used for visualization. The Pearson correlation between TRPV4 expression and various biomarkers, soluble factors, or geneset signature scores was calculated (supplemantary table 1). The mean value of log2(TPM + 1) was used as the signature score for each geneset. The following genesets were included in the analysis: epithelial-mesenchymal transition (EMT) genesets (HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION, KOHN_EMT_MESENCHYMAL, KOHN_EMT_EPITHELIAL) and the TGF-B signaling geneset from MSigDB (HALLMARK_TGF_BETA_SIGNALING) (38). GEPIA2 webtool were used for the analysis and visualization (39).

Survival Analysis

Kaplan-Meier analysis was used to study how TRPV4 gene expression affects survival in the microarray data sets collected and normalized by Balázs Győrffy . Both overall survival (OS) and disease-free survival (DFS) were analyzed. Patients were divided into low and high TRPV4 expression groups based on the median expression level. Differences between the groups were tested using the log-rank test (Mantel-Cox test), and exact p-values were provided. Hazard ratios (HR) were calculated using the Cox Proportional Hazards (Cox PH) model. Kaplan-Meier curves were created, with different colors showing low and high TRPV4 expression groups, and p-values were included on the plots (40).

Cell Culture

Colorectal cancer cell lines, HCT-116 (ATCC® CCL-247), HT-29 (ATCC® HTB-38), and LOVO (ATCC® CCL-229), were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS). The cells were maintained in a humidified incubator at 37°C with 5% CO_2. The culture medium was replaced every 2-3 days, and cells were passaged when they reached approximately 70-80% confluence.

Immunofluorescence Staining

EMT markers N-cadherin and E-cadherin, and TRPV4 channel were stained. Cells were fixed with 4% paraformaldehyde for 20 minutes at room temperature. After fixation, cells were permeabilized with 0.3% Triton X-100 in PBS and incubated overnight at 4°C with primary antibodies: anti-mouse monoclonal anti-E-cadherin (Thermofisher Sc., USA) and anti-rabbit monoclonal anti-N-cadherin (Thermofisher Sc., USA) (dilution according to manufacturer's recommendation) for EMT, and anti-rabbit polyclonal anti-TRPV4 antibody (Thermofisher Sc., USA) for TRPV4 expression. Following incubation, cells were washed twice with PBS. Secondary antibodies Alexa Fluor-488 goat anti-mouse (Thermofisher Sc., USA) and Alexa Fluor-568 goat anti-rabbit (Thermofisher Sc., USA) were applied for 2 hours at room temperature. DNA was stained with DAPI for 5 minutes. After washing, cells were visualized using a fluorescence microscope (Zeiss, AxioVert.A1, Germany) with appropriate wavelengths (Alexa Fluor-488 488 nm, Alexa Fluor-568 568 nm).

TGF-β Treatment and Translocation Scoring

To investigate the effect of TRPV4 on the TGF-β-induced EMT process, HCT-116 were seeded at a density of 10,000 cells per 100 µl in a 96-well plate. The cells were incubated with 10 ng/ml TGF-β (R&D, USA) for 18 hours. Following the incubation, five images per well were captured, and the experiments were repeated in triplicates. The following groups were analyzed using immunofluorescence staining: TRPV4 control group, EMT control group, TGF-β and GSK101 (SelleckChem, USA) co-stimulation group (EMT group), TGF-β and HC-067047 (SelleckChem, USA) treatment group (EMT group), and TGF-β-only

treatment group (TRPV4 & EMT group). After immunofluorescence staining, fluorescence intensity was measured for both nuclear and non-nuclear regions. The nuclear region was identified based on the DAPI signal.

Statistical Analysis

The data generated from in vitro experiments were analyzed using IBM SPSS 24.0 software. The measurements were expressed as standard deviation, median, and minimum-maximum values. For comparing the measurement data, if at least 30 replicates per variable were collected in the experimental groups, the normality of the data was assessed using the Kolmogorov-Smirnov test. For experimental groups with fewer than 30 replicates per variable, non-parametric methods were used without testing for normality. In this case, the Kruskal-Wallis test was used to assess significant differences between multiple groups, and the Mann-Whitney U test was used to evaluate differences between two groups. A p-value of <0.05 was considered statistically significant.

RESULTS

TRPV4 have higher expression level in colorectal cancer patients beginning with early stages

The transcriptomic data of colorectal cancer patients were compared with healthy tissue. Significant changes in the expression of seven ion channels among the 17 analyzed were observed. Notably, the expression of CSCL1 (0.97-fold), FAM155B (1.44-fold), and TRPV4 (1.42-fold) was significantly upregulated, while PIEZO2 (-1.18-fold), TMC4 (-1.17-fold), CSCL2 (-1.06-fold), and TMC8 (-0.5-fold) showed significant downregulation. Furthermore, TMC7, FAM155A, and TRPV4 expressions increase with fold changes of 0.59, 0.7, and 0.8, respectively, in stage IV (metastatic disease primer region) compared to stage II (not spread to any lymph nodes yet) (Figure 1a). Considering mechanisms influencing the metastatic characteristics of cancer are expressed from the onset of carcinogenesis, the ion channels TMC7, CSCL1, FAM155B, FAM155A, and TRPV4 might play a role in supporting colorectal cancer progression and metastasis. Many of these upregulated ion channels are associated with upregulating calcium signaling, a secondary messenger pathway linked to metastatic malignancies and TGF-β signaling.

TRPV4 mRNA expression was found to be elevated in colorectal cancer compared to healthy tissue. TRPV4 has six isoforms, and identifying the isoforms specific to colorectal cancer is crucial for predicting functionality and ensuring selectivity in drug development. RNA sequencing data revealed that the TRPV4-001 isoform (ENST00000261740.6) predominates over the other five isoforms in colorectal cancer (Figure 1b).

Given the principles of cancer evolution, these expression changes are likely to provide a selective advantage to cancer cells. To investigate the impact of TRPV4 on cancer progression or metastasis, Kaplan-Meier survival curves were generated using TCGA data to correlate mRNA expression levels with overall survival and disease-free survival. High TRPV4 expression was inversely associated with both overall, post-progression and disease-free survival (Figure 1c). Hazard ratios suggest that TRPV4 high patients have 1.35 (1.1 - 1.65, FDR = 0.5) times higher risk of death, 1.58 (1.17-2.14, FDR=0.2) times higher risk of progress, and 1.58 (1.27-1.97, FDR=0.01) times higher risk for recurrent disease.

Expression of TRPV4 correlated with EMT and TGF-β signaling

Next, we investigated the correlation of mesenchymal phenotype, infamous transition required for metastasis, and TRPV4 expression (supplemantary table 1). TRPV4 correlates moderately with mesenchymal markers such as N-cadherin ($r = 0.44$, $p = 3.1e-14$), Vimentin ($r = 0.48$, $p < 0.001$), Fibronectin ($r = 0.48$, $p < 0.001$), and Laminin V ($r = 0.49$, $p < 0.001$), all showing moderate positive correlations ($r > 0.4$) and significant p-values. Nevertheless, both the kohn mesenchymal geneset ($r = 0.46$, $p = 1.1e-15$) (Figure 2c) and hallmarks of epithelial to mesenchymal transition geneset (Figure 2a) also show a moderate positive correlation with TRPV4 ($r=0.47$, $p < 0.001$), supporting the link between TRPV4 and mesenchymal features. On the other hand, TRPV4 expression is negatively correlated with epithelial markers like E-Cadherin ($r = -0.12$, $p = 0.043$) and EPCAM ($r = -0.17$, $p = 0.002$). Even though the correlations are weak ($r < 0.3$), the p-values are statistically significant. The epithelial geneset (Figure 2e) also shows a weak negative correlation with TRPV4 ($r = -0.15$, $p = 0.013$), further reinforcing the trend that TRPV4 could be associated with mesenchymal rather than epithelial traits.

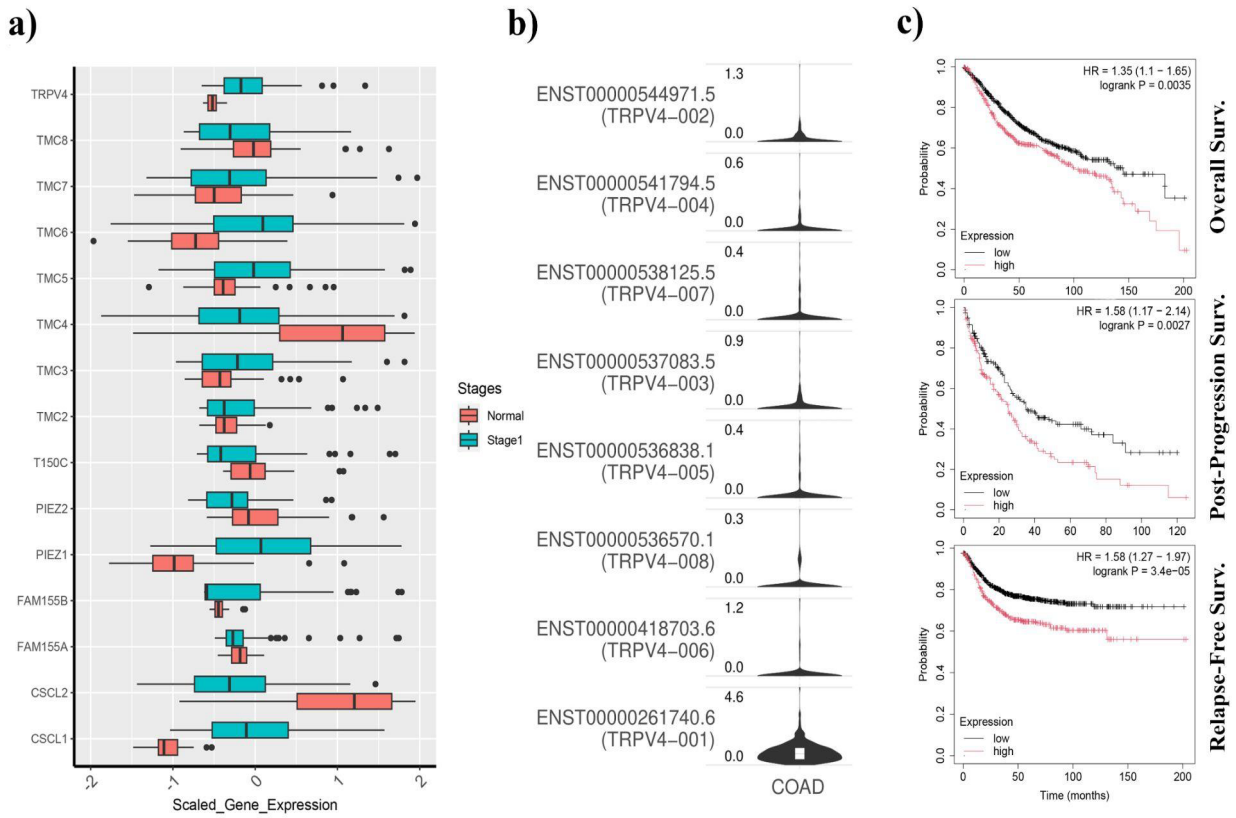

Figure 1. Expression levels of 17 mechanosensitive ion channels in normal colon tissue and stage 1 colorectal cancer (CRC) are shown (a), along with various TRPV4 isoforms (b). Kaplan-Meier survival analysis (c) compares low TRPV4 expression (black) and high TRPV4 expression (red).

To understand the mechanism by which TRPV4 influences EMT, we investigated the relationship between EMT inducer soluble factors and TRPV4 in the tumor microenvironment. The expression of TRPV4 shows a significant positive correlation with TGF-β1 expression (r = 0.5, p = 0) (Figure 2b), TGF-β receptor expression (r = 0.43, p = 1.3e-13) (Figure 2d), and TGF-β signaling (r = 0.41, p = 1.1e-12) (Figure 2f), suggesting a moderate positive relationship with these key components of TGF-β pathways. This indicates that TRPV4 may have a role in modulating TGF-β-related signaling in the context of tumor progression.

TGF-β treatment induce translocation of TRPV4

According to TCGA data, mRNA expression of TRPV4 isoforms in colorectal cancer has been shown. However, to discuss the impact of TRPV4 on colorectal cancer, it is necessary to demonstrate the translation of mRNA expression levels into protein expression, verify membrane localization, and show functionality upon activation. To this end, immunofluorescent methodology was used to visualize the TRPV4 channel through confocal microscopy, allowing for the simultaneous demonstration of expression and localization.

TRPV4 expression was significantly expressed in Duke grade 4 colorectal cancer cell lines HCT-116 and HT-29, as well as in LOVO cells originating from lymphatic metastasis of colorectal cancer (Supplemantary Figure 1). However, in all three colorectal cancer cell types, TRPV4 expression was primarily localized in the nucleus and vesicular structures surrounding the nucleus. While the accumulation around the nucleus could potentially be related to the Golgi apparatus or endoplasmic reticulum, no direct evidence for this is available.

Functional TRPV4 is expected to localize outside the nucleus, as cells regulate its activity through post-translational modifications that control its trafficking from intracellular compartments to the membrane (41,42). Given the increased expression levels observed in stage IV colorectal cancer and its correlation with the mesenchymal phenotype and TGF-β signaling, we hypothesized that TRPV4 trafficking might be regulated by TGF-β.

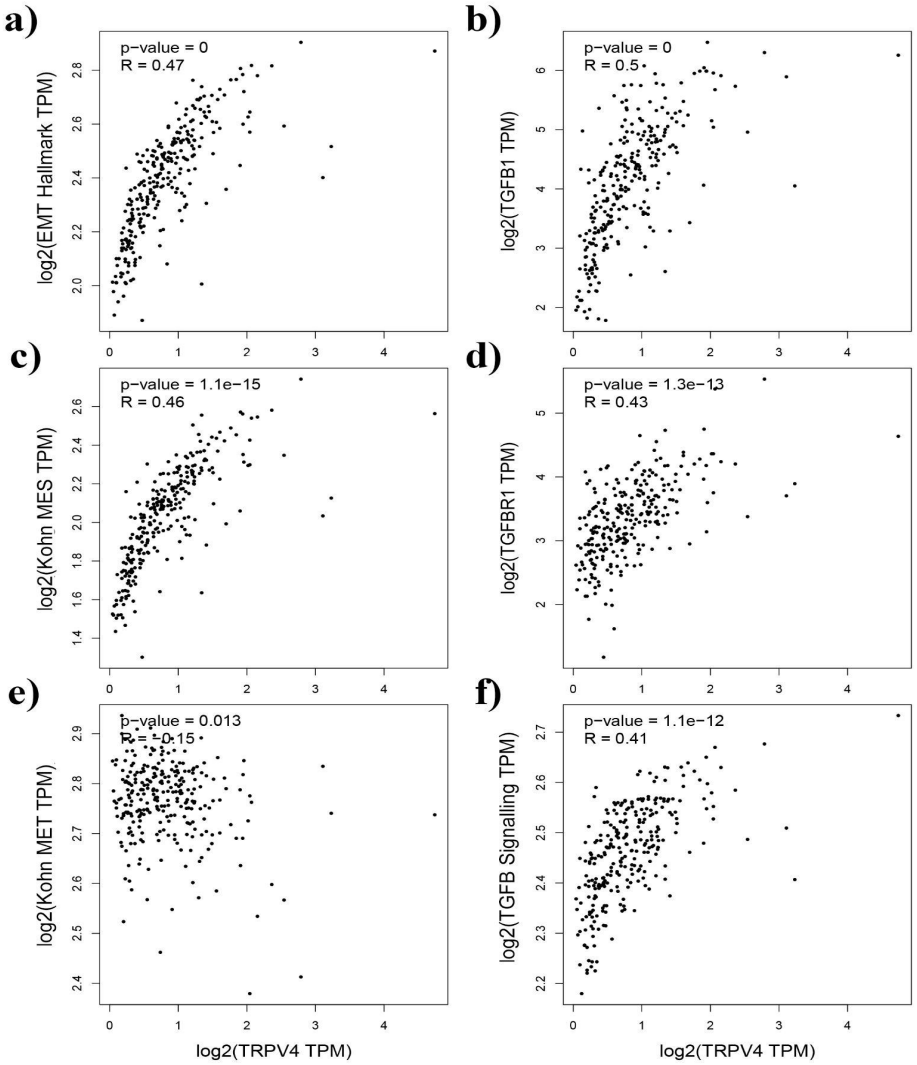

Figure 2. Correlation analysis between TRPV4 expression (log2 TPM) and EMT-associated signatures, mesenchymal markers, and TGF-β signaling pathways. Scatter plot showing a positive correlation between TRPV4 expression and the EMT hallmark gene signature (a), TGFB1 expression (b), the Kohn mesenchymal (MES) signature (c), TGFBR1 expression (d), the Kohn MET signature (e), and TRPV4 expression and the TGF-β signaling gene signature (f).

To test this, the HCT-116 cell line was treated with 10 ng/mL TGF-β1 for 18 hours prior to fixation. A significant decrease in nuclear TRPV4 levels was observed, accompanied by increased cytosolic and membrane expression (Figure 3a, 3b, 3e). These findings suggest that TGF-β signaling plays a critical role in controlling TRPV4 trafficking and potentially contributes to a gain-of-function.

TRPV4 inhibition attuned TGF-β dependent N-Cadherin expression

In TGF-β-stimulated HCT-116 cells, TRPV4 expression was observed to decrease around the perinuclear region while redistributing throughout the cytoplasm and membrane.

To investigate whether this trafficking of TRPV4 plays a role in the epithelial-mesenchymal transition (EMT) process, two experimental groups were subjected to co-stimulation with TGF-β. One group was treated with the TRPV4 activator GSK101, and the other with the TRPV4 inhibitor HC-067047. Following incubation, it was observed that the TRPV4 inhibitor reduced N-cadherin expression (Figure 3d) compared to the TGF-β-only stimulated control group (Figure 3c). These results suggest that TRPV4 contributes to the TGF-β-mediated mesenchymal transition mechanism in colon cancer.

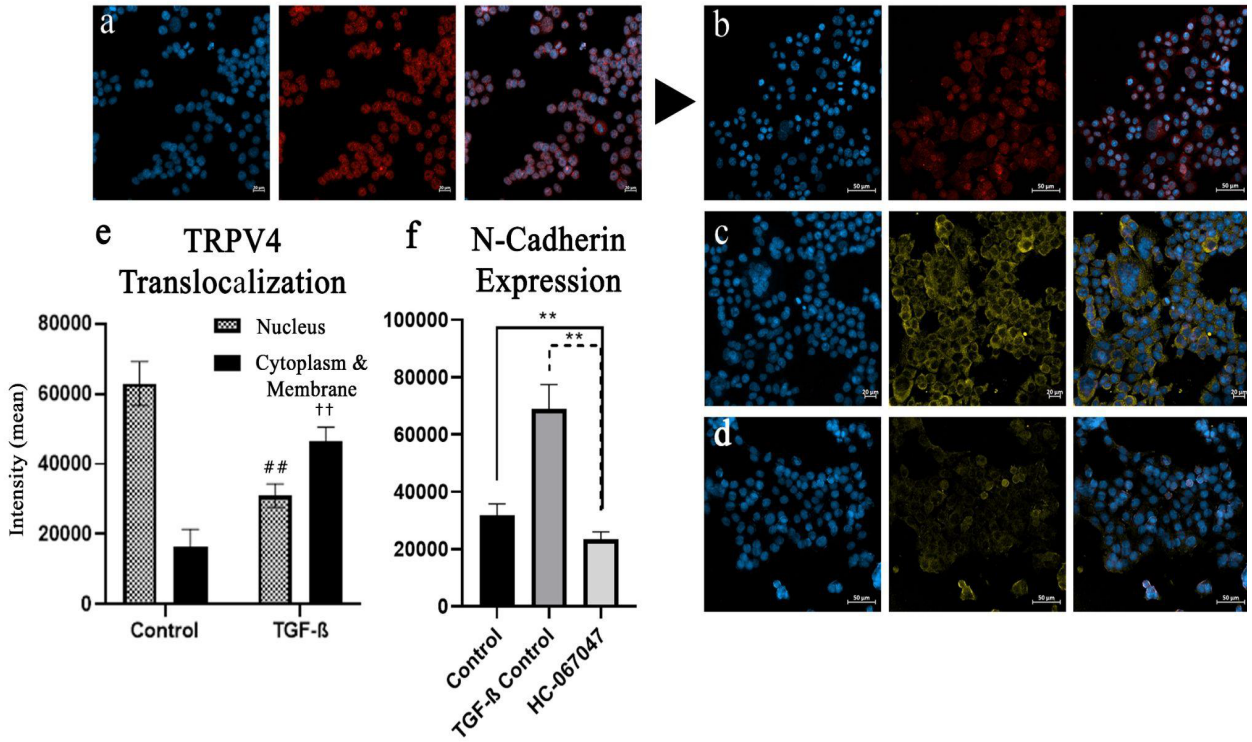

Figure 3. TRPV4 expression & localization in native HCT-116 (a) and TGF-β induced condition (b) TRPV4 translocalization is graphed in (e). All nucleus are blue, TRPV4 is red, and N-Cadherin is green. # and † symbols respectively indicate that statistical significance comparing to corresponding location in control group. N-cadherin expression in TGF-β treated (c) and HC-067047 & TGF-β co-treated cells (d). Alterations in N-Cadherin expression have been showed in (f). ##, ††, ** mean p<0.01.

DISCUSSION

This study investigated the mechanosensitive relationship between TGF-β-induced epithelial-mesenchymal transition (EMT) and 17 mechanosensitive ion channels, identifying TRPV4 as a key candidate. TRPV4 was found to have higher expression in progressive colon cancer and exhibited correlations with EMT and TGF-β signaling pathways. While predominantly localized in the nucleus in grade 3 and 4 colon cancer cell lines, TRPV4 shifted to the cytoplasm and membrane in TGF-β-stimulated HCT-116 cells, indicating that TGF-β regulates TRPV4 trafficking, potentially enhancing its functional activity. Moreover, inhibiting TRPV4 significantly reduced TGF-β-induced N-cadherin expression compared to TGF-β treatment alone. These findings suggest that TRPV4 responds early to TGF-β signaling, contributing to its mechanistic role in promoting mesenchymal transition.

TRPV4 has been a regulator in cancer progression through EMT and cytoskeletal remodeling across various cancer types. In colorectal cancer, TRPV4 contributes to invasiveness by stimulating ZEB1 expression and promoting the EMT process via AKT signaling, while its inhibition activates the PTEN pathway, suppressing tumor development (43). Similarly, TRPV4 regulates cytoskeletal dynamics through the RhoA/ROCK1 pathway, enhancing metastasis in endometrial cancer (44). In nasopharyngeal carcinoma, TRPV4 activation enhances tumorigenesis via NFAT4 signaling and its pharmacological inhibition reduces EMT and ERK activation, demonstrating antitumor effects in hepatocellular carcinoma (45,46). There are various other studies showing similar results in several cancers (47,48). These findings align with our study, however, while studying the effect of TRPV4 activation, these studies focused on exogenous activators rather than pathophysiologic channel activity.

TRPV4 function is regulated by endogenous factors like arachidonic acid, endocannabinoids, mechanical forces (e.g., increased matrix stiffness, osmolarity, and shear stress), and cell crowding in the microenvironment (49,50). It also responds to physiologic movements, such as bladder stretch, due to its localization in adherence junctions of urothelial cell membranes. Similarly, the channel expressed on

the basolateral side of epithelial cells and its over-expression related to chronic constipation (51,52). Importantly, TRPV4 translocation appears to be a key regulatory mechanism for its activity (41,42,53). Our study further demonstrates that TRPV4 trafficking as a player regulated by TGF-β and promotes EMT, which could explain a mechanism for its pathophysiological activation in colon cancer.

TRPV4 crosstalks and interacts with soluble factors in both pro-inflammatory and anti-inflammatory in various tissues and pathologies (54–56). The channel mediates MAPK downstream to regulate cytokines and potentiate EGF response (54,57,58). Similarly, TRPV4 involves TGF-β signaling regulation in non-cancer contexts, such as ventricular fibroblasts and chondrocytes (59,60). Our findings indicate that TRPV4 plays a key role in TGF-β-induced epithelial-mesenchymal transition (EMT), with trafficking to the cell membrane enhancing its functional activity in colon cancer. Our observation of TRPV4's role in EMT and TGF-β signaling expands on these findings by a mechanistic link between TRPV4 out-of-nucleus trafficking and its regulatory role in TGF-β-induced mesenchymal transition in colon cancer.

TRPV4 emerges as a promising mechanoreceptor capable of mediating TGF-β1 regulation and function. While our results establish a TGF-β1 dependence on TRPV4, the role of physical forces such as matrix stiffness or shear stress in this mechanism was not investigated. Nonetheless, evidence from non-cancer contexts suggests that physical forces can regulate both the function and trafficking of TRPV4, providing indirect support for our hypothesis (61,62). Notably, in HUVEC cells, TRPV4 activation in response to 12 dyne/cm² shear stress led to a reduction in the VE-cadherin layer, which forms adherens junctions, demonstrating mechanosensitive TRPV4 translocation and functional activation (63). This aligns with our observation of TRPV4 translocation to gain function and supports the notion that physical forces may modulate TGF-β1 activity via TRPV4. Further investigation is needed to explore this mechanistic link in cancer-related contexts, particularly under conditions of altered matrix stiffness and shear stress.

CONCLUSION

Our findings highlight TRPV4 as a critical regulator of TGF-β1-mediated mesenchymal transition in the colon microenvironment through an induced translocation mechanism. The channel's function is facilitated by TGF-β1 induced translocation out-of-nucleus upon stimulation. In this context, we demonstrate that TRPV4 inhibition reduces TGF-β-induced EMT, suggesting potential anti-metastatic benefits. Although not explored in this study, TRPV4's regulation by lipid metabolites and its mechanosensitive properties present new research interest in how tumor cells respond to TGF-β signaling under pathophysiological conditions. Furthermore, its correlation with measurable ionized calcium levels in the blood may provide personalized treatment to calcium signaling (64). Additionally, TRPV4's potential therapeutic relevance is supported by its roles in pain treatment and anti-angiogenic therapies (65,66). Pharmacophore studies targeting active isoforms and splice variants of TRPV4 could support individualized treatments to slow EMT in patients with high TRPV4 expression, paving the way for tailored therapeutic strategies.

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Author contribution(s): CK contributed conception, design of study, data collection and processing, analysis-interpretion, literature review, and writing. YB contributed conception, design of study, supervision, literature review, and writing.

Conflict of interest: There is no conflict of interest between authors

Ethical approval: In compliance with the Declaration of Helsinki, Dokuz Eylul University Non-Interventional Clinical Research Ethics Committee approved the study (Date: 31.07.2019, Decision No: 2019/19-08)

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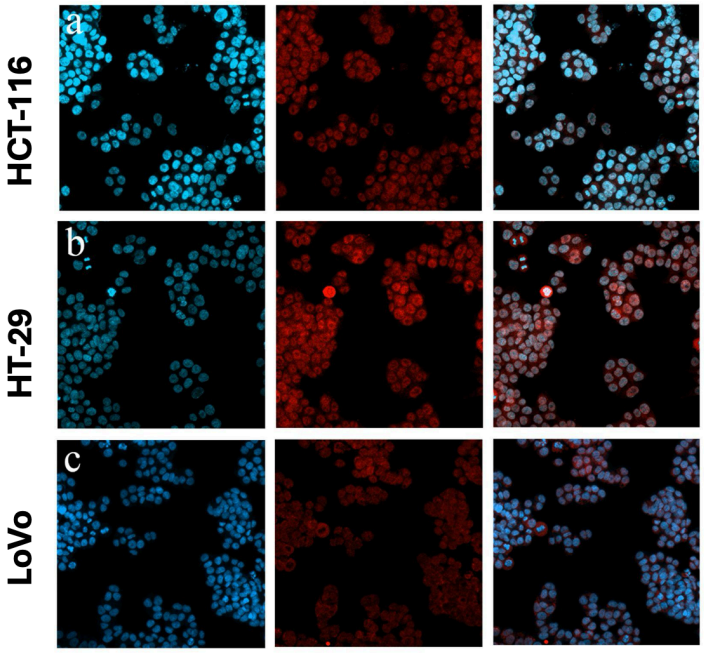
SUPPLEMENTARY MATERIALS



Supplemantary Figure 1. TRPV4 expression and localization in HCT-116 (a), HT-29 (b), LOVO (c). Nuclei are stained blue, and TRPV4 expression is marked in red. Images were captured at 20x magnification.

Supplemantary Table 1. TRPV4 and Gene/Geneset correlations

Category	Gene/Geneset	Pearson (r)	p-value
Epithelial Markers	E-Cadherin	-0.12	0.043
	EPCAM	-0.17	0.0039
Mesenchymal Markers	N-cadherin	0.44	3.1e-14
	Vimentin	0.48	0
	Fibronectin	0.48	0
	Laminin V	0.49	0
EMT Genesets	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.47	0
	KOHN_EMT_MESENCHYMAL	0.46	1.1e-15
	KOHN_EMT_EPITHELIAL	-0.15	0.013
TGF-B1 Signaling	HALLMARK_TGF_BETA_SIGNALING	0.41	1.1e-12
Soluble Factors	TGF-B1	0.5	0
	TGF-B1 receptor	0.43	1.3e-13
	CCL2	0.35	3.3e-09
	CXCL12	0.31	2.2e-07
	IL-1B	0.21	0
	WNT1	0.045	0.45
	WNT2	0.35	1.7e-09
	CXCL8	0.28	3.2e-06
	CXCL6	0.34	5.5e-09
	EGF	0.05	0.4
	HGF	0.24	4.2e-05
	FGF9	0.12	0.056
	FGF10	0.29	1.0e-06
	FGF18	2,70E+01	0.65