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Treatment of cancer stem cells from human colon adenocarcinoma cell line HT-29 with resveratrol and sulindac induced mesenchymal-endothelial transition rate

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Abstract

In the current experiment, the combined regime of resveratrol and a Wnt-3a inhibitor, sulindac, were examined on the angiogenic potential of cancer stem cells from human colon adenocarcinoma cell line HT-29 during 7 days. Cancer stem cells were enriched via a magnetic-activated cell sorter technique and cultured in endothelial induction medium containing sulindac and resveratrol. Expression of endothelial markers such as the von Willebrand factor (vWF) and vascular endothelial cadherin (VE-cadherin) and genes participating in mesenchymal-to-epithelial transition was studied by real-time PCR assay. Protein levels of Wnt-3a and angiogenic factor YKL-40 were examined by western blotting. ELISA was used to determine the level of N-acetylgalactosaminyltransferase 11 (GALNT11) during mesenchymal-endothelial transition. Autophagy status was monitored by PCR array under treatment with the resveratrol plus sulindac. Results showed that resveratrol and sulindac had the potential to decrease the cell survival of HT-29 cancer cells and the clonogenic capacity of cancer stem cells compared with the control ($p < 0.05$). The expression of VE-cadherin and vWF was induced in cancer stem cells incubated with endothelial differentiation medium enriched with resveratrol ($p < 0.05$). Interestingly, the Wnt-3a level was increased in the presence of resveratrol and sulindac ($p < 0.05$). YKL-40 was reduced after cell exposure to sulindac and resveratrol. The intracellular content of resistance factor GALNT11 was diminished after treatment with resveratrol ($p < 0.05$). Resveratrol had the potential to induce the transcription of autophagy signaling genes in cancer stem cells during endothelial differentiation ($p < 0.05$). These data show that resveratrol could increase cancer stem cell trans-differentiation toward endothelial lineage while decrease cell resistance by modulation of autophagy signaling and GALNT11 synthesis.

Keywords Human cancer stem cells · Sulindac · Resveratrol · Endothelial differentiation · Autophagy

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Introduction

Colon cancer is thought to be one of the most common cancers that occur worldwide. Based on statistics, this tumor is the third and second most common malignant tumors seen in male and females, respectively (Evans et al. 2006; Jia et al. 2010; Roselló et al. 2018). Recent investigations revealed the existence of cancer-associated stem cells, termed as cancer stem cells (CSCs), inside the tumor niches (Desiderio et al. 2014). Similar to normal stem cells, CSCs possess two distinct features: self-renewal activity and trans-differentiation capacity to various lineages (Bomken et al. 2010). Considering the unique dynamic of CSCs, these cells are highly resistant to various insults and stimuli such as anti-proliferative drugs and ionizing irradiation (Harrison and Lerner 1991). One explanation would be that the expression of anti-apoptotic factors and ABC transporters is highly conserved in CSCs as compared with normal stem cells or neighboring cancer cells (Zhou et al. 2001). Different groups previously stated that the gene expression pattern of CSC-related pathways is involved in epithelial-mesenchymal transition (EMT), playing a key role in the progression of the tumor and escape from the immune response (Druzhkova et al. 2018; Fan et al. 2012). The critical role of various growth factors such as hepatocyte growth factors was found to affect EMT in cancers (Della Corte et al. 2014).

The tumor ability to augment angiogenesis and neovascularization is an essential factor to expand and metastasize to remote sites (Fan et al. 2012). Endothelial cells (ECs) are key cells participating in vascular bed formation. In support of vascularization inside the tumor niche, CSCs could trans-differentiate into the endothelial lineage, thereby facilitating the nourishment of tumor mass and further expansion (Fan et al. 2012). Different signaling pathways and effectors participate in the endothelial differentiation of CSCs (Yao et al. 2011). For instance, the crucial role of the signaling pathway Wnt/β-catenin containing WNT ligands and cell surface receptors frizzled and lipoprotein receptor–related protein 5/6 has been confirmed in different cell behaviors and tumor metastases (Huang et al. 2014).

In line with routine and classical therapeutic approaches, many attempts have been made to use the natural compounds for the treatment of different cancers by restricting neovascularization and expansion. One of the natural ingredients associated with the anti-cancer activity is resveratrol (Res) (Huang et al. 2014). This phyto-compound with a molecular formula of 3,5,4′-trihydroxystilbene ($C_{14}H_{12}O_3$) has been identified in different plants (Fig. 1a) (Signorelli and Ghidoni 2005). Previous experiments revealed that various anti-cancer activities of Res were related to the expression and function of ABC carriers and activation of apoptotic signaling pathways inside the tumor cells (Huang et al. 2014). Buhrmann and co-workers identified an increased chemosensitization of colorectal cancer cells (HCT116 and HCT116R cell lines) after

treatment with Res via the modulation of the TNF-β signaling pathway (Buhrmann et al. 2018).

In the current experiment, we aim to investigate the potential effect of Res on endothelial differentiation of CSCs derived from a human intestinal adenocarcinoma cell line, HT-29. CSCs were isolated and exposed to Res over a period of 48 h. The effect of Res on the expression of endothelial-specific genes such as the von Willebrand factor (vWF) and vascular endothelial cadherin (VE-cadherin) was monitored after CSC exposure to endothelial induction medium. The potent inhibition of canonical Wnt signaling by sulindac (Sulin; $C_{20}H_{17}FO_3S$; a canonical Wnt signaling blocker) (Fig. 1b) was also investigated during trans-differentiation into the endothelial lineage. We also monitored the CSC autophagy response by PCR array during EMT transition.

Material and methods

Cell culture and expansion protocol

In this study, we examined the combined effect of Res and Sulin on cancer intestinal epithelial cell HT-29. For this purpose, the human-derived epithelial colorectal cancer cell line HT-29 (NCBI code: C466) was obtained from the National Cell Bank of Iran (Pasteur Institute). The cells were expanded in Roswell Park Memorial Institute medium (RPMI, Gibco) enriched with 10% fetal bovine serum (FBS, Gibco), 100 IU/ml penicillin and 100 mg/ml streptomycin (Cat No. 15140122, Gibco). We transferred the cells to a humidified atmosphere at 37 °C with 5% CO_2. After reaching 70% confluence, the cells were subcultured using 0.25% Trypsin-EDTA solution (Cat No. 25200-056, Gibco). We used HT-29 cells at passage 3 for different analyses. Three sets of experiments were performed in this study.

Treatment protocol

The combined effect of Res and Sulin was investigated on different features of cancer cells and CSCs in HT-29 cell line over a period of 48 h. First, we determined the rate of cytotoxicity in HT-29 cancer cells. The cells were exposed to different doses of Res (100, 200, 400, 600 and 800 μM) and Sulin (100, 200, 400 and 600 μM) for 48 h. In our experiment, cells were randomly divided into four different groups as follows: control non-treated cells, Res group ($IC_{50} = 57$ μM), Sulin group ($IC_{50} = 64$ μM) and cells that received the combination of Res and Sulin. After completion of cytotoxicity, IC_{50} values were calculated and normal cancer cells and CSCs were subjected to other analyses.

Fig. 1 The molecular structure of Res and Wnt-3a inhibitor Sulin (**a**, **b**). Measuring the survival rate of the HT-29 cell line with different concentrations of Res and Sulin for 24 h (**c**, **d**). Data confirmed a dose-dependent cytotoxicity of both Res and Sulin on HT-29 cells. This experiment was performed in triplicate (each in octuplicate)
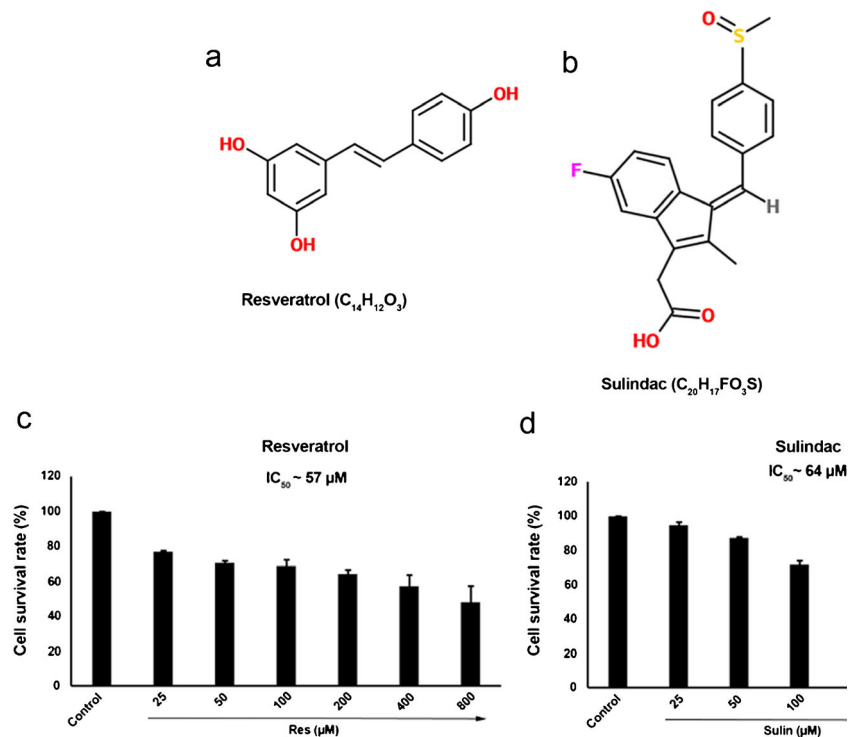
MTT assay

The effects of Res and Sulin were measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) (Cat No. M-2128, Sigma). We plated 2×10^4 cells on each well of 96-well plates and maintained for 24 h. HT-29 cells were treated with different concentrations of Res and Sulin for 24 h. After completion of incubation time, 20 μl of MTT solution (5 mg/ml in phosphate-buffered saline (PBS), Cat No. 18912-014, Sigma) was added to each well and kept for 4 h. Then, we removed the MTT solution and added 100 μl dimethyl sulfoxide (CAS No. 67-68-5, Merck Millipore). The absorbance of each sample was measured at 570 nm using a microplate reader (Model: ELx808, BioTek). Cell viability was expressed as percent of control HT-29 cells. This experiment was performed in triplicate. To calculate IC_{50} values, we used IC_{50} Tool Kit software, accessible online at URL: http://www.ic50.tk/

Isolation of HT-29 CSCs by magnetic-activated cell sorter

To harvest CSCs, we enriched HT-29 CSCs by a magnetic-activated cell sorter (MACS) technique according to our previous published experiments (Akbarzadeh et al. 2017). In brief, HT-29 cells were resuspended in PBS containing 1% bovine serum albumin (BSA, Sigma) and maintained for 20 min. To isolate CSCs, CD133-coated magnetic beads (Miltenyi Biotec) were added to the cell suspension and

rotated gently at 4 °C for 40 min. Samples were passed through LS columns (Miltenyi Biotec) according to the manufacturer's recommendations.

Immunophenotyping of enriched CSCs

Flow cytometry analysis

To confirm the purity of enriched CSCs, we performed flow cytometry analysis based on the detection of total $CD133^+$ cells. The samples were washed with PBS and incubated with the FITC-conjugated anti-human CD133 antibody (Clone: AC133; Cat No. 130-113-673; Miltenyi Biotec) at 4 °C for 30 min. After twice washing with PBS, cells were fixed with pre-chilled 4% paraformaldehyde (PFA) solution. For flow cytometry analysis, BD FACSCalibur system and FlowJo software (version 7.6.1) were used. The purity of cells was compared with control HT-29 cells prior to MACS procedure.

Immunofluorescence imaging

In order to validate the quality of the enrichment procedure, the stemness feature was also monitored by immunofluorescence (IF) imaging. For this purpose, HT-29 CSCs were plated at an initial density of 1×10^4 cells/well on an 8-well Chambered Cell Culture Slide (SPL) and cultured for 8 h. Next, cells were fixed by 4% PFA for 15 min, permeabilized by 0.05% Triton X-100 (Sigma) and blocked with 1% BSA solution for 30 min. One hundred microliters of PBS

containing 1 µg/ml anti-human CD133 antibody conjugated with FITC was added to each well and kept for 1 h. For background staining, we incubated the cells with 100 µl DAPI solution (1 µg/ml, Sigma) for 30 s. Finally, images were taken by a fluorescence microscopy (Model: BX51, Olympus) and processed by CellSense imaging software.

Clonogenic assay

To evaluate the clonogenicity of CSCs after being exposed to Res and Sulin, we performed a clonogenic assay. In brief, 1×10^5 CSCs and HT-29 cancer cells were transferred to each well of 24-well plates and incubated with Res and Sulin for 7 days. Next, the number of colonies was scored at 16 high-power fields and compared with control-matched groups.

Endothelial differentiation of HT-29 CSCs

To better understand the inhibitory/stimulatory effect of Res and Sulin on CSC differentiation toward endothelial lineage (MendT/EndMT), isolated HT-29 CSCs were cultured in medium containing endothelial cell growth medium 2 (EGM-2; Promocell) with 2% FBS and Res and/or Sulin for 7 days. The medium was replenished every 3–4 days.

Real-time PCR

For EndMT and MendT in vitro, we measured the transcription level of vWF, VE-cadherin, Snail1, ZEB1, ZEB2, and vimentin. Total RNA was extracted by using an RNAX plus solution (CinnaGen) according to the manufacturer's recommendations. cDNA was synthesized by using a reverse transcription kit (Bioneer). The expression of each gene was measured by SYRB Green dye (TaKaRa) and Rotor-Gene 6000 (Corbett Life, Austria). We used glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene to normalize the expression of each gene. Three sets of experiments were conducted. Primers used for real-time PCR are listed in Table 1.

Measuring the level of Wnt-3 by western blotting

The level of both Wnt-3 and YKL-40 peptides was studied during endothelial differentiation in cells from different groups according to the protocol above-mentioned. On day 7, cell lysates were obtained by resuspending cells in an ice-cold lysis buffer (NP-40, NaCl, Tris–HCl, and Nonidet) with cocktail enzyme inhibitors. Protein samples were separated on 12% SDS polyacrylamide gel electrophoresis followed by a transfer to a polyvinylidene difluoride (PVDF) membrane (Millipore). The membranes were incubated with antibodies against Wnt-3 (Cat No. ab32249, Abcam) and YKL-40 (Cat No. ab77528, Abcam) at a dilution of 1:1000 overnight at

Table 1 List of primers used in the current experiment

Gene	Sequence	Tm (°C)
vWF	F: 5′-GATGAGAACGGAGCCAATGAC-3′	61
	R: 5′-CAAACAGTGGTAAGAGGAGGAC-3′	
VE-Cadherin	F: 5′-TGAAGAAGGACGAGGCGGAC-3′	62
	R: 5′-CAGAGTCGGATGAGTCGGTGC-3′	
Vimentin	F: 5′-CAGATGCGTGAAATGGAAGAGAA	62
	R: 5′-TAGGTGGCAATCTCAATGTCAA	
ZEB1	F: 5′-CTGGAGAAAAGCCCTATCAATGT-3′	61
	R: 5′-CTGTCTTCATCCTCTTCCCTTGT-3′	
ZEB2	F: 5′-CAGCCATTACCCAGTTAAGA-3′	60
	R: 5′-CCCGTGTGTAGCCATAAGA-3′	
GAPDH	F: 5′-CAAGTTCAACGGCACAGTCAAG-3′	60
	R: 5′-ATACTCAGCACCAGCATCACC-3′	

4 °C. Blots were then incubated with HRP-conjugated anti-rabbit IgG (Cat No. ab6721; Abcam) for 1 h at RT. For visualizing the immunoreactive bands, ECL plus solution (BioRad) was used. Densitometric analysis of immunoblots was performed with Image software ver.1.44p (NIH). β-Actin (Cell Signaling) was used as a loading control.

Detecting the level of polypeptide N-acetylgalactosaminyltransferase 11 in CSCs by ELISA

It was determined that N-acetylgalactosaminyltransferase 11 (GALNT11) has a distinct role in the malignancy behavior of tumor cells. To evaluate the potential of Res and Sulin on the expression of GALNT11, we performed an ELISA assay (Cat No. CSB-EL009205HU; CUSABIO) according to the manufacturer's recommendation. Cell lysates (10 µg) were poured into each well pre-coated with a biotin-conjugated antibody specific for GALNT11 and incubated for 1 h. After washing with PBS, avidin-conjugated HRP was poured onto the wells followed by the addition of chromogenic substance TMB (3,3′,5,5′-tetramethylbenzidine). Upon the addition of stop solution, we scored the OD of each sample with an ELISA reader and compared with the values from the standard curve.

PCR array

The status of the CSC autophagy response was evaluated by PCR array analysis 7 days after induction of MendT as previously described by our group (Rezabakhsh et al. 2017). The transcription level of genes in autophagy signaling pathways was monitored by human autophagy RT2 profiler™ PCR Array (Cat No. PAHS-084Z) and LightCycler 480 Instrument II system (Roche). Data were analyzed using the comparative $2^{-\Delta\Delta CT}$ method and normalized to the mean of control housekeeping genes (ACTB, B2M, GAPDH, HPRT1 and RPLP0).

Statistical analysis

Data are represented as mean ± SD for each experiment. Statistical analysis was done using Instat GraphPad software ver. 2.02. The significant differences between groups were performed via the one-way analysis of variance (ANOVA) and Tukey (post hoc) test. $p < 0.05$ was considered as statistically significant.

Results

Res and Sulin inhibited the growth of HT-29 cells

In order to analyze the inhibitory effect of Res and Sulin on the human colon cancer cell line HT-29, MTT assay was conducted. Data demonstrated a dose-dependent activity of Res and Sulin on HT-29 cell inhibition rate (Fig. 1c, d). Compared with non-treated control cells, we observed ~ 1.29-fold decrease in the survival rate of HT-29 cells exposed to 25 μM Res while this value reached ~ 2.1-fold in group Res 800 μM. According to the results, Res IC_{50} was 57 μM. Similar to cells from 800 μM Res, a downward trend was also recorded in viability the rate for Sulin-treated HT-29 cells over a period of 24 h (Fig. 1c). HT-29 cells also showed sensitivity to Sulin with a 1.05-fold decrease in cell survival rate after exposure to a lower dose of 25 μM. The inhibition rate for cells that received the highest doses of Sulin, including 200 and 400 μM, was 1.49- and 1.55-fold, respectively, as compared with the control (Fig. 1d). These data support a notion that both Sulin and Res could inhibit and/or decrease the survival rate of HT-29 cells in vitro, which are important in regulating the growth and expansion of human colon cancer cells.

CSC isolation and characterization

Micrographs from in vitro analysis showed that the enriched cells tended to form colonies that are one of the CSC features (Fig. 2a, a′). The diameter of colonies reached 100 to 150 μm. Flow cytometry analysis showed that nearly 90% of isolated cells expressed stemness marker CD133 post-MACS procedure (Fig. 2b, b′). Similar to data from the flow cytometry panel, IF imaging confirmed the cell contribution of CD133 in the isolated cell membrane (Fig. 2c). These data showed the successful isolation of HT-29 CSCs and stemness maintenance prior to the various experiments.

CSC clonogenic capacity was reduced by the combination of Res and Sulin

Based on our data, the numbers of CSC colonies were profoundly diminished in the Res and Sulin groups compared with the non-treated control ($p_{\text{Control vs. Res}} < 0.01$; $p_{\text{Control vs.}}$ $_{\text{Sulin}} < 0.01$; and $p_{\text{Control vs. Res + Sulin}} < 0.0001$) (Fig. 2d). No significant differences were observed between the number of CSCs in groups Res + Sulin and Res or Sulin alone $p > 0.05$). These data showed that the inhibition of Wnt-3a could abort the formation of CSC colonies. Res also inhibited CSC stability by reducing the clonogenic capacity.

### Sulin decreased Res-induced endothelial differentiation of CSCs

Real-time PCR analysis was performed to check the potential differentiation of CSCs into endothelial-like phenotype after being exposed to the combined regime of Sulin and Res. Data demonstrated HT-29 CSCs had the potential to express VE-cadherin in endothelial induction medium (Fig. 3a). The expression of VE-cadherin was also observed in CSCs from the EGM-2 medium with RES compared with other groups ($p < 0.05$). Notably, the highest expression level of VE-cadherin was seen in CSCs from the Res group ($p_{\text{Control vs.}}$ $_{\text{Res}} < 0.0001$).

Addition of 57 μM Sulin to EGM-2 medium decreased the rate of MendT induced by Res compared with CSCs that received only Sulin ($p_{\text{Sulin vs. Res}} < 0.001$; $p_{\text{Sulin vs. Res +}}$ $_{\text{Sulin}} < 0.01$). These data may indicate that Res could induce MendT in HT-29 CSCs with collaboration of Wnt signaling in which the inhibition of Wnt signaling by Sulin leads to reduced CSCs in endothelial differentiation rate. In line with this statement, the use of Res plus Sulin in EGM-2 medium contributed to a higher VE-cadherin expression level compared with the Sulin-treated group. Compared with CSCs cultured in an EGM-2 medium, a very slight expression of VE-cadherin was obtained in CSCs after the incubation with the differentiation cocktail. In HT-29 cancer cells expanded either in condition with or without endothelial differentiation factors, the expression of VE-cadherin was slight. However, non-statistically significant differences were evident between groups ($p > 0.05$; Fig. 3a).

In contrast to changes of VE-cadherin, the mRNA expression of vWF was significantly induced in control HT-29 cancer cells after 7-day incubation with EGM-2 enriched with Res ($p_{\text{Res vs. Control}} < 0.05$; $p_{\text{Sulin vs. Res}} < 0.05$; $p_{\text{Sulin vs. Res +}}$ $_{\text{Res}} < 0.01$) (Fig. 3b). In both EGM-2$^+$ and EGM-2$^-$ media, CSCs had the potential to express a high rate of vWF compared with other groups (EGM-2-free medium: $p_{\text{Res vs.}}$ $_{\text{Control}} < 0.05$, $p_{\text{Res vs. Res + Sulin}} < 0.05$; EGM-2 medium: $p_{\text{Res vs. Control}} < 0.05$, $p_{\text{Res vs. Sulin}} < 0.05$).

In conjunction with data, CSCs rather than HT-29 cancer cells showed a better differentiation capacity into the endothelial lineage. Monitoring the expression, ZEB-1 and ZEB-2 showed a high transcription level in CSCs that received Sulin or the combined regime of Sulin and Res (ZEB-1$_{\text{CSCs}}$: $p_{\text{Sulin vs. Control}} < 0.001$, $p_{\text{Sulin vs. Res}} < 0.001$, $p_{\text{Sulin vs. Res +}}$ $_{\text{Sulin}} < 0.001$; ZEB-2$_{\text{CSCs}}$ (EGM-2$^-$): $p_{\text{Sulin vs. Control}} < 0.05$; ZEB-2$_{\text{CSCs}}$ (EGM-2$^+$): $p_{\text{Sulin + Res vs. Control}} < 0.05$) (Fig. 3c,

Fig. 2 Micrograph of HT-29 CSC colonies enriched by MACS (**a**, **a′**). Flow cytometric characterization of isolated CSCs by using an antibody against CD133 ($n = 3$; **b**, **b′**). Immunofluorescence imaging revealed the existence of CD133-positive cells after enrichment (**c**). Effect of Res and Sulin on CSC clonogenicity (**d**). The number of CSC colonies was counted at 20 high-power fields. Res, Sulin and their combinations could significantly reduce the number colonies/microscopic field as compared with the non-treated control. One-way ANOVA and Tukey post hoc analysis. $^{**}p < 0.01$, $^{***}p < 0.001$
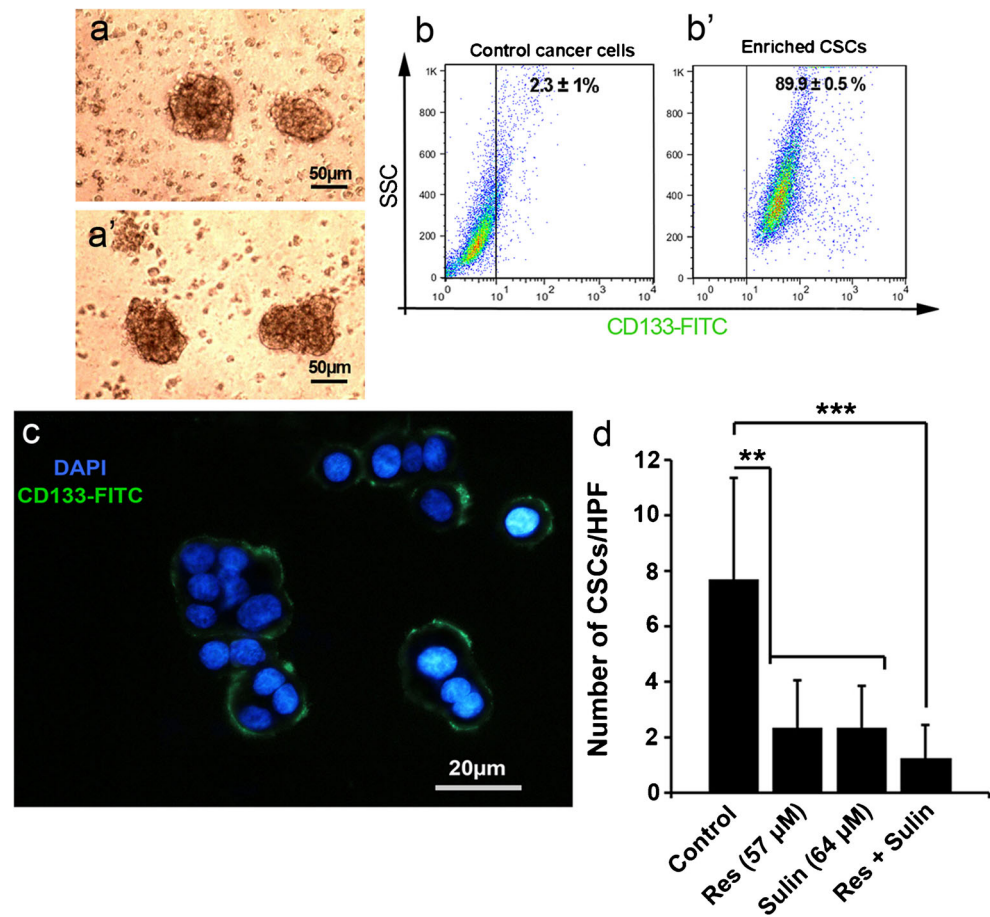
d). We further found no significant differences in the transcription level of vimentin in both normal cancer cells and CSCs in various conditions compared with the matched control ($p > 0.05$) (Fig. 3e).

In the current experiment, no significant difference was detected in CSCs and HT-29 cancer cells either in normal or in EGM-2 media ($p > 0.05$). Collectively, these data indicated the possible modulatory effect of Sulin and Res on intestinal CSCS in regulating the MendT rate in vitro.

CSC Wnt-3a protein levels were changed after treatment with Sulin and Res

According to data from western blot analysis, the intracellular contents of Wnt-3a were changed in both CSCs and HT-29 cells after exposure to Sulin and Res ($p < 0.05$; Fig. 4a, a′). We showed a significant increase in the level of CSC Wnt-3a after being exposed to EGM-2 with Sulin ($p < 0.05$). Noteworthy, the protein level of Wnt-3a was decreased in normal medium with the combined regime of Res and Sulin compared with CSCs cultured in EGM-2 medium with Res and Sulin ($p < 0.001$; Fig. 4a, a′). These data showed that CSC treatment with Res and Sulin in endothelial induction medium could increase the distribution of Wnt-3a in CSCs.

Proteomic analysis of non-cancer stem cells showed the inherent capacity of Res to induce factor Wnt-3a ($p < 0.001$) while no significant differences were indicated in the level of this factor in cells from Sulin ($p > 0.05$; Fig. 4a, a′). Interestingly, the combination of Sulin and Res had the ability to suppress the synthesis of Wnt-3a in EGM-2 medium compared with Res- and Sulin-free EGM-2 condition ($p < 0.001$; Fig. 4a, a′). Our results also confirmed an increasing level of YKL-40 factor in CSCs exposed to endothelial induction medium ($p < 0.05$; Fig. 4b, b′).

Compared with Res-free conditions and CSCs from the Sulin group, Res alone or in combination with Sulin decreased the YKL-40 factor (Fig. 4b, b′). Similar to data obtained from CSCs, it seems that human HT-29 cells were able to synthesize the YKL-40 factor after being exposed to Res, Sulin and their combination ($p < 0.05$). However, these changes were less in comparison with parallel-matched groups. These features support a notion that CSCs have the potential to express a large amount of the angiogenic factor YKL-40 in condition with pro-angiogenic factors. The administration of Res but not Sulin, decreased YKL-40 and the angiogenic activity of human CSCs. Based on the characteristic of non-CSCs, these cells showed an inherent resistance to MendT and secretion of pro-angiogenic factors such as YKL-40.
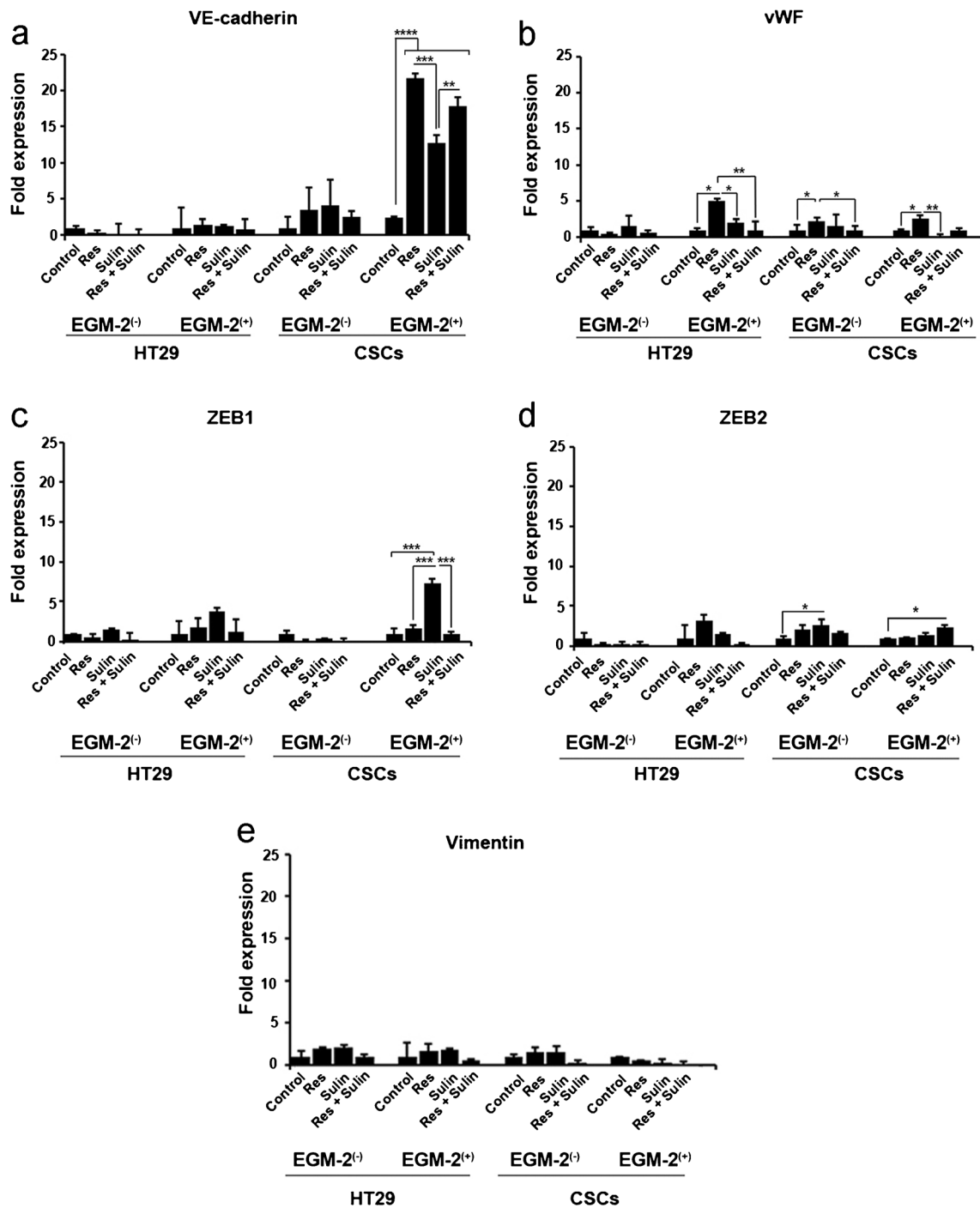
Fig. 3 Detecting the transcription level of VE-cadherin (**a**), vWF (**b**), ZEB-1 (**c**), ZEB-2 (**d**) and vimentin (**e**) by real-time PCR ($n = 3$). One-way ANOVA and Tukey post hoc analysis. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$

GALNT11 synthesis was reduced in CSCs exposed to Res

GALNT11 is associated with malignancies and tumor recurrence in various cancer cell types. We found that the exposure of CSCs to differentiation medium supplemented with Res or Sulin and their combination caused a reduction in the level of GALNT11 as compared with CSCs maintained in normal medium (Fig. 4c). CSC treatment with Res suppressed the synthesis of GALNT11 ($p < 0.05$). Similar to data from Res-treated cells, CSC culture in EGM-2 medium with Sulin contributed to the decrease of intracellular GALNT11 ($p < 0.05$; Fig. 4c). These data confirmed the reduction of CSC resistance after exposure to Res, especially after entering MendT.
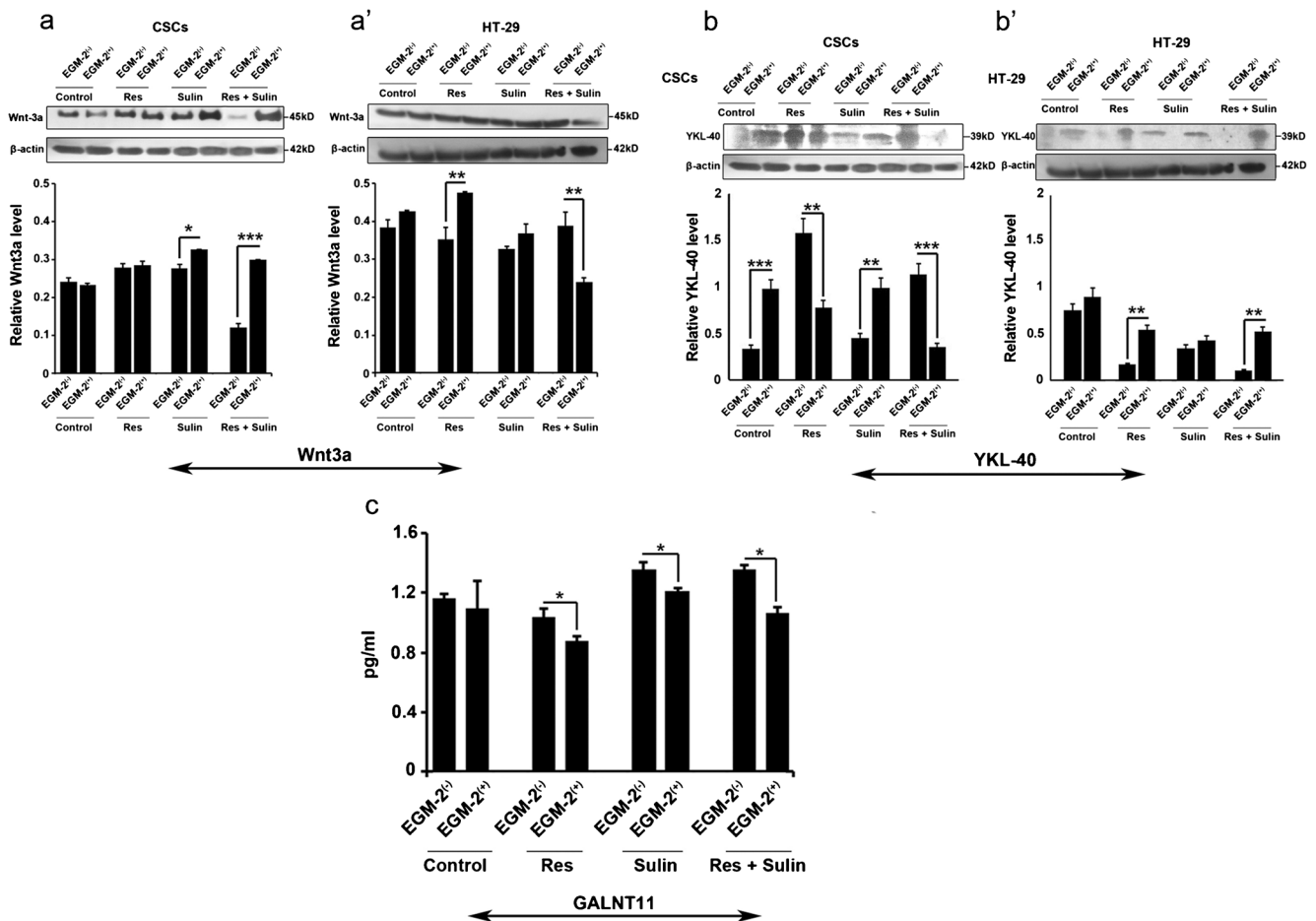
Fig. 4 Measuring the protein level of Wnt-3a (**a**, **a′**) and YKL-40 (**b**, **b′**) in CSCs after exposure to the combined regime of Res and Sulin with and/or without endothelial induction medium over a period of 7 days ($n =$ 3; **a**, **b**). GALNT11 levels were also detected by ELISA assay (**c**). One-way ANOVA and Tukey post hoc analysis. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Res induced autophagy signaling in CSCs exposed to EGM-2 and normal conditions

Dynamic promotion of autophagy is important in cancer cells against different therapeutic agents. In line with this comment, we aimed at determining the autophagy response in HT-29 CSCs exposed to Res and Sulin with and/or without endothelial differentiation medium (Table 2). PCR array showed that the treatment of CSCs with Sulin in normal condition did not stimulate the expression of genes related to the autophagy signaling pathway compared with the control CSCs ($p > 0.05$; Table 2).

We found a significant reduction in the transcription level of ATG10, ATG9B, DAPK1, FADD, HGS and SNCA in CSCs from Sulin-treated CSCs ($p < 0.05$); meanwhile, the exposure of CSCs with Res caused more than a 2-fold increase in the expression of genes APP, ATG10, ATG9A, DAPK1, ESR1, FAS, HTT, IFNG, IRGM, NPC1, PIK3CG, SNCA and TGM2 ($p < 0.05$; Table 2), indicating an inherent capacity of Res to induce autophagy signaling in CSCs. A combined regime of Res

and Sulin promoted the expression of APP and NPC1. Based on data, it seems that endothelial differentiation of CSCs in the presence of Sulin and/or Res could efficiently upregulate a large number of genes involved in autophagy response in comparison with CSCs from normal condition ($p < 0.05$; Table 2).

In CSCs exposed to EGM-2 medium with Res, Sulin and their combination, numerous genes participating in autophagy signaling pathways were activated compared with the control CSCs ($p < 0.05$). Upon treatment of CSCs with EGM-2 medium containing Res and Sulin, we determined upregulation of genes APP (4.2-fold), ATG4C (5.07-fold), ATG9A (3.19-fold), BAD (2.21-fold), BCL2 (5.04-fold), CTSB (2.51-fold), DRAM1 (2.28-fold), ESR1 (16.41-fold), FAS (2.32-fold), HGS (2.03-fold), HTT (3.31-fold), IFNG (4.48-fold), INS (3.59-fold), MAP1LC3B (4.39-fold), RAB24 (2.46-fold), SNCA (7.24-fold) and TMEM74 (2.39-fold) (Table 2). These data support a notion that CSCs under MendT are more susceptible to activate autophagy signaling, presumably showing the sensitivity of differentiating cells to Res and Sulin.

Table 2 Gene expression profiling of autophagy signaling in CSCs form HT-29 cell line after treatment with Sulin and Res in condition with/without endothelial differentiation growth factors

Gene	CSCs treated with endothelial induction medium				CSCS maintained in normal condition		
	Fold regulation						
	Control	Sulin	Sulin + Res	Res	Res	Sulin	Sulin + Res
AKT1	− 1.8213	− 1.1824	− 1.8361	**− 2.1092**	− 1.8899	− 1.217	**− 2.2217**
AMBRA1	− 1.2731	1.3488	− 1.4506	− 1.5764	− 1.1961	1.0116	− 1.0681
APP	1.7404	1.6567	*4.2397*	*4.868*	3.7974	1.5024	*1.0349*
ATG10	− 1.1394	− 1.0558	1.0281	**− 4.122**	2.8979	**− 2.3457**	− 1.9207
ATG12	*2.4256*	2.9519	1.6702	1.6208	− 1.1394	1.2198	− 1.2241
ATG16L1	− 1.1961	− 1.5351	1.1567	− 1.0425	− 1.6003	1.0329	− 1.2938
ATG16L2	− 1.4028	− 1.321	− 1.8618	− 1.9954	**− 2.4368**	− 1.5619	− 1.2968
ATG3	1.8256	1.0151	− 1.544	1.4044	1.6226	1.0187	− 1.6491
ATG4A	− 1.0705	1.3931	1.2983	1.3915	− 1.0656	− 1.0668	1.0534
ATG4B	**− 2.0209**	1.0582	− 1.9679	− 1.1251	− 1.116	1.1594	1.1368
ATG4C	*3.5186*	*3.1565*	*5.0747*	*2.7258*	1.4323	1.3674	1.459
ATG4D	**− 2.035**	2.3757	1.9274	− 1.4044	**− 4.2624**	− 1.0163	− 1.8171
ATG5	1.788	1.5458	1.1892	1.5655	− 1.4126	1.2426	− 1.1527
ATG7	1.3931	*2.0634*	1.8489	1.4607	1.6339	1.9588	1.129
ATG9A	*2.0491*	*4.7513*	*3.1895*	1.7492	*3.3288*	1.3295	1.3001
ATG9B	**− 2.3538**	− 1.6799	**− 2.308**	− 2.5609	− 1.9977	**− 2.4116**	**− 2.0023**
BAD	− 1.2643	*2.9248*	*2.214*	− 1.0792	1.4323	1.192	− 1.0389
BAK1	− 1.3519	1.7674	1.6818	− 1.1303	− 1.8088	1.3441	− 1.1264
BAX	− 1.1634	1.1032	1.3104	− 1.1408	**− 2.7226**	− 1.0521	− 1.1607
BCL2	− 1.5035	*5.8226*	*5.0397*	− 1.6208	1.5783	− 1.3503	− 1.5929
BCL2L1	− 1.0705	− 1.4658	**− 5.7891**	− 1.5874	− 1.374	− 1.7695	− 1.2968
BECN1	− 1.0558	− 1.0956	1.2983	1.181	− 1.4828	1.1837	− 1.3519
BID	− 1.6876	1.7072	1.3379	− 1.007	− 1.5529	1.0116	1.0681
BNIP3	− 1.1715	− 1.3996	− 1.162	− 1.5874	− 1.4523	− 1.6245	− 1.8812
CASP3	*3.6935*	− 1.1212	− 1.1019	*2.0232*	1.9702	1.5728	1.1769
CASP8	1.8383	1.6799	1.3195	1.4914	**− 2.6851**	1.9498	− 1.0755
CDKN1B	*2.5817*	1.5601	1.1647	1.8108	1.9566	1.7695	1.9747
CDKN2A	1.4828	1.2556	1.2226	− 1.0867	− 1.9566	− 1.0892	1.1057
CLN3	− 1.1715	− 1.088	1.9141	− 1.1487	− 1.0485	1.0994	− 1.3488
CTSB	*4.1267*	1.7674	*2.5082*	*2.3403*	1.2241	1.7581	1.8213
CTSD	− 1.4126	1.0081	− 1.014	− 1.8747	**− 2.8186**	− 1.4948	− 1.7072
CTSS	*2.4938*	1.1989	1.4044	1.7171	1.0656	1.4607	1.2354
CXCR4	1.2938	1.5565	− 1.5874	− 1.3348	− 1.7471	1.2454	1.1527
DAPK1	− 1.1394	1.7431	1.7942	**− 2.9417**	*2.8979*	**− 2.3457**	− 1.5966
DRAM1	1.3964	1.3089	*2.2763*	1.2226	**− 2.4033**	1.154	− 1.2326
DRAM2	1.8639	1.6377	1.4811	1.2658	− 1.9163	1.154	− 1.5387
EIF2AK3	1.318	1.3803	− 1.0425	1.4607	1.9793	− 1.1837	− 1.1688
EIF4G1	− 1.1006	− 1.2156	1.007	− 1.6021	− 1.2556	− 1.2953	− 1.1607
ESR1	*2.7606*	*7.6653*	*16.4119*	1.9009	*3.3288*	1.2658	1.7674
FADD	− 1.8856	− 1.0317	**− 2.3403**	− 1.8618	− 1.6003	**− 3.5227**	1.2184
FAS	1.9977	1.459	*2.3241*	*2.308*	*2.1263*	1.3074	1.3059
GAA	− 1.0558	1.0246	− 1.3441	− 1.4607	1.3089	− 1.0377	1.4489
GABARAP	− 1.0198	− 1.0293	− 1.1728	− 1.057	**− 2.2894**	− 1.192	− 1.6491
GABARAPL1	− 1.1796	− 1.4224	1.0693	− 1.1096	− 1.5674	1.1045	− 1.2184
GABARAPL2	**− 2.1962**	1.4624	1.0792	**− 2.4737**	**− 2.1116**	− 1.651	− 1.3996

Table 2 (continued)

Gene	CSCs treated with endothelial induction medium				CSCS maintained in normal condition		
	Fold regulation						
	Control	Sulin	Sulin + Res	Res	Res	Sulin	Sulin + Res
HDAC1	− 1.0705	− 1.129	1.2924	− 1.0281	− 1.8004	1.1225	− 1.0461
HDAC6	− 1.6114	− 1.4158	**− 2.4566**	**− 3.0035**	**− 2.2736**	− 1.5511	− 1.7674
HGS	**− 2.8186**	*2.8252*	*2.0373*	− 2.8415	1.15	**− 3.4822**	**− 2.3161**
HSP90AA1	1.1109	− 1.1109	1.7492	1.3947	**− 3.2603**	1.3692	1.0105
HSPA8	1.0956	− 1.6189	1.1251	− 1.3535	− 1.4028	− 1.1674	1.0534
HTT	1.2326	1.9119	*3.3096*	*2.308*	2.6666	1.7959	1.3484
IFNG	2.9248	1.3059	*4.4795*	2.9214	2.4938	1.3404	1.3161
IGF1	1.4028	− 1.1688	− 1.6133	− 1.4241	1.4323	1.0449	− 1.0175
INS	− 1.7151	1.604	*3.5884*	− 1.6434	1.5892	− 1.1355	− 1.1933
IRGM	− 1.1394	− 1.0509	1.0281	− 1.2086	*2.8979*	1.3287	− 1.4794
LAMP1	− 1.0341	1.0855	1.6245	− 1.0497	1.5458	1.0693	1.1607
MAP1LC3A	− 1.5637	1.0081	− 1.0994	− 1.9816	− 1.9931	− 1.4044	− 1.158
MAP1LC3B	**− 2.2372**	1.6114	*4.3873*	− 1.5018	1.476	− 1.2512	1.5856
MAPK14	− 1.2643	− 1.0509	1.1096	− 1.2226	**− 2.2894**	− 1.3883	− 1.0681
MAPK8	*2.3107*	1.1742	1.7983	1.4845	− 1.3835	1.2894	1.1057
MTOR	− 1.1554	− 1.8639	**− 2.9349**	− 1.1408	− 1.8856	− 1.3226	− 1.279
NFKB1	**− 2.0777**	− 1.4658	**− 2.1535**	− 1.3348	− 1.374	− 1.4743	− 1.5929
NPC1	*2.7798*	1.4456	1.3441	*2.1238*	2.2894	1.639	*2.1165*
PIK3C3	1.9566	1.3426	− 1.6548	1.4109	1.1878	1.5837	− 1.5929
PIK3CG	*2.035*	1.1342	− 1.2834	1.7132	*2.8186*	1.0668	1.0035
PIK3R4	1.4126	1.0293	1.181	− 1.0425	1.3457	− 1.1755	**− 2.0162**
PRKAA1	1.6339	1.1032	− 1.4811	1.1251	− 1.3457	− 1.0305	− 1.1933
PTEN	1.1634	1.2128	− 1.0497	1.021	− 1.4828	− 1.4077	− 1.507
RAB24	− 1.0558	1.5746	*2.4566*	− 1.0644	1.3645	− 1.2512	− 1.1368
RB1	1.9386	1.2128	1.9274	1.6434	1.2731	1.5511	− 1.0607
RGS19	− 1.9793	− 1.788	**− 2.3511**	**− 3.1895**	**− 2.2736**	− 1.7777	− 1.528
RPS6KB1	1.3835	1.088	1.8361	1.1096	− 1.2556	1.0187	− 1.528
SNCA	1.1554	1.5929	*7.2434*	*4*	*3.208*	**− 2.3457**	1.5175
SQSTM1	− 1.429	− 1.5783	**− 2.2868**	− 1.2454	**− 2.0777**	− 1.2397	− 1.276
TGFB1	1.2297	1.5783	1.6548	1.0644	1.4224	1.4175	1.2354
TGM2	1.6339	1.0317	1.3629	1.2805	*2.0634*	1.2974	1.7431
TMEM74	1.4557	1.2731	*2.3894*	1.5801	− 1.6077	1.8834	1.3333
TNF	− 1.8987	− 1.2241	− 1.0994	− 1.4208	− 1.318	− 1.0521	1.0246
TNFSF10	− 1.1715	− 1.2819	**− 5.439**	− 1.8234	**− 2.7606**	− 1.7092	**− 2.5521**
TP53	− 1.2731	− 1.0956	− 1.021	− 1.5157	**− 6.1831**	− 1.0743	− 1.5819
ULK1	− 1.9119	1.0855	− 1.057	**− 2.3241**	**− 2.0922**	− 1.4983	− 1.6606
ULK2	− 1.5245	*2.3977*	− 1.1329	1.1303	− 1.5892	1.1199	1.3676
UVRAG	− 1.6453	1.4389	1.2114	− 1.1892	− 1.9977	1.5404	− 1.1688
WIPI1	− 1.0198	1.5458	1.6548	− 1.1487	− 1.4624	− 1.0234	1.0534

The p values are calculated based on Student's t test of the replicate $2^{(-\text{Delta Ct})}$ values for each gene in the control group and treatment groups and p values less than 0.05 are indicated in italics for genes with 2-fold increase and bold for genes with − 2-fold decrease ($n = 3$)

Discussion

In the current experiment, we aimed to investigate the combined effect of Res and Sulin on the angiogenesis potential of CSCs over a period of 7 days. We also monitored the activity of the Wnt-3a signaling effector on endothelial differentiation of CSCs. Based on data from MTT assay, a decreased cell survival rate was found after cell exposure to Sulin and Res. Consistent with our results, Yamamoto and colleagues showed reversible cytotoxic effects of Sulin on colon cancers. They reported that Sulin could inhibit the cancer cell proliferation rate governed by inducing death receptor TNF-α and expression of NF-κB gene (Yamamoto et al. 1999). Evidence points to short-term effects of Sulin on the progression of familial adenomatous polyposis. The inhibition of COX-2 and β-catenin coinciding with the dysregulation of KRAS factor could yield these effects (Rezabakhsh et al. 2017). Commensurate with these descriptions, we used the combined regime of Sulin and Res to control the dynamics growth and angiogenesis potential of colon CSCs. In a pioneer work done by Reddivari et al., the dynamic growth of CSCs was blunted after exposure to the mixture of Sulin and grape Res. These effects were promoted by the modulation of the Wnt/β-catenin signaling pathway, pGSK3-β (cytoplasmic) and cell cycle checkpoints, notably cyclin D1 (Reddivari et al. 2016). They also stated that the proliferation rate and clonogenic property of colon cancer CSCs were profoundly aborted after transplantation into mouse model. Noteworthy, a cell type–specific response has been shown against Res in in vitro condition, showing the selective tissue and cell phenotype–dependent activity of Res. For instance, Res potently could hamper the lipogenesis pathways in different human cancer cell lines named MDA-MB231, MCF7 and 231LM, but no inhibitory effects were found in HMEC and MCF10A cell lines (Pandey et al. 2011; Wang et al. 2012). The metabolic activity of different glioma stem cell lines was decreased after exposure to Res in a dose-dependent manner (Cilibrasi et al. 2017). Despite the potent effect of Res on ovarian CSCs, from human ovarian cancer cell line A2780, it was reported that Res was unable to exert any detrimental effect on IMR90 normal human fibroblasts (Fu et al. 2014; Seino et al. 2015). We further investigated the combined regime of Res and Sulin on endothelial differentiation of HT-29 CSCs. Compared with HT-29 cancer cells, the incubation of HT-29 CSCs with endothelial induction medium enriched with Res and Sulin promoted endothelial-like phenotype by upregulating VE-cadherin and vWF. These data support a notion that the multipotentiality of CSCs facilitates the endothelial differentiation compared with HT-29 cancer cells. In line with our observations, Campagnolo et al. showed an increased endothelial differentiation in Res-treated vascular progenitor cells by engaging via modulation of the MiR-21/Akt/β-Catenin signaling pathway

(Campagnolo et al. 2015). It seems that mesenchymal-epithelial transition could possibly decrease CSC resistance in response to different phyto-compounds such as Res (Du and Shim 2016). The great potential of Res and its combination with Sulin decreased the transcription of ZEB1, ZEB2 and vimentin genes as compared with non-stem cancer HT-29 cells. These modulations point to the great potential of Res in reducing cancer cell multipotentiality by modulation of stemness-related genes playing a critical role in EMT transition and invasive behavior (Zhang et al. 2015). The difference in the expression pattern of ZEB genes with vimentin could be related to different signaling pathways activated after exposure to Res or the conditions supplemented with Res and Sulin. The lack of significant differences in cancer HT-29 cells pre- and post-treatment with Res or in condition with Res and Sulin showed the cell resistance response even after incubation with endothelial induction medium, EGM-2. Consistent with the results from western blotting, we found that the combined regime of Res and Sulin reduced the intracellular content of Wnt-3a in CSCs and HT-29 cells in the normal medium. However, a high level of Wnt-3a factor was observed in the endothelial-specific medium while most inhibition effects were observed in CSCs under normal condition. Hope and colleagues found that a low concentration of Res could blunt the expression of Wnt-3a (Hope et al. 2008). Another finding in the current experiment is related to the induction of angiogenic factor YKL-40 in CSCs undergone the differentiation procedure with Res, indicating the potent role of angiogenic response in cancer stem cells by an increased mesenchymal transition to endothelial-like cells. Similar to the dynamic of factors and panel analysis, these features were not prominent in HT-29 cancer cells described for CSCs. Interestingly, Res and Sulin were able to suppress the synthesis of YKL-40 in an EGM-2-free condition. It seems that there is a close relationship between the dynamic of Wnt-3a and YKL-40 in CSCs.

Conclusion

Consistent with our results, Zhang and co-workers described an anti-angiogenic property of Res on human glioma cell tumor by repressing YKL-40 (Zhang et al. 2010). We also found that the treatment of CSCs with Res reduced cell distribution of GALNT11 after 7 days but was more evident in cells from the EGM-2 medium. According to data, the GALNT11 enzyme is correlated with cancer cell resistance to chemotherapeutic agents (Krushkal et al. 2017). The suppression of GALNT11 in CSCs after exposure to Res could infer the reduction of CSC resistance to chemotherapeutic agents. These data support a notion that the CSC resistance was decreased during endothelial differentiation and exposure to Res.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Akbarzadeh M, Movassaghpour AA, Ghanbari H, Kheirandish M, Maroufi NF, Rahbarghazi R, Nouri M, Samadi N (2017) The potential therapeutic effect of melatonin on human ovarian cancer by inhibition of invasion and migration of cancer stem cells. Sci Rep 7:17062

Bomken S, Fišer K, Heidenreich O, Vormoor J (2010) Understanding the cancer stem cell. Br J Cancer 103:439–445

Buhrmann C, Yazdi M, Popper B, Shayan P, Goel A, Aggarwal BB, Shakibaei M (2018) Resveratrol chemosensitizes TNF-β-induced survival of 5-FU-treated colorectal cancer cells. Nutrients 10:888

Campagnolo P, Hong X, di Bernardini E, Smyrnias I, Hu Y, Xu Q (2015) Resveratrol-induced vascular progenitor differentiation towards endothelial lineage via MiR-21/Akt/β-catenin is protective in vessel graft models. PLoS One 10:e0125122

Cilibrasi C, Riva G, Romano G, Cadamuro M, Bazzoni R, Butta V, Paoletta L, Dalprà L, Strazzabosco M, Lavitrano M (2017) Resveratrol impairs glioma stem cells proliferation and motility by modulating the Wnt signaling pathway. PLoS One 12:e0169854

Della Corte CM, Fasano M, Papaccio F, Ciardiello F, Morgillo F (2014) Role of HGF-MET signaling in primary and acquired resistance to targeted therapies in cancer. Biomedicines 2:345–358

Desiderio V, Papagerakis P, Tirino V, Zheng L, Matossian M, Prince ME, Paino F, Mele L, Papaccio F, Montella R, Papaccio G, Papagerakis S (2014) Increased fucosylation has a pivotal role in invasive and metastatic properties of head and neck cancer stem cells. Oncotarget 6:71–84

Druzhkova I, Ignatova N, Prodanets N, Kiselev N, Zhukov I, Shirmanova M, Zagainov V, Zagaynova E (2018) E-cadherin in colorectal cancer: relation to chemosensitivity. Clin Colorectal Cancer. https://doi.org/10.1016/j.clcc.2018.10.003

Du B, Shim JS (2016) Targeting epithelial–mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules 21:965

Evans C, Morrison I, Heriot A, Bartlett J, Finlayson C, Dalgleish A, Kumar D (2006) The correlation between colorectal cancer rates of proliferation and apoptosis and systemic cytokine levels; plus their influence upon survival. Br J Cancer 94:1412–1419

Fan F, Samuel S, Evans KW, Lu J, Xia L, Zhou Y, Sceusi E, Tozzi F, Ye XC, Mani SA (2012) Overexpression of Snail induces epithelial–mesenchymal transition and a cancer stem cell-like phenotype in human colorectal cancer cells. Cancer Med 1:5–16

Fu Y, Chang H, Peng X, Bai Q, Yi L, Zhou Y, Zhu J, Mi M (2014) Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/β-catenin signaling pathway. PLoS One 9:e102535

Harrison DE, Lerner CP (1991) Most primitive hematopoietic stem cells are stimulated to cycle rapidly after treatment with 5-fluorouracil. Blood 78:1237–1240

Hope C, Kestutis P, Marina P, Moyer MP, Johal KS, Woo J, Santoso C, Hanson JA, Holcombe RF (2008) Low concentrations of resveratrol inhibit Wnt signal throughput in colon-derived cells: implications for colon cancer prevention. Mol Nutr Food Res 52:S52–S61

Huang F, Wu X-N, Chen J, Wang W-X, Lu ZF (2014) Resveratrol reverses multidrug resistance in human breast cancer doxorubicin-resistant cells. Exp Ther Med 7:1611–1616

Jia Z-f, Huang Q, C-s K, W-d Y, G-x W, S-z Y, Jiang H, P-y P (2010) Overexpression of septin 7 suppresses glioma cell growth. J Neuro-Oncol 98:329–340

Krushkal J, Zhao Y, Hose C, Monks A, Doroshow JH, Simon R (2017) Longitudinal transcriptional response of glycosylation-related genes, regulators, and targets in cancer cell lines treated with 11 antitumor agents. Cancer Informat 16:1176935117747259

Pandey PR, Okuda H, Watabe M, Pai SK, Liu W, Kobayashi A, Xing F, Fukuda K, Hirota S, Sugai T (2011) Resveratrol suppresses growth of cancer stem-like cells by inhibiting fatty acid synthase. Breast Cancer Res Treat 130:387–398

Reddivari L, Charepalli V, Radhakrishnan S, Vadde R, Elias RJ, Lambert JD, Vanamala JK (2016) Grape compounds suppress colon cancer stem cells in vitro and in a rodent model of colon carcinogenesis. BMC Complement Altern Med 16:278

Rezabakhsh A, Cheraghi O, Nourazarian A, Hassanpour M, Kazemi M, Ghaderi S, Faraji E, Rahbarghazi R, Avci ÇB, Bagca BG (2017) Type 2 diabetes inhibited human mesenchymal stem cells angiogenic response by over-activity of the autophagic pathway. J Cell Biochem 118:1518–1530

Roselló S, Papaccio F, Roda D, Tarazona N, Cervantes A (2018) The role of chemotherapy in localized and locally advanced rectal cancer: a systematic revision. Cancer Treat Rev 63:156–171

Seino M, Okada M, Shibuya K, Seino S, Suzuki S, Takeda H, Ohta T, Kurachi H, Kitanaka C (2015) Differential contribution of ROS to resveratrol-induced cell death and loss of self-renewal capacity of ovarian cancer stem cells. Anticancer Res 35:85–96

Signorelli P, Ghidoni R (2005) Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. J Nutr Biochem 16:449–466

Wang S, Kanojia D, Lo P, Chandrashekaran V, Duan X (2012) Enrichment and selective targeting of cancer stem cells in colorectal cancer cell lines. Human Genet Embryol S2:2161–0436

Yamamoto Y, Yin M-J, Lin K-M, Gaynor RB (1999) Sulindac inhibits activation of the NF-κB pathway. J Biol Chem 274:27307–27314

Yao D, Dai C, Peng S (2011) Mechanism of the mesenchymal–epithelial transition and its relationship with metastatic tumor formation. Mol Cancer Res 9:1608–1620

Zhang W, Murao K, Zhang X, Matsumoto K, Diah S, Okada M, Miyake K, Kawai N, Fei Z, Tamiya T (2010) Resveratrol represses YKL-40 expression in human glioma U87 cells. BMC Cancer 10:593

Zhang R, Zhang P, Wang H, Hou D, Li W, Xiao G, Li C (2015) Inhibitory effects of metformin at low concentration on epithelial–mesenchymal transition of CD44(+)CD117(+) ovarian cancer stem cells. Stem Cell Res Ther 6:262

Zhou S, Schuetz JD, Bunting KD, Colapietro A-M, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7:1028–1034