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Original Article

The effects of valproic acid on the mRNA expression of Natriuretic Peptide Receptor A and KQT-like subfamily Q-1 in human colon cancer cell lines

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ABSTRACT

Aim and objectives: The histone deacetylase (HDAC) inhibitor, Valproic Acid (VPA), causes growth inhibition and apoptosis in colorectal cancer cells. HDAC inhibition is associated with the transcriptional regulation of Natriuretic Peptide Receptor-A (NPR-A). NPR-A regulates voltage-gated potassium channel, KQT-like subfamily Q, member 1 (KCNQ1). NPR-A and KCNQ1 are also involved in the initiation and propagation of cancer cells. In this study, we investigated the simultaneous expressional changes of NPR-A and KCNQ1 among VPA-treated colon cancer cells.

Materials and methods: Human colorectal cancer cells were cultured and treated with increasing concentrations of VPA at different time points. MTT viability test was conducted to evaluate the growth inhibition. Real Time RT-PCR was used to quantify differential mRNA expression of NPR-A and KCNQ1 genes. Two-way ANOVA and bonferroni post-tests were used to analyze data statistically.

Results: We showed that VPA treatment inhibits the growth of SW-480 cells more efficiently compared to HT-29. NPR-A and KCNQ1 genes were significantly upregulated upon VPA treatment in both cell lines ($P < 0.0001$).

Conclusion: The alteration of NPR-A and KCNQ1 genes were more ordered among SW-480 cancer cells. The expressional changes of KCNQ1 and NPR-A among VPA treated human colon cancer cells follow the same pattern in similar combinations. VPA could regulate the expression of KCNQ1 through altering the mRNA expression of NPR-A.

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1. Introduction

Colorectal cancer (CRC) encompasses a heterogeneous and tumor specific complex of diseases resulting from different alterations in genetic and epigenetic molecular pathways.[1] It is the third most common cancer and third leading cause of cancer death in the United States.[1] Modification of histones is an epigenetic process which is involved in gene regulation while inhibitors of Histone deacetylases (HDACs) are introduced as a novel therapeutic class of drugs against different cancer types.[2,3] HDAC inhibitors regulate the expression of genes which are involved in specific biological responses such as apoptosis, immune regulation and angiogenesis.[4–6] Valproic Acid (VPA) is a branched, short-chain fatty acid which acts as a HDAC1 and HDAC2a inhibitor. VPA is an established anti-epileptic drug[7] which is shown to induce differentiation and apoptosis in a variety of carcinoma cells.[8,9] VPA has been also introduced as an antiproliferative compound in CRC, alone or in combination with other therapeutic strategies.[10]

HDAC inhibitors modulate the transcriptional regulation of Natriuretic Peptide Receptor-A (NPR-A).[11,12] NPR-A is overexpressed in numerous human cancer cells including colon adenocarcinoma[13–17] and could be introduced as a novel anticancer target.[15,18] Atrial Natriuretic Peptide (ANP) is the specific ligand for NPR-A[19] which regulates the proliferation of human gastric cancer cells via K+ voltage-gated channel subfamily Q member 1 (KCNQ1; also known as LQT1).[20] KCNQ1 has been also introduced as a novel regulator of cancer cell proliferation and migration in different cancer types.[20–22] Moreover, HDAC inhibitors are reported to be capable of regulating the transcription of ATP sensitive K+ (KATP) channels,[23] which has not been reported among

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human cancer cells previously. In the present study, we investigated the effect of VPA as a HDAC inhibitor on the expression of NPR-A and KCNQ1 and also the possible simultaneous alterations of these genes in response to VPA in colon adenocarcinoma cancer cell lines.

2. Materials and methods

2.1. Cell culture and treatments

HT-29 and SW-480 human colon adenocarcinoma cells were purchased from National Cell Bank of Iran (Pasteur Institute, Iran) and cultured in RPMI 1640 (Gibco, Life Technologies, USA). HCT-116 and Caco-2 colon adenocarcinoma cells were also purchased from National Cell Bank of Iran (Pasteur Institute, Iran) and cultured in DMEM (Gibco, Life Technologies, USA). All cell culture media were supplemented with 10% FBS, 100U/ml penicillin and 100 μg/ml streptomycin. Tissue culture flasks were incubated in a fully humidified atmosphere at 37 °C with 5% CO_2. 0.5×10^6 cells/mL were counted and seeded in each well of 6-well tissue culture plates followed by resuspension in complete growth media and incubated for 24 h in order to keep cells in logarithmic phase growth. HT-29 and SW-480 cells were treated with the most antiproliferative concentrations of VPA (Sigma-Aldrich, USA) (1 mM, 2 mM and 4 mM) with the least cytotoxicity effects on normal cells.[24] Treated and non-treated cells were then incubated for different time points (24, 48 and 72 h). Finally, the cells were detached using 0.025% trypsin-EDTA (Gibco, Life Technologies, USA) for consequent RNA extraction.

2.2. Total RNA extraction and Real Time RT-PCR

Total RNA was extracted using Biozol (Bioer, China) according to the manufacturer's protocol. To remove any genomic DNA contamination, 1 microgram of total RNA was used for DNase I (CinnaGen, Iran) digestion and were reverse transcribed to cDNA using random hexamer primers (Bioron, Germany). Real-time RT-PCR was performed with Bioer Real-time PCR detection system (Bioer Technology, China) and Bioron SYBR green master mix (Bioron, Germany). Human 18S ribosomal RNA (18s rRNA) was used as a consistent internal control for gene expression normalization.[25] Gene specific primers which were designed to span exons for KCNQ1, NPR-A and 18s rRNA are summarized in Table 1. All data are presented as fold changes compared to non-treated cells.

2.3. Cell viability (MTT) assay

The cells were counted, seeded in 96-well plate (10^4 cells/well) and incubated for 24 h in complete culture media. The cells were then treated with different increasing concentrations of VPA (1 mM, 2 mM and 4 mM) at different time intervals (24, 48 and 72 h). After each time point, the complete culture media was removed and a final volume of 80 μl of Phenol Red free RPMI 1640 (Gibco, Life Technologies, USA) with 20 μl of MTT solution (5 mg/mL) were added to each well and incubated for 4 h at 37 °C with 5% CO_2. 100 μl DMSO was then added to each well as a cell lysis solution. Percentage of cell viability was assessed by spectrophotometry at 570 nm using ELx800 Absorbance Reader (Biotek, USA).

Table1
Gene specific primers used for Real Time RT-PCR.

Primer (accession)	Sequence (5′ > 3′)	T_m	Amplicon size (bp)
KCNQ1 (NM_000218.2)	TGTCCACCATCGAGCAGTATG CCGTCCCGAAGAACACCAC	61.5	84
NPRA (NM_000906.3)	GTCAACACAGCCTCAAGA CCTTTGCCCTTCATTTCTAC	60.1	136
18srRNA (M10098)	CAGCCACCCGAGATTGAGCA TAGTAGCGACGGGCGGTGTG	60.7	252

2.4. Statistical analysis

All of the experiments for each sample were repeated in triplicates and data were demonstrated as means ± SE (Standard Error). Statistical software SPSS22.0 and Graphpad Prism 5.04 were used for data analysis. Two-Way ANOVA with Bonferroni post hoc test was used for comparing means of multiple samples. P-values lower than 0.05 were considered as statistically significant.

3. Results

3.1. The mRNA expression level of NPR-A and KCNQ1 among different human colon cancer cell lines

The expression levels of NPR-A and KCNQ1 were quantified among four different human colon adenocarcinoma cell lines including HCT-116, SW-480, HT-29 and Caco-2. NPR-A was overexpressed in SW-480 and HT-29 cells in comparison to HCT-116

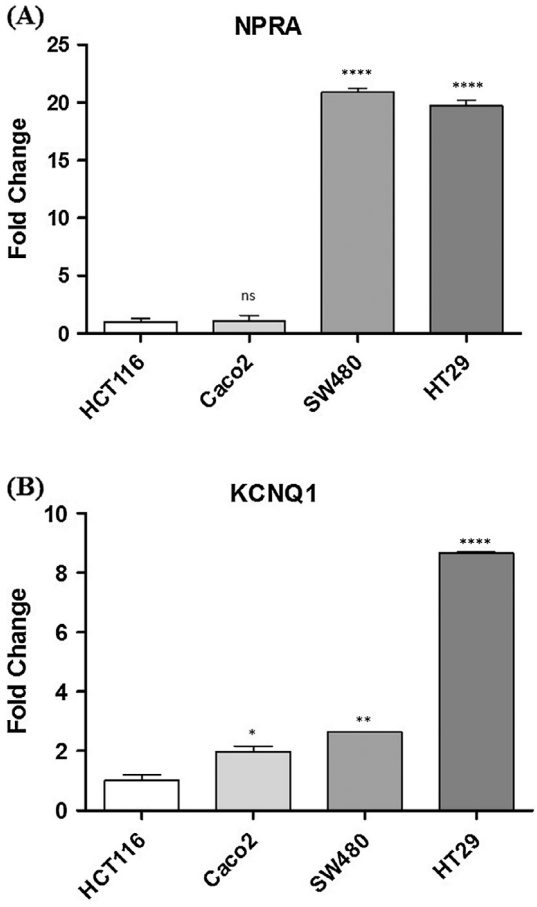

Fig. 1. The mRNA expression level of NPRA and KCNQ1. NPRA and KCNQ1 are overexpressed in HT-29 (A) and SW-480 (B) cell lines in comparison to other human colon cancer cells. Data are expressed relatively to mRNA levels in HCT 116 cell line, arbitrarily set at the value of 1. Data are obtained from experiments in triplicates and presented as means ± SE. Significant differences are evaluated using independent samples *t*-test. P-values lower than 0.05 are considered statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns: not significant.

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