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Original article

A novel all-trans retinoid acid derivatives inhibits the migration of breast cancer cell lines MDA-MB-231 via myosin light chain kinase involving p38-MAPK pathway☆

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ABSTRACT

Objective: To explore the effect and its probable mechanism of a synthetic retinoid 4-amino-2-trifluoromethyl-phenyl ester (ATPR) on the migration of human breast cancer MDA-MB-231 cells.
Methods: MTT assay was performed to measure the proliferation of MDA-MB-231 cells treated with different concentrations of all-trans retinoic acid (ATRA) and ATPR. The effect of ATPR and ML-7, a selective inhibitor of myosin light chain kinase (MLCK), and SB203580, an inhibitor of p38, on the migration of MDA-MB-231 cells were analyzed by wound healing assay. The expression of MLCK and phosphorylation of myosin light chain (MLC), ERK, JNK, p38 proteins were detected by western blot
Results: After the cells were treated by ATRA and ATPR, the proliferation and migration of breast cancer MDA-MB-231 cells were inhibited significantly. The IC 50 of ATRA and ATPR is 34.08 μmol/l and 18.06 μmol/l respectively. The relative migration rate of MDA-MB-231 cells treated with ATPR reached 50% at 48 h while the ATRA group is over 90%. The relative migration rate of ML-7 group and SB group had significant decrease compared with control group. The expression level of MLCK and phosphorylation of MLC of breast cancer cells was reduced when the cells were treated by ATPR with 48 h, the phosphorylation of ERK, JNK and p38 in breast cancer also reduced when cells were treated by ATPR with 2 h. In addition, ML-7 (50 μmol/l) could inhibit the phosphorylation of p38 and SB (50 μmol/l) could inhibit the expression of MLCK and phosphorylation of MLC.
Conclusions: ATPR had a better inhibition on the proliferation and the migration of breast cancer MDA-MB-231 cells than ATRA, and its probable mechanism was associated with the down regulation of expression of MLCK and phosphorylation of MLC protein involving p38-MAPK pathway.

1. Introduction

Breast cancer is the most common cancer among women around world. Each year, it is diagnosed at an estimated 1 million women worldwide, and is the cause of death of over 400,000 [1]. The incidence of breast cancer increases with ages and doubles every 10 years until the menopause, supporting a link with hormonal status [2]. Vitamin A and retinoids are essential nutrients for a large variety of biological processes, particularly the differentiation of epithelia [3]. Cell migration is a biological process that contributes crucially to a variety of physiological, wound healing and the inflammatory reaction. Moreover, cell migration is also responsible for the malignance of cancer as it allows tumor cells to invade the surrounding tissues, thereby forming metastases [4]. Recent studies demonstrated that MLCK dependent increases in myosin ATPase activity to play a critical role in non-muscle cell protrusion, contraction, and migration [5]. MLCK is a key Ca^{2+}/Calmodulin (CaM)-dependent effector that is responsible for smooth muscle cell and non-muscle cell migration via phosphorylation of Ser19, Thr18 on myosin light chains (MLC), an event that facilitates myosin interaction with actin filaments [6]. Resent studies have shown that both activated myosin II and its activator MLCK are enriched in lamellar protrusive structures in several cell types during migration [7]. Reduction in MLCK expression via antisense techniques produces fibroblast cell rounding and decreased proliferation and attenuates chemo-attractant-stimulated cell locomotion [8]. These observations directly implicate that MLCK in the signaling pathways controls the non-muscle cell motility.

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However, the exact mechanical of roles of MLCK during cell migration remain remarkably poorly understood.

MAPK pathways play key roles in cell proliferation, differentiation, and survival [9]. The closely related MAPK pathways are regulated through a series of phosphorylation steps in a three-component module: MAPKs are activated by MAPK kinases (MAPKK) on dual residues of threonine and tyrosine, and MAPKKs are in turn phosphorylated by MAPKK kinases (MAPKKK) on dual residues of serine/threonine. MAPKs have been divided into three main groups: the extracellular-regulated kinases 1/2 (ERK1/2 or MAPK p44/42), MAP 38, and the c-jun-N-terminal kinases (JNK). ERK1/2 is typically activated by the growth factors and cytokines via TKR and its downstream effectors following Ras, Raf, and mitogen-activated protein kinase kinase (MAPKK or MER) [10]. This group is known to mediate mitogenic and antiapoptotic effects [11]. p38 and JNK respond to the pro-inflammatory cytokines, cellular stress, heat shock, and hyper-osmolarity and are usually connected with anti-proliferation and promotion of apoptosis [12].

Retinoids, synthetic and natural analogs of vitamin A, play a well-characterized role in cancer development, cell differentiation, and cell growth [13]. ATRA has been reported to inhibit cell cycle progression and induce apoptosis in many tumor cell lines [14]. Retinoids have also been used in a number of clinical studies to investigate the therapeutic effect in a variety of tumors, including human breast cancer, and have been described to inhibit the growth of several human hormone-dependent breast cancer cell lines [15].

However, whether some ATRA retiamide derivatives affect the migration of breast cancer via MLCK and through which pathway is unknown. Therefore, the present study was undertaken to investigate the effect of a novel ATRA retiamide derivative, 4-amino-2-tri-fluoromethyl-phenyl ester (ATPR), on the migration of breast cancer and assess the expression of MLCK and the function of p38/MAPK signal transduction pathway.

2. Material and methods

2.1. Materials

ATRA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical (USA). Dulbeccos modified Eagles medium (DMEM) was obtained from Gibco BRL life Technologies (USA). ATPR was provided by school of Pharmacy, Anhui Medical University (Anhui, China). ML-7 and SB203580 were purchased from Cayman Chemical (USA). MDA-MB-231 cells were obtained from the American Type Culture Collection (USA). Bovine serum was purchased from the Zhejiang Tianhang Biological Technology Co (China). Primary antibodies (anti-MLCK, anti-p-MLC, anti-MLC, anti-p-ERK, anti-ERK, anti-p-JNK, anti-JNK, anti-p-p38, anti-p38, anti-β-actin) were purchased from Santa Cruz Biotechnology (USA). All secondary antibodies were purchased from MILLIPORE (USA).

2.2. Cell lines and cell culture

Breast cancer cell lines MDA-MB-231 was maintained in DMEM medium supplemented with 10% fetal bovine serum and 100 U/mL penicillin, 100 U/mL streptomycin in humidified 5% CO_2 at 37 °C. The media were changed every two or three days.

2.3. Cell viability assay

Cell viability was measured using the MTT assay. MDA-MB-231 cells (5×10^3cells/well) were seeded into 96-well plates and cultured. The cells were treated with different times (24 h, 48 h, 72 h) and different concentration of ATRA and ATPR (10, 20, 30, 40, 50, 60, 70 μmol/L), then incubation with MTT solution for 4 h, finally, the cells were exposed to an MTT-formazan dissolving solution (DMSO) for 30 minutes. The optical density (OD) was measured using an absorbance microplate reader (Bio-Tek, ELX800) at a wavelength of 490 nm. The cell viability was expressed as a percentage of the OD value of the control cultures.

Table 1
The effect of different concentrations of ATRA and ATPR on the proliferation of MDA-MB-231 CELL in 2 day.

	$OD_{570\ nm}$	Inhibition rate
Cell control	0.499 ± 0.025	–
DMSO control	0.396 ± 0.034	0.205
ATRA		
10	0.439 ± 0.014	0.119*
20	0.446 ± 0.025	0.107*
30	0.389 ± 0.028	0.219*
40	0.146 ± 0.018	0.708*
50	0.104 ± 0.013	0.791
60	0.116 ± 0.006	0.767*
70	0.115 ± 0.006	0.769*
Cell control	0.554 ± 0.033	–
DMSO control	0.413 ± 0.018	0.254
ATFMPE		
10	0.474 ± 0.025	0.145*
20	0.128 ± 0.009	0.768*
30	0.115 ± 0.005	0.793*
40	0.109 ± 0.011	0.802*
50	0.124 ± 0.004	0.776
60	0.113 ± 0.007	0.795*
70	0.133 ± 0.010	0.759*

Compared with control group, $^*P < 0.05$.

2.4. Wound healing assay

Migration of MDA-MB-231 Cells was measured using the in vitro wound-healing assay. Cells were seeded into 24-well plates and grown to 100% confluence. Wounds were created by scraping monolayer cells with a sterile pipette tip. At 0, 24, 48 h after the creation of wounds, cells were observed with 10 × objective in an Olympus (Olympus Corporation, Tokyo, Japan) photomicroscope. Images were acquired with a Nikon (Tokyo, Japan) color digital camera. Wound distances were measured at each time point and expressed as the average percent of wound closure by comparing the zero time.

Table 2
The effect of different concentrations of ATRA and ATPR on the proliferation of MDA-MB-231 CELL in 2 day.

	μmol/L	OD ($\bar{x} \pm s$)	Inhibition ratio
Cell control		0.838 ± 0.014	–
DMSO control		0.798 ± 0.011	0.048
ATRA			
	10	0.786 ± 0.011	0.041*
	12.5	0.804 ± 0.003	0.040*
	15	0.803 ± 0.009	0.042*
	17.5	0.855 ± 0.007	−0.020*
	20	0.844 ± 0.019	−0.007
ATPR			
	10	0.855 ± 0.007	−0.020*
	12.5	0.691 ± 0.032	0.176*
	15	0.351 ± 0.016	0.582*
	17.5	0.199 ± 0.003	0.763*
	20	0.187 ± 0.002	0.777*

Compared with control group, $^*P < 0.05$.