Experimental study of retinoic acid on improving iodide uptake in MCF-7 breast cancer cells

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Abstract The study aims to investigate the effect of retinoic acid on the iodide uptake of MCF-7 cells and its mechanism. The iodide uptake and expression of hNIS(human sodium/iodide symporter)mRNA in the breast cancer MCF-7 cells were compared individually before and after the intervention of all-trans retinoic acid (ATRA) with the iodide uptake assay and RT-PCR. The following results are obtained: (1) when treated with all-trans retinoic acid in the concentration of 1.0 μmol/L, the capacity of iodide uptake of MCF-7 cells reached about 1.5 times of the basal state; (2) 12 h after the intervention of 1.0 μmol/L ATRA, the hNISmRNA expression of the MCF-7 cells reached maximum. The study shows that all-trans retinoic acid has the effect to improve the iodide uptake of the MCF-7 cells and this effect may result from its up-regulation of the hNISmRNA expression.

Key words Breast cancer, Iodide uptake, hNIS, All-trans retinoic acid

CLC numbers R817.8, R737.9

1 Introduction

Breast cancer, which has a high incidence, is a malignant tumor that threatens the health of woman heavily. The phenomenon of iodide uptake in the breast cancer cells was found already. But the low uptake of iodide by the breast cancer cells cannot be used in the clinical diagnosis or radioiodine therapy. In this study, all-trans retinoic acid was used to test whether it can improve the iodide uptake in the breast cancer MCF-7 cells.

2 Materials and methods

2.1 Materials

2.1.1 Cells and culture conditions

MCF-7 cell lines, supplied by Institute of Biochemistry and Cell Biology (IBCB), Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, were adopted in this study. This cell line was an estrogen receptor-positive human breast cancer cell line which could grow at 37℃ in a humidified atmosphere of 5% CO_2 as a monolayer in RPMI 1640 medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen), 1% penicillin-streptomycin (Invitrogen) and insulin. MCF-7 cells (1×10^4cells/cm^2) were placed in plastic dishes. Culture media was replaced every 2 to 3 days. Cells could reach confluence in about 4 days. Confluent culture was then split 1:2 or 1:4 using 0.25% trypsin/EDTA.

2.1.2 Reagents

The major reagents were supplied by Shanghai Huamei Company. All-trans retinoic acid was supplied by Sigma Company. $Na^{125}I$ solution was supplied by Shanghai Institute of Radioimmuno Assay Technology. The RT-PCR primer was synthesized by Shanghai Hujin Biological Technological Co. Ltd.

2.1.3 Instruments

CO_2 cell incubator, SN-695 automatic γ counter, TC-96/T/H(a) gene amplifier, DY-501B electrophoresis and GIS-2008 gel imaging system were used in this study.

2.2 Methods

2.2.1 Experimental protocol

MCF-7 cells were divided into different experimental groups and control group according to the

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ATRA concentration in the culture media. The concentration of the ATRA in the culture media of the experimental groups was 1.0 μmol/L. The experimental groups were classified into 3, 6 ,12 ,24 ,48 and 72 h sub-groups according to the interfering time of the ATRA. The concentration of the ATRA in the culture media of the control group was 0 μmol/L.

2.2.2 Iodine uptake study

The MCF-7 cells grew in culture in the presence of ATRA (1.0 μmol/L) for 0, 6, 12, 24, 48 and 72h (experimental group) or in the absence of ATRA (control group). For the assay, the cells were harvested using 0.25% trypsin/EDTA and washed with 0.5 mL HBSS containing 3.7kBq carrier-free $Na^{125}I$ and 10 μmol/L NaI, and incubated for 2 h at 37℃. The specific activity under these conditions was 740 MBq/mmol. After incubation for different periods of time (5, 30, 60, 90 and 120 min), the cells were washed twice with 2 mL ice-cold HBSS and digested with 1 mL PBS containing 0.25% trypsin/EDTA without Ca^{++} and Mg^{++}. At last, radioactivity was measured with a γ-counter. Results were expressed as the amount of accumulated iodide in "pmol/10^6 cells".

2.2.3 RT-PCR

(1) Primer

The primer for hNIS gene was designed according to the hNIS cDNA sequence reported by Smanik et al. The upstream sequence of hNIS primer is 5'-CTC CTC CCT GCT AAC GAC TC-3' and the downstream sequence is 5'-GAC CAC CAT CAT GTC CAA CA-3'. The product of the amplification is a 415bp polypeptide. The primer for GAPDH was purchased from the manufacturers and used according to their recommendation. The upstream sequence of GAPDH primer is 5'-ACC ACA GTC CAT GCC ATC AC-3' and the downstream is 5'-TCC ACC ACC CTG TTG CTG TA-3'. The product of the amplification is a 452bp polypeptide.

(2) Sample collecting and total RNA isolation

The cells were harvested using 0.25% trypsin/EDTA and washed with PBS for twice. Total RNA from the MVF-7 cell culture was isolated by the acid-guanidinium-phenol-chloroform method and stored at −70℃.

(3) RT-PCR

Complementary DNA (cDNA) was synthesized from 2.0 μg total RNA using Meloney murine leukemia virus reverse-transcriptase with random primers. Each PCR vessel contained 39.5 μL sterile water, 5 μL 10×buffer, 1 μL dNTP, 2 μL primer (1 μL for upstream and 1 μL for downstream), 2 μL template and 0.6 μL Taq DNA polymerase. Amplification conditions for hNIS were denaturation at 94℃ for 2 min, 29 cycles of 94℃ for 30 s at 94℃ and 45 s at 56℃ and 1 min at 72℃, followed by extension at 72℃ for 6 min. Amplification conditions for GAPDH were denaturation at 94℃ for 2 min, 29 cycles of 94℃ for 30 s at 94℃ and 45 s at 61℃ and 1 min at 72℃, followed by extension at 72℃ for 6 min. The RT-PCR products (10 μL) were resolved on 1.5% agarose gels and visualized by ethidium bromide staining.

(4) Semi-quantitaive analysis

The relative content of hNIS gene was calculated through the correction of GAPDH gene content.

3 Results

3.1 Growth state of breast cancer MCF-7 cells

The MCF-7 cells started to adhere to the bottom of the flask 12 h later after the cells were seeded. 24 h later, the major part of the cells became adhered. The cells became larger, thin cytoplasmic dendritic prolongations were shown because of the proliferation of the spindle cells, and the cells changed from spindle to polygonal in shape morphologically (Fig.1).

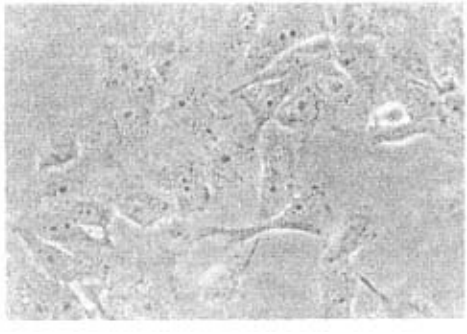


Fig.1 The growth state of the breast cancer MCF-7 cells.

3.2 Basal iodine uptake of MCF-7 cells

In the basal state which means having no intervention of all-trans retinoic acid, the breast cancer MCF-7 cells had the ability to uptake iodide. But this kind of uptake is very low. When the MCF-7 cells were cultured in the media containing iodide, the

MCF-7 cells began to uptake iodide from the 5th min. The uptake of iodide of MCF-7 cells reached maximum at the 30th min. The uptake content began to decrease at the 60th min. (see Table 1).

Table 1 Basal iodine uptake of MCF-7 cells

Time (min)	$Na^{125}I$ uptake (min^{-1})	NaI uptake (pmol/10^6cells)
5	127.60±7.23	10.63±0.60
30	536.00±14.27	44.67±1.19
60	425.20±18.63	35.43±1.55
90	388.80±15.55	32.40±1.29
120	277.00±10.68	23.08±0.89

3.3 Effect of all-trans retinoic acid on iodide uptake of breast cancer MCF-7 cells

When the breast cancer MCF-7 cells were treated with all-trans retinoic acid in the concentration of 1.0 μmol/L, the capacity of iodide uptake of MCF-7 cells began to increase after 6 h. Its iodide uptake reached about 1.5 times that of the basal state after 48 h (Fig.2).

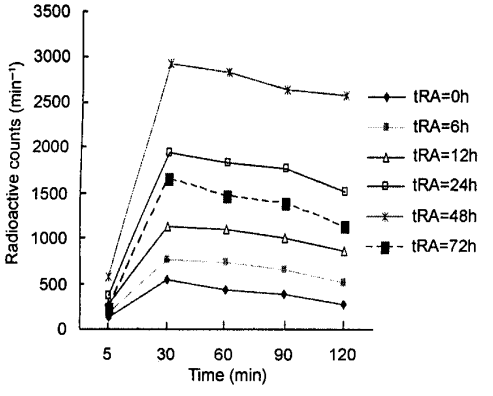
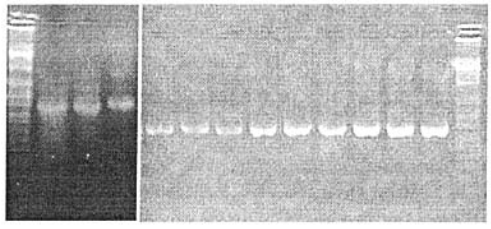


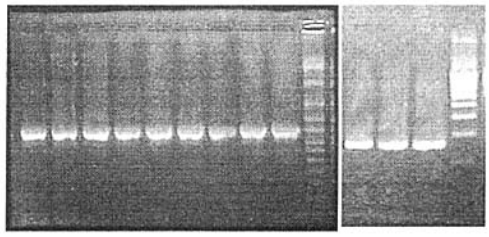
Fig.2 Effect of all-trans retinoic acid on iodide uptake of MCF-7 cells.

3.4 Effect of all-trans retinoic acid on the hNIS mRNA expression of MCF-7 cells

Three hours after the intervention of 1.0 μmol/L ATRA, the hNISmRNA expression of the MCF-7 cells began to rise, reached maximum after 12 h, began to decrease after 24 h, and reached about half of the maximum after 72 h (Fig.3 and Table 2).



Note: lanes 2-4, control group; lanes 5-7, 3h sub-group; lanes 8-10, 6h sub-group; lanes11-13, 12h sub-group; lanes 1and14, marker;



Note: lanes 1-3, 24h sub-group; lanes 4-6, 48h sub-group; lanes 7-9, 72h sub-group; lanes 10 and 14 ,marker; lanes 11-13, GAPDH

Fig.3 Gel electrophoresis of hNIS mRNA after ATRA intervention on MCF-7 cells.

Table 2 The maximum iodide uptake and hNISmRNA expression in different groups of MCF-7 cells

Group	Maximum uptake(pmol/10^6 cells)	hNISmRNA
0 h	44.67±1.19	0.1024±0.0148
3 h		0.2950±0.0149
6 h	64.07±1.45	0.5603±0.0393
12 h	94.23±1.89	0.7954±0.0186
24 h	161.53±1.97	0.6488±0.0213
48 h	244.57±2.46	0.5219±0.0237
72 h	137.45±1.82	0.3665±0.0266

Note: The maximum iodide uptake and hNISmRNA expression of the experimental groups were higher than that of the control group, $p<0.01$.

4 Discussion

The thyroid is not the unique tissue that has the ability of radioiodide uptake. This ability has also been demonstrated in some other extrathyroidal tissues. It was reported 40 years ago that the concentration of iodide in the lactating breast tissue of the mice is 6-15 times more than that in the blood plasma, only inferior to that in the thyroid gland[1]. It was also reported years ago that hormone dependent breast cancer also has the ability of iodide uptake. The concentration of iodide in this kind of breast cancer tissue can be 5-7 times of that in the blood plasma.[2,3]

Transport of iodide into thyroid cells is mediated

by a transmembrane carrier protein, the sodium/iodide symporter (NIS) first reported by Dai et al. in 1996.[4] NIS protein simultaneously transports two different ions, sodium and iodide, in the same direction across the plasma membrane of thyroid cells. Tazebay[5] indicated that the NIS is responsible for iodide transport into epithelial cells of the normal lactating breast and breast cancer cells.

It is found in this study that all-trans retinoic acid can upregulate the NIS mRNA expression and thus improve the ability of iodide uptake in estrogen receptor positive human breast cancer cell line (MCF-7). The iodide uptake ability can be improved about 5.5 times maximal in MCF-7 cells when they have been induced by all-trans retinoic acid for 48 h with the concentration of 1 μmol/L.

Retinoic acid (RA) plays an important role in development, differentiation, and cell growth. The action of RA is mediated through two families of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs).[6] Both RARs and RXRs are expressed in MCF-7 cells, an estrogen receptor (ER)-positive human breast cancer cell line. In clinical studies, tRA and its analogues have proved useful for treatment of a number of cancers.

In this study, we have found that all-trans retinoic acid has the effect to improve the iodide uptake of the MCF-7 cells and this effect may result from its up-regulation of the hNISmRNA expression. The effect of all-trans retinoic acid on the normal breast cells has not been studied in this study. So, much work is needed to testify whether uptake of iodide in breast tumors will be of clinical use. Unlike thyroid cancer patients, most breast cancer patients have functioning thyroids. The presence of a patient's normal thyroid will pose a challenge to the use of radioiodide to detect or treat breast tumors although the all-trans retinoic acid can improve the tumor cells' iodide uptake, because the thyroid will sequester nearly all the radioiodide until itself is destroyed.

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