THE EXPRESSION OF CONNEXIN GENES IN NASOPHARYNGEAL CARCINOMA CELLS AND THE EFFECT OF RETINOIC ACID ON THE REGULATION OF THOSE GENES

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ABSTRACT

Objective: To detect which members in the connexin gene family are expressed in nasopharyngeal carcinoma (NPC) cell line HNE_1, and the mechanism by which those genes are specifically switched on and off during retinoic acid (RA) induction. Methods: Establishing the cell growth curves of NPC cells. Observing the effect of RA on connexin genes by Northern hybridization. Results: Two genes Cx46 and Cx37, belonging to the connexin gene family, were expressed in HNE_1. The down-regulation of Cx46 and Cx37, up-regulation of RARa and growth inhibition was observed in HNE_1 after exposure to RA. The gene expression and cell growth in HNE_1 cells was restored after removal of RA. Conclusion: Two members of the connexin gene family: Cx37 and Cx46 were expressed in HNE_1 cells, RA can inhibit the expression of those two genes mediated by RARa, and the effects of RA on HNE_1 are reversible.

Key words: Connexin gene, NPC cell, Gene expression, Retinoic acid.

Gap junctional intercellular communication (GJIC) is a kind of intercellular communication system by which cells acquire information and energy from their surroundings and by which cells exchanged molecules and ions directly from the inside of one cell to that of neighboring cells. GJIC is mediated by gap junction channels, which are composed of connexin molecules. So far, 12 cDNAs coding for different connexin species have been cloned in rodent animals, and most of them have been found to be homologous in human [1].

Connexin gene expression has a certain tissue and developing stage specificity[2]. During recent years, researchers have focused on the relationship between the GJIC, connexin gene and the carcinogenesis. The lack or reduction of GJIC was found between tumor cells or between the tumor cell and the normal cell. Moreover, the expression of the connexin gene was much reduced in tumor cells in comparison with matched normal cells[3,4] and carcinogens can inhibit GJIC[5]. After transfecting connexin genes into GJIC-deficient cancer cells, the transfectants showed an increase in communication capacity and a decrease in tumorigenicity[6]. Similar phenomenon was found in some treated tumor cells, which were induced by a differential agent. The connexin gene was regarded as a second kind of tumor suppressor gene, since in tumor cells, there is less alteration in their structure but not in their expression.

Lee's study showed a more complicated picture with TPA treated MCF-10; TPA could up-regulate the expression of Cx26[3].

We know nothing about the expression and regulation of the connexin gene in nasophareyngeal carcinoma. In this study we aim to find the family members of the connexin gene that are expressed in nasophareyngeal carcinoma, and to investigate the effects of retinoic acids on the expression and regulation of the connexin gene in nasopharyngeal carcinoma cells. The study might shed a light on the role of the connexin gene in the etiology of nasophareyngeal carcinoma.

MATERIALS AND METHODS

Cell Culture and Treatment

The nasopharyngeal carcinoma cell line, HNE_1, a poorly differentiated squamous epithelium, was maintained in RPMI-1640 with 10% heat-inactivated fetal calf serum. In the RA-treated group, cells were incubated in the standard medium containing RA (Sigma, Saint Louis, MO, USA) (day 1). RA stock solution (10^{-2} mol/L RA in DMSO) was added to a final concentration of 10^{-6} mol/L–10^{-4} mol/L. Medium was changed every 72 h.

Cell Growth Kinetics

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Cell growth curves were established by seeding 2×10^5 cells in 25 ml glass culture bottles in 5 ml of standard medium with or without RA. Cells were treated with trypsin and counted under the microscope daily.

In Vitro Morphology

The RA-treated cellular morphology and growth patterns were examined every day. Using an IB-2 Olympus microscope (Olympus, Tokyo, Japan), we compared the induced cell phenotypes with untreated control cultures.

RNA Isolation and Gel Blot Analysis

Total cellular RNA was isolated using TRIzol Reagent (Life Technologies, Gaithersburg, MD, USA). For RNA gel blot analysis, 30 μg RNA was transferred to nylon filters and then hybridized sequentially with radio-labeled probes [7].

Probe

The probes: Cx26, Cx31.1, Cx32, Cx33, Cx37, Cx42, Cx43, Cx46 were granted by Dr. David Kiang (Breast Cancer Research Laboratory, Department of Medicine, University of Minnesota Medical School, Minneapolis, MN 55455, USA), and the RARa probe was granted by Professor Zhu Chen (Shanghai Institute of Hematology, Ruijin Hospital, Shanghai Second Medical University, People's Republic of China).

RESULTS

Members of the Connexin Gene Family: Cx37 and Cx46 Were Expressed in HNE_1 Cell

A 1.5 kb and a 1.6 kb of hybridization band were shown after the HNE_1 mRNA hybridized with Cx37 and Cx46 probe respectively. The same membrane re-hybridized with the GAPDH probe after the connexin gene probes were stripped (Figure 1).

Growth Kinetics

Cellular growth curves revealed that RA inhibited the HNE_1 cell growth depending on time. After being treated with 10^{-4} mmol/L RA for 5 days, the cell numbers decreased to 50% of the untreated cell numbers. At concentrations of 10^{-5} mmol/L and 10^{-6} mmol/L, RA had no effect on the growth of HNE_1 cell (Figure 2).

RA Could Inhibit the Expression of Cx37 and Cx46

RA could inhibit the expression of Cx37 and Cx46 in different ways. RA could inhibit the expression of Cx46 completely after RA treated HNE_1 cells for 1 day, but RA inhibited expression of Cx37 depending on time (Figure 3).

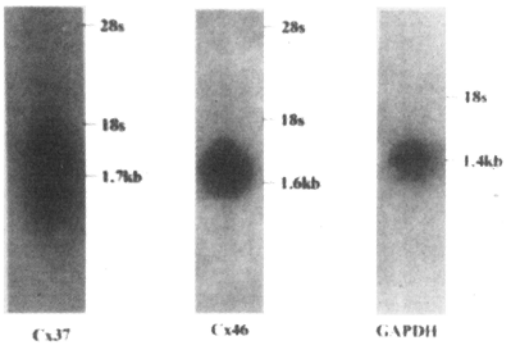

Fig. 1. The expression of connexin genes in HNE_1 cells

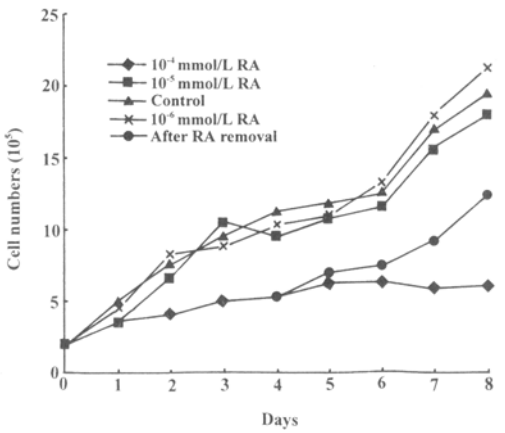

Fig. 2. Cell growth curve of HNE_1 cells exposure to RA

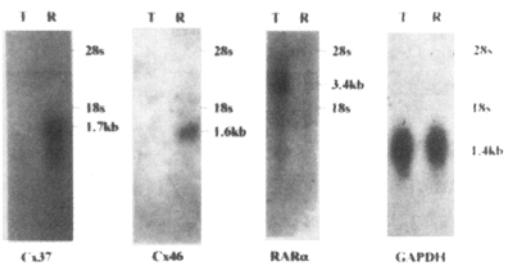

Fig. 3. The effect of RA on gene expression of HNE_1 cells
T1, T2, T8: HNE_1 treated with RA on day 1, 2, 8
C1: control

RA Induced the Expression of RAR α

Northern hybridization showed that RA could induce the expression RAR α. After HNE_1 cells were treated by RA for 1 day, RA could highly increase the expression of RAR α with a stable level in the following several days (Figure 3).

The Gene Expression and Cell Growth in HNE_1 Cell Were Restored after Removal of RA

After HNE_1 cells were treated by RA for 4 days, the standard medium containing RA was replaced by the standard medium in an aliquot of cell. Cell growth curve and Northern hybridization showed that cell growth in HNE_1 cell and the expression of Cx37, Cx46 an RAR α were restored (Figure 4).

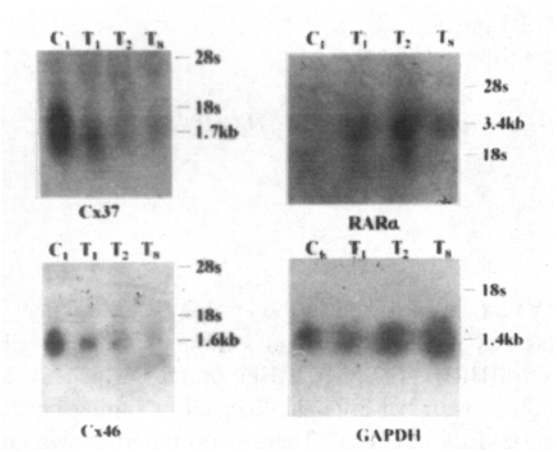

Fig. 4. Resumption of gene expression in HNE_1 after RA removal

T: HNE_1 cell treated with RA

R: HNE_1 cell after RA removal

DISCUSSION

Nasopharyngeal carcinoma cell line HNE_1 expressed two different connexin genes: Cx37 and Cx46, which showed a different expression pattern from other kinds of cancer cells. The expression of the connexin gene has the specificity of tissues, of which the mechanism is not well understood.

The expression of the connexin genes could be regulated by external stimuli. Our result showed that differentiation agent could down-regulate the expression of Cx46 and Cx37 in HNE_1 cells, and this down-regulation was related with the up-regulation of RAR α. Our result also indicated that the modulation of Cx46 and Cx37 by retinoid acid is via the up-regulation of RAR α, possibly through the RA-RAR-RARE pathway.[8]

Cell growth in HNE_1 cells and the expression of Cx37, Cx46 and RAR α were restored after RA removal. This result showed that the effects of RA on HNE_1 cells were reversible and the inhibition of Cx37 and Cx46 genes needs the consistent stimuli of a differentiation agent.

The differentiation agent can inhibit the expression of Cx37 and Cx46, and this result is in contrast to the other researcher's reports. We think that there are two reasons: (1) It seems that the increase of GJIC and the connexin gene expression could provide the advantage of growth to some cancer cells, which was reported in bladder carcinoma[9]. The differential agent could let the cancer cell lose this advantage through the down-regulation of the connexin genes; (2) Some cancer cell could express some connexin genes, which do not exist in their matched normal cells, and these aberrant localized connexin proteins could not form gap junction channels or normal gap junction channels. The differentiation agent can correct this aberrant localization and restore or partly restore the normal function of gap junction[10]. It is certainly true that there are other members of the connexin gene playing a role in the GJIC of the normal nasopharygeal epithelial cells and RA-treated nasopharygeal carcinoma cells.

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