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Basic Investigations

EXPRESSION OF THE RETINOIC ACID RECEPTORS (RARs) AND RETINOID X RECEPTOR (RXRs) GENES IN BREAST CANCER

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A number of studies have been shown that retiniods can inhibit malignant cell growth including certain breast carcinoma cells. Its inhibitory effort is observed only in ER-positive but not in ER-negative breast cancer cells. We examined retinoic acid receptors (RARs) and retinoids X receptors (RXRs) levels in 6 breast carcinoma cell lines and 18 breast cancer biopsy specimens. We found that RAR-α mRNA level was significantly higher in ER positive cell lines and samples. RAR-γ mRNA was expressed at relatively high levels in majority of tumor samples independent of the ER-status while RAR-β mRNA was expressed at low levels. We also found high RXR-α mRNA levels in all of the tumor samples examined while RXR-γ mRNA could not be detected. Our study suggests a possibility that retinoids inhibit tumor cell growth through RAR-α and RAR-α levels may serve as a potential marker to determine responsiveness of patients to retinoids therapy.

Key words: Breast tumor, RARs genes, RXRs genes, Gene expression.

The vitamin A derived retinoids have important effects on mediating cell growth and differentiation.[1] A number of studies have shown that retinoids are highly effective in preventing the development of epithelial carcinoma including breast cancer.[2] Retinoids act by binding to specific nuclear receptor which belong to the steroid/thyroid hormone nuclear receptor superfamily. So far, two classes of retinoid receptor, the RARs and RXRs, have been identified. Each class is further subdivided into three distinct types namely RARα, β, γ and RXRα, β, γ.[1,3] They all act by binding to specific DNA sequence called RARE and RXRE respectively. There are data showing that retinoids selectively inhibited the growth of the ER positive human breast carcinoma (HBC) cells and ER negative cells were refractory to the inhibitory effects of retinoids.[4] The mechanism by which retinoids inhibit the growth of breast carcinoma cells remains unclear. The study of the expression of retinoid nuclear receptors in human breast carcinoma will be of great help to explore the mechanism by which retinoids act.

In this study we used the techniques of molecular biology to study the expression and distribution of RARs and RXRs genes in human breast carcinoma cell lines and human breast carcinoma specimens.

MATERIALS AND METHODS

Cell Line and Tumor Samples

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The MCF7, ZR75, BT74, T47D, Hs578T, MDA-MB-231 and MDA-MB-468 cell lines were a generous gifts of Dr. Fontana. (Univ. of Maryland Cancer Center), Eighteen specimens of infiltrating ductal carcinoma obtained from patients at Bowman-Gray School of Medicine, Winston-Salem, NC.

Steroid Receptor Assay

Steroid receptor assays were performed using traditional DCC method,[5] 10 fmol/mg protein or greater of estradiol binding was considered positive.

Flow Cytometry Assay

Flow cytometry was performed utilizing Kute method,[6] breast cancer tissue was made into single cell suspension, then incubated with PI (50 mg/ml) in a 3.4 M citrate buffer (pH 7.6) containing 10 mM Nacl, RNAs (5.6 mg/ml). After being filtered, the resulting nuclei were analyzed on MODFIT for cell kinetics.

cDNA Probes, RNA Isolation and Northern Analysis

RARα, β, γ and RXRα, β, γ cDNA probes were kindly provided by Dr. Chambon. The plasmids carrying these probes were digested with appropriate restriction enzymes and the probes were gel purified. The probes were made according to the method of Fienberg and Vogelstein.[7] Total cellular RNA was isolated by the method of Chomczyski and Sacchi.[8] Thirty five μg RNA of each sample were size fractionated onto a 1.2% formaldehyde/agrose gel and transferred to supported nitrocellulose membranes. The membrane was baked at 80 ℃ for two h under vacuum and prehybridized in prehybridization solution (containing 50% Formamide, 5×SSPE, 0.5 SDS, 5×Denhardt) at 42 ℃ for at least six h. Then the membrane was hybridized in hybridization solution (containing prehybridization solution plus 10% dextran and specific ^{32}P labeled probes), washed in 2×SSPE, 0.2% SDS twice, every time for 15 min. at room temperature, washed in 0.1×SSPE, 0.2% SDS twice, every time for 30 min. at 65 ℃. After washing, the membranes were exposed to Kodak X-Omat AR film.

Statistical Analysis

Comparison of the RARs and RXRs mRNA levels between the ER-positive and ER-negative tumor was made using *t*-test.

RESULTS

RAR α mRNA Expression in HBC Cell Lines

RAR α mRNA expression was significantly higher in ER-positive HBC cell lines than in ER-negative HBC cell lines (Figure 1).

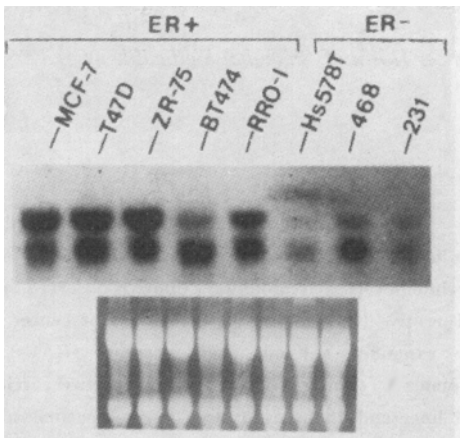

Fig. 1. RARα mRNA levels in ER-positive and ER-negative breast carcinoma cell lines.

RARα mRNA Expression in HBC Samples and the Relationship between RARα mRNA Level and ER, PR Status

We studied the expression of RARα mRNA in 18 HBC samples and the relationship between RARα mRNA level and ER, PR status and DNA content (Figure 2). We found that RARα mRNA level were significantly higher in ER-positive and PR-positive samples than in ER-negative and PR-negative ones ($P<0.05$). There was no relationship between RARα mRNA level and DNA content and S-phase (Table 1).

RARβ and RARγ mRNA Expression in HBC Samples

Relatively higher levels of RARγ mRNA were observed in a majority of the samples while RARβ mRNA was only detected in one of the eighteen samples. No correlation was observed between the RARβ and RARγ expression pattern and ER status.

RXRs mRNA Expression in HBC Cell Lines and Samples

RXRs belong to another family of receptors which were discovered only recently. Little information is available on the function of RXRs. We also examined RXRα , β,γ mRNA levels in the same 18 HBC samples and cell lines (Figure 3). RXRα mRNA was expressed at high levels in all samples and cell lines. RXRγ mRNA level was relatively low. We could not adequately asses RXRβ mRNA level because the strong cross hybridization of the RXRβ cDNA probe with 18s and 28s RNAs.

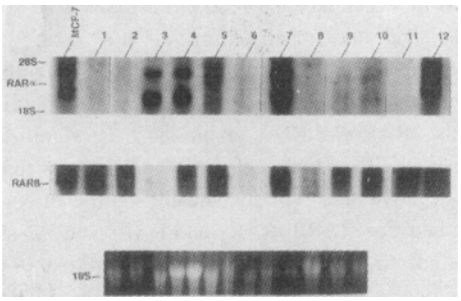

Fig. 2. RARα and RARγ mRNA levels in ER-positive and ER-negative breast carcinoma samples.

Table 1. Estrogen and progesterone receptor levels, S-phase fraction and RARα mRNA levels in breast carcinoma biopsy specimens.

Patient	ER (fmol/ml of protein)	PR (fmol/mg of protein)	% S	% G2+M	RARα mRNA % of MCF7 signal
1	0	0	9	3	1.19
2	0	0			0.41
3	25	27	1	4	45.0
4	27	194	10	7	41.38
5	94	64			50.32
6	0	0	7	1	3.10
7	50	75	1	1	226.59
8	32	53	4	4	5.30
9	52	167	7	6	4.2
10	79	178	5	3	28.53
11	0	0	11	16	4.39
12	58	20	7	15	132.56
13	0	0	11	12	28.56
14	101	317	14	9	118.62
15	0	0	11	11	17.02
16	0	0	13	13	11.67
17	0	0	6	11	6.52
18	0	0	6	4	56.34

DISCUSSION

Retinoids are highly effective in inhibiting the growth of tumor cells. In human breast carcinoma, retinoids selectively inhibited the growth of the ER-positive cells and ER-negative cells were refractory to

the inhibition effect of retinoids.[4] Retinoids mediate their action via nuclear receptors RARs and RXRs. Each class of these nuclear retinoid receptors is further subdivided into three species namely α, β and γ. To know by which receptor retinoids act is very helpful to understand the manchenism of it's action. Our experiment demonstrated that a statistically significant correlation exit between ER status and RARα mRNA levels. RARα mRNA expression is significantly higher in ER-positive cell lines and samples than in ER-negative ones. A statistically significant correlation between RARβ, RARγ and RXRs and ER-status was not found. These results suggest that RARα may play a major role in mediating the inhibitory effect of retinoids on cell growth. Some data showed that estradiol could strongly increase RARα mRNA level in ER-positive breast cancer cell lines, and retinoids can inhibit this effect and prevent the stimulation of cell growth by estradiol.[9] All these results further support a role for RARα in the modulation of cell growth inhibitory effect of retinoids. In order to prove this hypothesis, we can use the technique of gene transfection to introduce RARα gene into ER-negative cells in which no RARα mRNA or very low RARα mRNA was expressed. Then we can observed the change of cell growth caused by retinoids after transfection. This is our ongoing project.

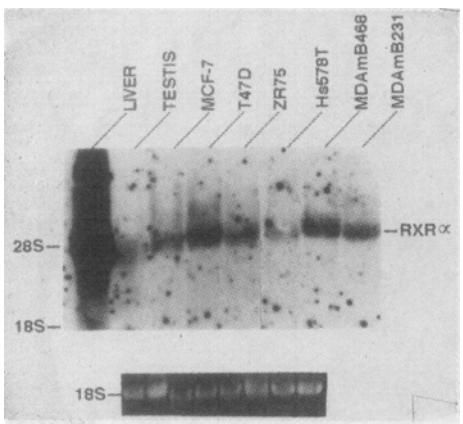

Fig. 3. RARα mRNA levels in ER-positive and ER-negative breast carcinoma cell lines.

It is reported that the expression of RARβ is higher in normal lung tissue than in tumor tissue. So was in colon cancer and malignant blood disease.[10] Our result showed that RARβ mRNA was only detected in one of the eighteen breast carcinoma samples. The positive rate is very low in breast carcinoma.

RXRs belong to another family of receptors which is a distinct but closely related to the RARs family. RXRs were discovered only recently and are believed to play important roles in the regulation of a variety of genes.[6] Our results demonstrated that RARα mRNA was expressed at high level and the RXRγ mRNA at low level in breast carcinoma. There were no statistically significant correlation between RARα, RXRγ mRNA levels and ER-status. It needs further study to explore the function of RXRs.

Retinoids selectively inhibited the growth of ER-positive breast cancer cells. Our experiment demonstrated that among retinoid nuclear receptors only RXRα is associated with ER-status. These suggest that retinoids inhibit tumor cell growth through RXRα. So detection of expression of RXRα in breast cancer samples is of great help to guide retinoids therapy in clinic.

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A CASE OF PIGMENTARY GRAPE-MEMBRANOUS CYST IN UNILATERAL OVARY

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The tumors derived from ovarian single germinal layer is not common and eyeball grape-membranous pigment lining in internal wall of cyst has not been reported. A case of this tumor which was hospitalized in our hospital is reported here.

A female patient at the age of 35 years was hospitalized because a mass in pelvic cavity was detected while a gynecological general survy in May 1994. She felt lower abdominal distension pain or slight implicative pain sometimes, but had a normal menstruation. The gynecological examination found a about 6×5 cm cystic mass in left posterior uterus, which was movable, nontenderness. The B-ultrasonic examination showed a 8×6 cm cyst with a clear surrounding in left uterus. T3, T4, and ECG examination were normal. The patient had been a known hyperthyroidism and hyperthyroid heart disease since 1990, the symptoms of which were controlled after therapy. A 6×6 cm, smooth, and nonattached cystic mass in left-posterior uterus was detected during operation.

Pathological Examination

Massive examination: A 5.5×4×3.5 cm, smooth, greyish-white cyst, which was full of light yellow and clear liquid, smooth and black and brown in internal wall, was observed. Microscopieal examination: The cystic wall was comprised of ovarian interval cells and fibrocytes, internal wall was lined single-layer cubic or cylindrical epitherlium, and these cellular plasmas involved a lot of black and brown granules. Immunohistochemistry: Melanoma (++). Pathological diagnosis: Left ovary pigmentary grape-membranous cyst.

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