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Suppression of Interferon-induced Oligo-2', 5'-adenylate Synthetase Induction in Human Hepatoma Cell Line, Li-7

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SUMMARY

Induction of oligo-2', 5'-adenylate synthetase (2-5AS) activity by interferon (IFN) was investigated in a human hepatoma cell line, Li-7 cells. Little induction of 2-5AS activity by IFN was demonstrated in Li-7 cells in comparison with other types of cell lines including Ramos, NC-37, FL, Co-3. Furthermore, failure to induce 2-5AS was much clearer in old-cultured cells. Cell growth inhibition by IFN was demonstrated in only high titers of IFN ($>10^4$ IU/m*l*), in which the enzyme had one hundred fold higher activity than that of untreated cells.

Poor induction of 2-5AS was in part the result of some inhibitor presented in cellular extracts of Li-7 cells and the decreased level of 2-5AS mRNA transcription.

Key words: Interferon, Oligo-2', 5'-adenylate synthetase, Hepatoma cell

INTRODUCTION

Interferons (IFNs) can inhibit cell growth and affect cell differentiation, therefore it is confirmed that IFN is an important group of naturally occurring regulatory molecules. These effects of IFN are not well difined at the molecular level, and the study of IFN as regulatory agents has been at a descriptive level. Effects of IFNs on cell growth have been largely studied using tumor cell lines and peripheral lymphocytes (10). Cell growth inhibition by IFN may be mediated in part by some enzymes involved in macromolecular metabolism, such as ornithine decarboxylase, protein kinase, oligo-2', 5'-adenylate synthetase (2-5AS) and by cyclic nucleotides (10).

There are some evidences that 2-5AS system, which is induced in IFN treated cells and plays an antiviral function, can act in a role in IFN-induced growth

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inhibition of peripheral lymphocytes and Daudi cells (3). In these systems, the proliferation of cells is closely associated with the expression of c-myc gene or the stability of c-myc mRNA. This transcript might be controlled by the 2-5AS system because the increased activity of this enzyme is a casual factor of the decreased level of c-myc mRNA (3, 9).

Furthermore, similar results for the expression of c-ras gene was also observed in rat liver regeneration after hepatectomy. Enhanced transcription of c-ras gene was found in rat regenerating liver cells, in which 2-5AS activity was suppressed by an unknown mechanism (4, 7). It is therefore interesting to investigate the inhibitor or suppressor of 2-5AS activity in tumor cells including hepatoma cells.

MATERIALS AND METHODS

Cells. All of the cell lines used in this experiment were cultured with RPMI 1640 medium containing 5% FBS (Gibco) at 37℃ in a humidified 5% CO_2 incubator.

Enzyme assay. Oligo-2', 5'-adenylate synthetase activity in cells was measured by the liquid phase reaction as previously described (6).

Inhibition of 2-5AS activity by cell lysate. The supernatant-I (S-I) of Li-7 cells was prepared by the method of Fujii *et al* (6). Aliquots of this supernatant or α-fetprotein (AFP) were added to the reaction mixture of 2-5AS prepared from FL cells treated with 10^3 units/m*l* of IFN-α (3×10^6 IU/vial, Lot 02-31, Japan Red Cross Society), and incubated for 4 hr at 33℃.

Degradation of 2-5A by AFP. Degradation of oligo-2', 5'-adenylate (2-5A) synthesized by the solid phase reaction and aceton precipitation was measured in the presence of the indicated concentration of AFP as previously described (6).

Growth inhibition by IFN. Cells were treated with IFN for 4 days. After which, the cells were washed with phosphate buffer saline (PBS), stained with gentian violet solution for 2 to 3 min, washed thoroghly with water, and dried at room temperature. The dye binding to the cells was dissolved with methylcellosolve for 2 to 3 hr, and absorbance was measured at a 550 nm.

Preparation of cytoplasmic RNA and northern blot hybridization. Northern blot analysis was carried out on mRNA preparations from Li-7 cells to determine the size and amount of transcript identified by human 2-5AS cDNA (pSP25R), which was kindly supplied by Dr. Y. Soukawa *et al.* Total cytoplasmic RNA (50 μg) was electrophoresed on formaldehyde agarose gels, blotted and hybridized to the ^{32}P-labelled probe as previously described (5, 6).

RESULTS

Human hepatoma cell line, Li-7 was treated with an indicated titer of IFN-α and IFN-β (10^2, 10^4 and10^5 IU/m*l*) for 24 hr, and 2-5AS activity was measured by

liquid phase reaction as described in the text. As shown in Table 1, the activity of 2-5AS hardly induced in Li-7 by both types of IFN, and a slight increase of this enzyme activity was demonstrated in the cells treated with high titer (10^5 IU/m*l*) of IFN. However the other cells have about one to three hundred fold higher activity than that of Li-7 cells. During the cultivation of cells without IFN, the activity of this enzyme in Li-7 cells gradually decreased. Furthermore, the induction of 2-5AS by IFN was also more suppressed in old-cultured cells (Table 2). It may be likely that some inhibitory factor will increasingly accumlate in cells after cultivation for several days.

Failure to induce activity of this enzyme was consistent with the result of cell growth inhibition of Li-7 cells by IFN. Cell growth was inhibited in IFN titer having a capacity to induce 2-5AS activity (above 10^4 IU/m*l* of IFN) (Table 3).

Table 1 *Induction of 2-5AS activity by IFN. Cells cultured for 3 days were treated with IFN for 24 hr at 37°C, and the enzyme activity was measured by the method described in the text.*

Cell lines	2-5AS (DPM×10^{-2}/μg protein)					
	Control	IFN-α (IU/m*l*)			IFN-β (IU/m*l*)	
		10^2	10^4	10^5	10^2	10^4	10^5
Li-7	1.04	20.7	69.6	107.5	11.3	100.0	118.0
Ramos	0.86	396.1	671.8	—	430.3	704.4	—
NC-37	1.15	118.4	245.6	—	178.9	303.8	—
FL	0.95	158.0	318.0	—	155.3	386.6	—
Co-3	0.76	171.8	360.9	—	137.6	191.8	—

Table 2 *Induction of 2-5AS activity in Li-7 cells. Li-7 cells cultured for an indicated number of days were treated with 10^3 IU/ml of IFN for 24 hr at 37°C.*

Days after cultivation	2-5AS (DPM×10^{-2}/μg protein)	
	IFN-α (IU/m*l*)	
	0	10^3
1	1.18	84.0
2	1.02	54.0
3	0.56	53.0
4	0.38	36.1
5	0.51	—

Table 3 *Effect of IFN on cell growth of Li-7 cells. Li-7 cells were cultured with IFN for 4 days, after which growing cells were stained with gentian violet solution.*

IFN (IU/m*l*)	ABS	
	IFN-α	IFN-β
0	554	
10	550	542
10^2	560	534
10^3	526	508
10^4	248	385
10^5	145	334
10^6	—	55

A slight increase of this enzyme activity suppressed the cell growth of Li-7 to half of untreated control cells.

As shown in Fig. 1, northern blot analysis revealed that three molecular size mRNA of 2-5AS was recognized in FL cells, however about 1.8 Kb mRNA of low molecular size was detectable in Li-7 cells. Furthermore, there was a drastic decrease in the amount of 2-5AS mRNA in Li-7 cells as compared with that in FL cells. Increase of the amount of this mRNA by IFN was also consistent with the induction of this enzyme activity.

Failure to induce 2-5AS was in part the result of some inhibitor present in Li-7 cell extracts. The supernatant obtained from the extracts suppressed the activity to about 50% of the enzyme prepared from FL cells (Table 4). In contract, the supernatants from Molt 4 and Raji cells, which had a mormal level of 2-5AS induction by IFN, did not show an inhibitory effect. Similar results were also obtained in an experiment using AFP (Table 5). Ten micrograms of AFP significantly inhibited the activity of this enzyme. This inhibition was not the result of the degradation of oligo-2', 5'-adenylate (2-5A), because AFP did not have a capacity to degrade 2-5A (Table 6).

DISCUSSION

The oligo-2', 5'-adenylate system including 2-5AS and RNase L plays an important role in cell growth regulation in addition to the establishment of antiviral state of cells treated with IFN (1). Recently, it has been reported that the activity of 2-5AS was reduced in several virus infected cell lines (2, 5, 6) and rat liver regeneration (4). Therefore, antiviral state or susceptibility to virus infection, and cell proliferation changed in these cells because of reduced enzyme activity.

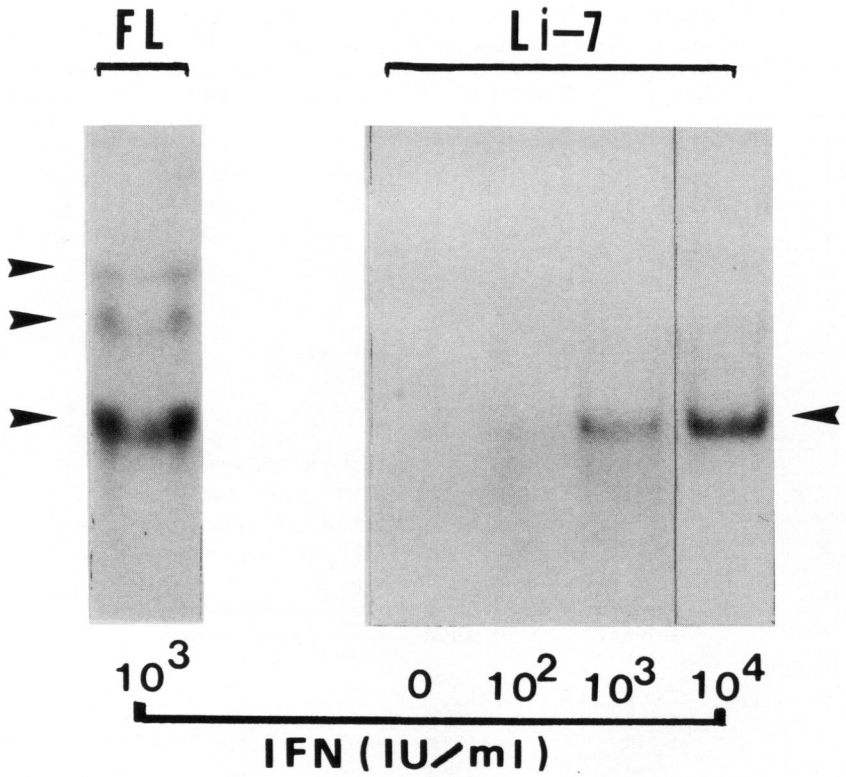

Fig. 1 Analysis of 2-5AS mRNA transcripts in Li-7 cells.
Total cytoplasmic RNA (50 μg) from cells treated indicated titer of IFN for 24 hr were electrophoresed on formaldehyde agarose gels and Northern blot hybridized to ^{32}P-labelled of pSR25R DNA.

Table 4 *Inhibitory effect of cell extract on the activity of 2-5AS. Li-7 cell extract of 5 μg, 10 μg and 15 μg were added to the liquid phase reaction mixture of 2-5AS prepared from FL cells treated with 10^3 IU/ml of IFN.*

Li-7 extract (μg)	FL (μl)	DPM	% of control
0	5	53456	100
5	5	39991	74.8
10	5	25986	48.6
15	5	23218	43.4
5	—	2079	
10	—	5953	
15	—	7603	

Table 5 *Effect of AFP on 2-5AS activity. Experiment was carried out in the same manner as described in table 4.*

FL (μl)	AFP (μg)	Molt-4 (μg)	Raji (μg)	DPM	% of control
10	—	—	—	83048	100
10	10	—	—	31360	37.8
5	—	—	—	60296	100
5	5	—	—	43145	71.6
5	10	—	—	29167	48.4
5	—	20	—	55713	92.4
5	—	—	20	45824	76.0

Table 6 *Degradation of oligo-2', 5'-adenylate. Indicated concentration of AFP and 2-5A were mixed and incubated, after which radioactivity of 2-5A was recovered by DEAE cellulose column.*

AFP (μg)	DPM	% of control
0	37829	100
5	43882	116
10	37327	100
20	40908	108

Suppressed induction of 2-5AS was observed in human hepatoma cell, Li-7 compared with that of other cell lines. Though it is not known whether the number of IFN receptor is changed, these results may be due to the presence of some inhibitor including AFP, and are also due to the suppression of transcription of 2-5AS mRNA or the change of stability of this mRNA.

As the correlation between the expression of c-ras mRNA and the activity of 2-5AS is inverted in rat liver regeneration, and furthermore c-ras mRNA also increased in rat hepatoma cell lines (4, 7, 8), it is likely that the reduced induction of 2-5AS in Li-7 may bring about the increase of c-ras mRNA and c-myc mRNA. The reduction of this enzyme may also be correlated with the promotion of oncogenesis.

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