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Regulation of the Expression of Epidermal Growth Factor Receptor mRNA with Thyroid Hormone L-3,5,3'-Triiodothyronine in Rat Hepatoma Cells

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The expression of epidermal growth factor (EGF) receptor mRNA was examined using thyroid hormone L-3,5,3'-triiodothyronine (T_3) by Northern blot analysis in rat hepatoma cells. The expression of full-length, but not truncated receptor mRNA was enhanced and destabilized with T_3. These results suggest that T_3 induces not only the expression of full-length EGF receptor mRNA but also accelerates their rate of turnover. The 3' terminal region of full-length EGF receptor mRNA may have a role in the destabilization of EGF receptor mRNA by T_3, because truncated EGF receptor mRNA lacks only the 3' terminal region of full-length EGF receptor mRNA.

Key words: EGF receptor; EGF receptor mRNA; mRNA stability; regulation of gene expression; thyroid hormone

The epidermal growth factor (EGF) receptor mediates cell proliferation by EGF or transforming growth factor α (Carpenter and Cohen, 1990). Normal rat liver cells express full-length and truncated EGF receptor messenger RNAs (mRNAs) (Petch et al., 1990; Satoh et al., 1996, 1997). Full-length EGF receptor mRNA encodes a transmembrane EGF receptor tyrosine kinase, c-ErbB1. Truncated EGF receptor mRNA encodes a secreted EGF-binding protein with a yet unknown physiological function (Ullrich et al., 1984). Regulation of the gene expression of EGF receptors has not been well understood. Full-length and truncated EGF receptor mRNAs initiate from the same 5' site (Ishii et al., 1985) and are subsequently processed into different forms by alternative splicing (Petch et al., 1990). Expression of EGF receptors has been reported to be regulated by thyroid hormone (Mukku, 1984; Vonderhaar et al., 1986). Thyroid hormone is a ligand of c-ErbA, a member of the steroid/nuclear receptor superfamily of ligand-regulated transcription factors (Tsai and O'Malley, 1994). However, the EGF receptor promoter has not been reported to contain the thyroid hormone-responsive element, while it was recently reported to have putative estrogen-responsive elements (Yarden et al., 1996). Thyroid hormone was also reported to decrease the length of the poly(A) tract of mRNA (Krane et al., 1991; Murphy et al., 1992).

Here we studied the effect of a thyroid hormone, L-3,5,3'-triiodothyronine (T_3) on the expressions of full-length and truncated EGF receptor mRNAs in rat hepatoma cells. We found that full-length EGF receptor mRNA was upregulated rapidly and subsequently destabilized with T_3.

Materials and Methods

Cell culture

AH66 cells provided by Dr. K. Sato (Department of Molecular Biology, Tottori University, Yonago, Japan) were cultured in Dulbecco's

Abbreviations: DCC-NCS, dextran-charcoal-treated NCS; DRB, 5,6-dichlororibofuranosylbenzimidazole; EGF, epidermal growth factor; kb, kilobase; mRNA, messenger RNA; NCS, newborn calf serum; T_3, L-3,5,3'-triiodothyronine

Modified Eagle's Medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) with 10% newborn calf serum (NCS; Filtron Ltd., Brooklyn, Australia) at 37˚C in a humidified atmosphere containing 5% CO_2.

Treatment of cells with thyroid hormone and 5,6-dichlororibofuranosylbenzimidazole (DRB)

Cells were inoculated at 5×10^5 cells/dish in 9 cm plastic dishes and cultured for 48 h. The cells were rinsed with serum-free medium and cultured in the medium containing 3% dextran-charcoal-treated NCS (DCC-NCS), which was free of thyroid hormone, with or without 100 nM T_3 (Nacalai Tesque, Inc., Kyoto, Japan) for 3 h. For the measurement of stability of mRNA, the culture medium was changed to the fresh medium containing 65 μM DRB (Harrold et al., 1991; Sigma-Aldrich Japan K.K., Tokyo) and 3% DCC-NCS with or without 100 nM T_3 and cells were cultured for an indicated time.

Northern blot analysis

RNA was extracted from the cells by the acid guanidinium thiocyanate-phenol-chloroform method (Chomozynski and Sacchi, 1987) and was analyzed by Northern blot analysis as described previously (Satoh et al., 1997). The RNA was denatured, electrophoresed and transferred to a nylon membrane. The membrane was hybridized with the probe, washed and exposed to an X-ray film. The rat EGF receptor cDNA encoding extracellular domain (Petch et al., 1990) was provided by Dr. H. Shelton Earp (University of North Carolina at Chapel Hill,

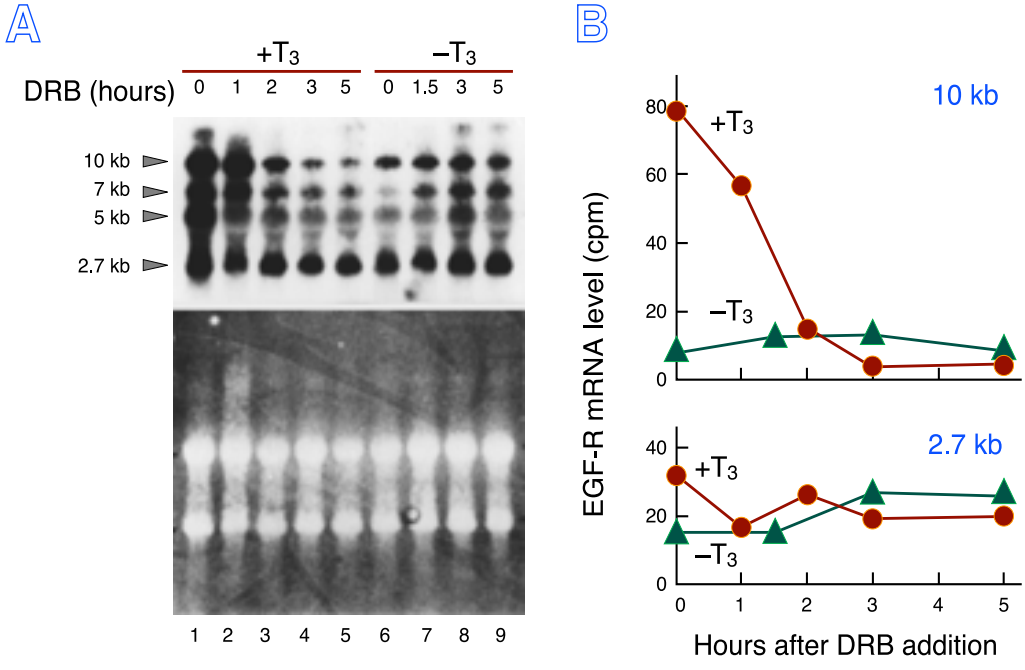

Fig. 1. Effect of T_3 on the expression of EGF receptor mRNAs in AH66 cells. Cells were cultured with (+T_3) or without (–T_3) 100 nM T_3 for 3 h at 37˚C and were then treated with DRB and cultured with or without T_3 for indicated times. RNA was extracted from the cells and was analyzed by Northern blot. **A:** Upper panel; EGF receptor mRNA proved with the cDNA probe. Sizes of the mRNA are shown on the left side. Lower panel; RNA (20 μg/lane) stained by ethidium bromide. **B:** The levels of 10 and 2.7 kb EGF receptor mRNAs were quantitated and shown as a function of time after the addition of DRB. The background level (13.9 cpm) of the same area was subtracted. Data were reproduced three times by repeated experiments.

North Carolina, USA) and used as the ^{32}P-labeled probe for EGF receptor mRNA. The EGF receptor mRNA level was quantitated by an AMBIS 4000 image analyzer (Japan Medical Dynamic Marketing Inc., Tokyo, Japan).

Results

AH66 cells were cultured in the presence or absence of T_3 for 3 h, and then RNA synthesis was stopped by the addition of DRB and cultured in the presence or absence of T_3. After the incubation, total cellular RNA was prepared at various time points and was subjected to Northern blot analysis with the EGF receptor cDNA probe (Fig. 1A). Ten, 7 and 5 kb full-length and 2.7 kb truncated EGF receptor mRNAs were detected in the cells as previously described (Satoh et al., 1996, 1997). The bands of the full-length EGF receptor mRNA were much darker in the presence of T_3 (lane 1) than in the absence of T_3 (lane 6). The mRNA levels of the 10 and 2.7 kb were quantitated by the image analyzer (Fig. 1B). The level of 10 kb mRNA with T_3 treatment was 10-fold higher than T_3-untreated cells at 0 h. However, the level reduced rapidly to the level of T_3-untreated cells by 2 h after the addition of DRB. On the other hand, in the absence of T_3, the low level of 10 kb mRNA was detected and did not change significantly up to 5 h after the addition of DRB. In contrast, the level of 2.7 kb mRNA was not affected by the T_3 treatment as shown at 0 h and did not change for 5 h in the presence of DRB. The effect of T_3 on the levels of 7 and 5 kb mRNAs was similar to those of 10 kb mRNA (data not shown). These results indicate that T_3 induced the expression of the mRNA of the full-length EGF receptor and had accelerated turnover. However it did not have such an effect on the truncated receptor mRNA.

Discussion

It has been reported that thyroid hormone upregulates EGF receptor protein in cells of rat liver (Mukku, 1984) and mouse mammary glands (Vonderhaar et al., 1986). We found that the level of full-length EGF receptor mRNA was upregulated with T_3 in rat hepatoma cell line, AH66 cells. This is consistent with the following reports: the enhancement of EGF binding to the membranes of liver and mammary glands of euthyroid animals as opposed to hypothyroid controls (Mukku, 1984; Vonderhaar et al., 1986), the enhancement of the transcription of EGF receptor in T_3-treated human mammary cells (Fernandez-Pol et al., 1989) and the stimulation of T_3 on the level of 5.6 kb EGF receptor mRNA in human epidermoid cells (Kesavan et al., 1991). The higher level of 10 kb mRNA in the T_3-treated cells may suggest that the expression of full-length EGF receptor mRNA is enhanced at transcriptional levels and/or alternative splicing steps in the presence of T_3. We also observed that the induced full-length EGF receptor mRNA was unstable in the treatment of T_3. It has been reported that rat mRNAs were unstable in the presence of a thyroid hormone than in its absence (Krane et al., 1991; Murphy et al., 1992). It has also been explained that the extension of polyadenylation in the cell nuclei is downregulated by thyroid hormone (Murphy et al., 1992; Sachs and Wahle, 1993).

Full-length and truncated EGF receptor mRNAs were produced by alternative splicing (Petch et al., 1990). The expression of truncated EGF receptor mRNA was not affected by the treatment of T_3 (Fig. 1). The mRNAs have a common 5' terminal region but truncated mRNA lacks a 3' terminal region of full-length form mRNA in rat hepatoma cells (Satoh et al., 1996). We hypothesize that the 3' terminal region of the full-length EGF receptor mRNA may be involved in the turnover acceleration by T_3 in the rat hepatoma cells.

T_3 exerts effects in hepatocellular regenerations during cirrhosis and partial hepatectomy (Knopp et al., 1992; Sasaki et al., 1992; Huang and Liaw, 1995; Oren et al., 1996). The experimental findings in the hepatocellular carcinoma cell line, AH66 may have clinical implications particularly relevant to liver diseases and hepatocellular carcinoma.

Our results suggest that the mode of action of thyroid hormone mediates the growth-promoting and developmental effects by the transient induction of full-length EGF receptor.

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References

1 Carpenter G, Cohen S. Epidermal growth factor. J Biol Chem 1990;265:7709–7712.
2 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–159.
3 Fernandez-Pol JA, Hamilton PD, Klos DJ. Transcriptional regulation of proto-oncogene expression by epidermal growth factor, transforming growth factor β1, and triiodothyronine in MDA-468 cells. J Biol Chem 1989;264:4151-4156.
4 Harrold S, Genovese C, Kobrin B, Morrison SL, Milcarek C. A comparison of apparent mRNA half-life using kinetic labeling techniques vs decay following administration of transcriptional inhibitors. Anal Biochem 1991;198:19–29.
5 Huang MJ, Liaw YF. Clinical associations between thyroid and liver diseases. J Gastroenterol Hepatol 1995;10:344–350.
6 Ishii S, Xu YH, Stratton RH, Roe BA, Merlino GT, Pastan I. Characterization and sequence of promoter region of the human epidermal growth factor receptor gene. Proc Natl Acad Sci USA 1985;82:4920–4924.
7 Kesavan P, Mukhopadhayay S, Murphy S, Rengaraju M, Lazar MA, Das M. Thyroid hormone decreases the expression of epidermal growth factor receptor. J Biol Chem 1991;266: 10282–10286.
8 Knopp J, Brtko J, Filipcik P. Effect of triiodothyronine on rat liver polysome profiles and translational activity of mRNA after partial hepatectomy. Endocr Regul 1992;26:67–72.
9 Krane IM, Spindel ER, Chin WW. Thyroid hormone decreases the stability and poly(A) tract length of rat thyrotropin β-subunit messenger RNA. Mol Endocrinol 1991;5:469–475.
10 Mukku VR. Regulation of epidermal growth factor receptor levels by thyroid hormone. J Biol Chem 1984;259:6543–6547.
11 Murphy D, Pardy K, Seah V, Carter D. Post-transcriptional regulation of rat growth hormone gene expression: increased message stability and nuclear polyadenylation accompany thyroid hormone depletion. Mol Cell Biol 1992;12: 2624–2632.
12 Oren R, Dotan I, Papa M, Marravi Y, Aeed H, Barg J, et al. Inhibition of experimentally induced cirrhosis in rats by hypothyroidism. Hepatology 1996;24:419–423.
13 Petch LA, Harris J, Raymond VW, Blasband A, Lee DC, Earp HS. A truncated, secreted form of the epidermal growth factor receptor is encoded by an alternatively spliced transcript in normal rat tissue. Mol Cell Biol 1990;10:2973–2982.
14 Sachs A, Wahle E. Poly(A) tail metabolism and function in eucaryotes. J Biol Chem 1993;268: 22955–22958.
15 Sasaki H, Matsumoto S, Shijyo H, Shimizu M, Tohara K, Tsutsu N, et al. Gross ascites as a first manifestation of primary hypothyroidism due to post-treatment of radioiodine therapy for Graves' disease. Intern Med 1992;31:256–259.
16 Satoh Y, Oomae M, Ichii S. Studies on rat epidermal growth factor (EGF) receptor: rapid amplification and cloning of cDNA ends corresponding to 3' termini of full-length EGF receptor mRNAs. Yonago Igaku Zasshi 1996; 47:218–227 (in Japanese with English abstract).
17 Satoh Y, Oomae M, Hoshikawa Y, Izawa M, Sairenji T, Ichii S. The stability of truncated epidermal growth factor receptor mRNA is higher than that of the full-length receptor mRNA in rat hepatoma cells. Endocrine J 1997;44:403–408.
18 Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 1994;63:451–486.
19 Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, Tam AW, et al. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 1984;309: 418–425.
20 Vonderhaar BK, Tang E, Lyster RR, Nascimento MCS. Thyroid hormone regulation of epidermal growth factor receptor levels in mouse mammary glands. Endocrinology 1986;119:580–585.
21 Yarden RI, Lauber AH, El-Ashry D, Chrysogelos SA. Bimodal regulation of epidermal growth factor receptor by estrogen in breast cancer cells. Endocrinology 1996;137:2739–2747.

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