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INCREASE OF ESTROGEN RECEPTOR LEVEL BY THYROXINE IN ESTROGEN DEPENDENT PITUITARY TUMOR (MTT/F84) IN RATS

Nariaki Fujimoto, Bidyut Roy, Hiromitsu Watanabe, and Akihiro Ito

Department of Cancer Research,
Research Institute for Nuclear Medicine and Biology,
Hiroshima University,
1-2-3 Kasumi, Minami-Ku, Hiroshima 734, Japan

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Summary. A rat transplantable pituitary tumor, MtT/F84, grows much faster in E_2 treated rats than in normal females, but is much retarded in thyroidectomized rats. Triiodothyronine (T_3) administration in a drinking water increased the tumor growth by the dose dependent manner. The tumor contained both estrogen receptor (ER) and T_3 receptor. ER levels both in the nuclei and cytosols elevated 2 to 3 times by the T_3 administration compared to those of control. E_2 administration promotes the growth of MtT/F84 through elevation of nuclear ER level. T_3 may directly elevate cellular ER level and thus it may enhance estrogenic actions including the tumor growth.

Thyroid hormone acts almost all organs to control cellular metabolism including the growth and developmental changes (1). It acts directly on the cell proliferation e.g.: the growth of rat pituitary tumor cell lines (GH_3 and GC) are highly dependent to thyroid hormone (2,3,4). These cell lines respond to physiological concentration of L-triiodothyronine (T_3) or thyroxine (T_4) at time of cell proliferation and synthesize growth hormone. Estrogen, on the other hand, has been known to be the most powerful chemical for the pituitary tumorigenesis. Numerous essential studies on the cellular growth mechanism of estrogen have been done mainly in mammary tumor cells (5). Among the studies, the role of autocrine growth factors has been most promising to explain the estrogen triggered tumor growth (5, 6).

A MtT/F84, transplantable rat pituitary tumor established and maintained in our laboratory since 1981, grows well in 17β-estradiol (E_2)

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treated rats with dose dependently (7). In contrast, its growth is much retarded in the thyroidectomized (Tx) rats. The present study was undertaken to correlate the growth effect of both E_2 and T_3 with estrogen receptor (ER) levels in MtT/F84 grown in thyroidectomized rats given T_3 and E_2.

Materials and Method

Animals Young female F344 rats (4 weeks of age) were purchased from Charles River Japan Co. (Tokyo). Ether-anesthetized rats were thyroidectomized a week before the experiment. Transplantation of MtT/F84 cells was described previously (7). Individual rats were implanted with cholesterol pellets containing 2.5 mg of E_2 (Sigma Chemicals E-9000). T_3 (Sigma Chemicals T-2877) was orally administered in the drinking water at the dose of 1.5 μg/l (T_3-low) or 300 μg/l (T_3-high).

T_3 receptor (TR) assay It was performed according to the method of Oppenheimer et al (8). Tissues were homogenized in 0.32 M sucrose-3 mM $MgCl_2$ and centrifuged at 700 x g for 10 min. The pellets were resuspended in 2.4 M sucrose, 3 mM $MgCl_2$ and centrifuged at 53,000 x g for 45 min. This resulting pellets were resuspended in TMDS buffer (2 mM Tris, 3 mM $MgCl_2$, 1 mM dithiothreitol, 0.32 M sucrose, pH 7.4).

Aliquots of suspended nuclei were incubated in [^{125}I]T_3 (L-3,5,3'-[^{125}I]T_3, specific activity = 781 Ci/mmol, NEN) by the range of 0.03-3 nM with and without 1,000-fold non-radioactive T_3 (3 μM of T_3) at 37 C for 30 min. After the incubation, equal volume of 2% Triton X-100 was added to each tube. The nuclei were washed 2 times by centrifugation (1,500 x g for 10 min) with TMDS buffer and counted. The data were analyzed by the method of Scatchard.

ER assay Procedure of ER assay was based on Bronzert et al (9) and Ginsberg et al (10). The tumor tissues were homogenized in TED buffer (10 mM Tris, 10 mM EDTA, 1 mM dithiothreitol, pH 7.4). The homogenates were centrifuged at 800 x g for 10 min to separate the nuclear pellets and supernatants. The supernatants were centrifuged at 105,000 x g for 60 min to get cytosol for assay. The nuclear pellets were washed in TED buffer and resuspended in KTED buffer (TED buffer pH 7.4 containing 0.6 M KCl). After sonication, they were incubated in ice bath for 60 min and centrifuged at 105,000 x g for 60 min to obtain the nuclear salt extract.

Aliquots of the cytosol and nuclear extract were incubated in [^{3}H]E_2 (2,4,6,7-[^{3}H]E_2, specific activity = 90.8 Ci/mmol, NEN) within the range of 0.03-3 nM with and without 1,000-fold excess non-radioactive E_2 (3 μM of 17β-estradiol). After incubation at 30 C for 30 min, unbounded E_2 was absorbed with the dextran coated charcoal method. Protein concentration of cytosol was measured by Lowry method. The DNA of nuclear salt extract was measured by the method of Labarca et al, using bisbenzimidazole (11).

Results

Growth of MtT/F84. 4.3 x 10^4 of MtT/F84 cells were implanted per site in the estrogenized F344 rats. Animals were divided into 3 groups (T_3-0, T_3-low and T_3-high) according to the amount of T_3 administered. The grafted sites were observed on every other day. The incidence of tumor take

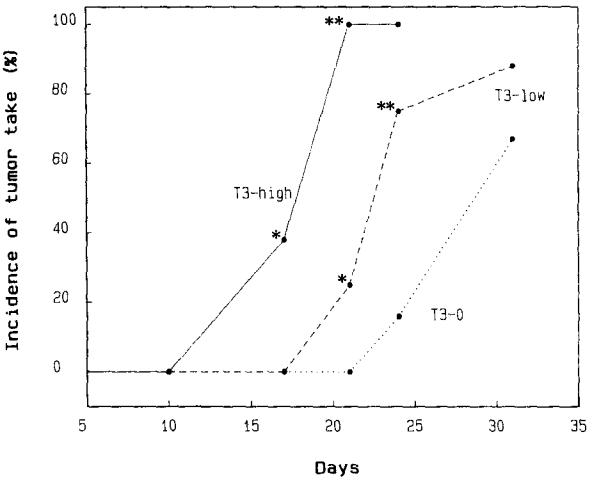

Fig 1. MtT/F84 growth in estrogenized F344 rat treated with various doses of T_3. *, ** : significantly different from control by $P<0.05$, 0.01 respectively.

was 0 % on day 10 in the all experimental groups. On day 17, tumor take was found only in T_3-high group and it was 100 % on day 21, 25 % in T_3-low and 0 % in T_3-0 (Fig. 1). The tumor take in T_3-0 was noted on day 24. Latency and incidence of tumor take as well as average tumor sizes were well correlated to the administered doses of T_3.

Existence of TR. MtT/F84 contained specific binding sites for T_3 (TR) (Maximum binding = 1.9±0.44 pmol/mg DNA, Kd = 2.3±0.42 nM).

ER levels. MtT/F84 contains ER as reported previously (7). We have measured ER levels both in cytosols and nuclear extracts of the tumor in the individual groups (Table 1). Cytosolic ER levels were lowest in T_3-0,

Table 1. ER levels in MtT/F84[a] grown in thyroidectomized female rats given E_2 and various doses of T_3

Treatments	No. of tumor examined	Cytosolic ER Maximum binding sites (fmol/mg protein)	Kd (nM)	Nuclear ER Maximum binding sites (fmol/mg DNA)	Kd (nM)
T_3-0	3	71±12[b]	0.57±0.05	237±73[e]	0.14±0.00
T_3-low	3	113± 8[c]	0.64±0.02	427± 8	0.15±0.02
T_3-high	3	184±23[d]	0.59±0.02	553±11[f]	0.18±0.02

[a] The range of the examined tumor sizes were from 5 to 15 mm in diameter.

[b] vs. c, c vs. d, and e vs. f: significantly different by $P<0.05$.

highest in T_3-high and moderate in T_3-low with significant difference. Nuclear ER levels were also increased with the increase of T_3 dose. Accordingly, total cellular ER levels in the existence of E_2 were increased by the administered dose of T_3.

Discussion

We observed that the growth of MtT/F84 became retarded in the thyroidectomized rats, but it was resumed with administration of thyroid powder. This observation may indicate the intimate relationship between estrogen and thyroid hormone on the growth of MtT/F84. Recent studies revealed that both genes of ER and TR belong to the same gene family (12,13,14), and there may be some relationships between ER and TR on their functions.

It is believed that thyroidal actions on the target cells are mediated through TR. It has been confirmed to be widely dispersed among the rat tissues and many cell lines obtained from rodent pituitary (1). We have also confirmed nuclear binding sites for T_3 (TR) in MtT/F84 using competition binding assay.

We have previously reported that the growth of MtT/F84 was dependent upon the administered dose of E_2 and accordingly nuclear ER level was elevated (7). In the present study, growth of MtT/F84 is dependent on the dose of T_3, and both cytosolic and nuclear ERs were increased. This T_3 promoted increase of ER is similar to the relationship between progesterone bindings and estrogen in which progesterone binding depends on the dose of E_2 (15). Thus, it could be considered that T_3 seems to enhance the growth through the elevation of ER level. In GH_4C_1 rat pituitary tumor cells, thyroid hormones induced an autocrine growth factor to promote cell division (6). In MtT/F84, however, the growth seems to be more directly related to the interaction of estrogen and ER, and the action of T_3 for the growth promotion may be secondarily through the control of ER level. In uterine and mammary tissues, the increase of specific estrodiol binding by prolactin have been reported (16).

Recent reports proposed the nuclear localization of ER in the cell and cytoplasmic ER is considered as an artifact by the cell fractionation (17,18). Nevertheless, the measurement of both cytosolic and nuclear ER may be important since cytosolic ER level could be interpreted as a week binding of ER to nuclear sites. After all, total amount of ER in a cell is important in this report.

If thyroid hormones directly regulate the expression of ER gene in MtT/F84, it could be a good model for the investigation of gene expression by T_3 or functional relationships between ER and TR.

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