Hiroshima J. Med. Sci.
Vol.46, No.4, 143～150, December, 1997
HIJM 46–19

Effect of 17 β-estradiol, Retinoic Acid and Tamoxifen upon Primary and Transplanted Thyroid Tumor in $B_6C_3F_1$ Mice Fed an Iodine Deficient Diet

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ABSTRACT

This study was aimed to establish TSH dependent, transplantable thyroid tumor (TT) in $B_6C_3F_1$ (BCF_1) mice. In addition, transplanted TT was examined for its growth in mice given 17β- estradiol (E_2), retinoic acid (RA), tamoxifen (TAM), T_3 and T_4. Both sexes of BCF_1 mice were observed for 12 months under IDD and distilled water (DW), starting at 4 weeks of age. Groups of mice received an i.p. injection of radioactive iodine (^{131}I) once at a dose of 60 μ Ci/head and/or given 0.25 mg E_2 pellet s.c. One piece of induced pituitary or thyroid tumor was individually dissected aseptically and s.c. grafted under the fat pad of one site of the neck in the same strain of mice at 5 weeks of age. All mice were sacrificed between 7.5 to 13.5 months after grafting the tumors depending on the experiments. The transplantability of both pituitary and thyroid tumor was 100% in IDD mice, but TT was about 50% with a combined treatment of IDD plus E_2. A supplement of thyroid hormones of T_3 or T_4 in mice with IDD completely inhibited the growth of *in situ* or grafted thyroid tumors. The growth of *in situ* thyroid gland was significantly promoted by the oral administration of RA in both sexes, whereas the growth of transplanted TT was significantly increased by RA in the female, but not in the male. Oral administration of TAM proved inhibitory upon in *in situ* and transplanted TT in the male, but not in the female. Thyroid tumor induced by IDD could grow only in mice with IDD and was partially regulated of its growth by RA and TAM.

Key words: *Thyroid tumors, TSH, Retinoic acid, BCF_1 Mice*

Although new tumorigenic chemicals have been produced abundantly in daily life, estrogens are still the best tumorinogen for the pituitary gland in rodents. An excessive amount of estrogen or an absence of physiological hormones such as T_4 and/or T_3 is also important for the development of pituitary tumor by positive or negative feedback. Various compounds that hamper the negative or positive feedback loop of the thyroid-pituitary axis have been shown to be tumorigenic on the pituitary gland in long and medium term two-stage rodent bioassay models[2,5,7,8,10,14,15,19,25]. Treatment by IDD or with a combination of radioactive iodine and E_2 promoted thyrotropic or mammotropic pituitary tumors via negative feedback. It was also postulated that curtailment in the secretion of thyroid hormones results in an excessive release of TSH, which produces a chronic hyper-stimulation and consequently hyperplasia of the thyroid gland through various possible mechanisms[2,3,16,17,21].

In this study, we examined the effect of IDD, ^{131}I and E_2 in leading to tumor formation in the pituitary and thyroid glands[1,2,7,8,13,14,19,25]. Since there were no TSH dependent, transplanted thyroid tumor available in BCF_1 mice, we first isolated TSH dependent pituitary and thyroid tumors in mice and assessed the influence of various chemicals such as RA, TAM, E_2, T_3 and T_4 on the growth of TSH dependent tumor. Estrogen has long been known to have a tumorigenic effect upon pituitary, breast, liver, kidney or other tissues[9,11,12]. The activity of estrogen has been evaluated on cellular growth in pituitary and thyroid tumors, but the effect of a supplement of T_4 and/or T_3 upon the growth of those two types of tumors was not known.

Retinoic acid has a role in preventing epithelial cell tumorigenesis and even a therapeutic effect on human malignant tumors[24]. In our previous study, however, it was found that the growth of transplanted pituitary tumor (MtT/F84) in rats

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Abbreviations used: IDD, iodine deficient diet; DW, distilled water; TT, thyroid tumor; T_4, l-thyroxine; T_3, triiodo-thyronine; E_2, 17 β-estradiol; RA, retinoic acid; TAM; tamoxifen; BCF_1, $B_6C_3F_1$.

was promoted by RA[23]. In this study, we examined transplanted TT in mice. TAM, a nonsteroidal anti-estrogen, has been widely used in the treatment of breast cancer and the low incidence of side effects has increased enthusiasm for using TAM as a preventive measure in women at risk of developing breast cancer[4,18,22]. Recently, however, TAM was evaluated to have a carcinogenicity in liver in rodent studies[9,28]. Since the effect of TAM is highly influential on hormone dependent tumors, it was also examined in transplanted TT in the present study.

MATERIALS AND METHODS

Animals and housing conditions: Both sexes of $B_6C_3F_1$ mice were raised in our laboratory by crossing female C_{57}BL/6NCrj and male C_3H/HeNCrj, who were purchased from Charles River Japan, Inc. (Kanagawa, Japan). When the offspring reached four weeks old, they were started on an iodine deficient diet and distilled water to avoid iodine intake. Some groups of mice were given a single i.p. injection of Na ^{131}I (10–60 μ Ci/head, Specific activity, 7.77 Ci/mg, Dupont, USA) or 0.25 mg of 17 β-estradiol melted in cholesterol powder. After injection, all offspring were housed with their mothers in a radio-active protected quarter in Radiation Facility of Research Institute for Radiation Biology and Medicine, Hiroshima University. At 4 weeks of age, about 7～8 animals were housed together in autoclaved cages with sterilized wood chips and were kept in a controlled room temperature (24±2°C) and humidity (55±10%) under a regular 12-h light and 12-h dark cycle. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* for Hiroshima University.

Tumor transplanting procedure: After sacrifice, each piece of pituitary or thyroid tumor was aseptically minced into a piece about 0.5 mm in size in Hank's solution and they were transplanted s.c. into one site of the neck fat pad of the isologous strain of mice at 5 weeks of age with a sterilized trocar needle.

IDD and Chemicals: From 4 weeks of age, mice were given an iodine deficient diet (iodine content less than 5 ppm, Oriental Co. Ltd., Tokyo) and distilled water (DW) until the end of the experiment. The control mice were given a MF diet (Oriental Co. Ltd., Tokyo) and tap water *ad libitum*. At 5 weeks of age, 0.25 mg of 17-β estradiol (E_2, Sigma) containing a cholesterol pellet was s.c. implanted on the back and was renewed every month. Mice grafted with thyroid tumors were orally given T_4 (L-thyroxine, T-2501, Sigma Chemical Co.) in doses of 2.5 mg (T_4-L) or 10 mg (T_4-H) dissolved in distilled water or T_3 (3,3′ 5' Triiodo L-thyronine, T-2752, Sigma Chemical Co.) 1 mg (T_3-L) or 5 mg (T_3-H) also dissolved in distilled water. RA (Sigma Chemical Co., R-2625) was given in doses of 25 mg, 50 mg and 100 mg mixed with MF based IDD powdered diet (Oriental Co. Ltd.). TAM (Sigma Chemical Co., T-9262) was given in doses of 1, 5 and 25 mg/kg mixed with MF based IDD powdered diet (Oriental Co. Ltd.). Both chemicals were given throughout the experimental periods.

Pathology: All mice were observed every day and weighed once a month. The mice in Table 1 were sacrificed 12 months after administration of IDD, and the mice in other experiments (Tables 2～5) were sacrificed when the size of the grafted tumors reached more than 0.5 cm in their longest diameter. Mice were autopsied under ether anesthesia and the net weights of the pituitary, thyroid and grafted tumors were measured. After fixation in 10% neutral formaline, all pituitary, thyroid and grafted tumors were stained with hematoxylin and eosin, periodic acid-Schiff, or by the van-Giesson method if necessary. Enlarged tissues were histologically classified as non-neoplastic (hyperplastic) or neoplastic (adenoma or carcinoma) lesions. Hyperplasia was defined by either a diffuse or focal lesion without any mitotic figures. Neoplastic lesion was usually focal among hyperplastic lesions with some mitotic figures. For BrdU staining, paraffin sections of tissues were incubated overnight at room temperature and stained with monoclonal mouse anti-bromodeoxyuridine (Dako-BrdUrd, Bu20a, Code No. M 744) at a dilution of 1:20. After staining, BrdU-incorporated cells were counted by observing a 10^3 square μm. area of each selected tissue section.

Serum TSH and T_4 levels; Blood samples were collected from the jugular vein under ether anesthesia and sera were stored at −20°C until assay[20]. Serum TSH levels were measured by RIA reagents of the NIADDK provided by the NIH. Serum T_4 levels were measured by a RIA kit (Spac) obtained from Amersham Co. Ltd.

Statistical analysis: The Student's t test was used for data analysis.

RESULTS

Induction of pituitary and thyroid tumors (Table 1): By the administration of IDD, the survival of mice was about 95% in all experimental groups, but some mice became moribund or died because of enlarged pituitary or thyroid glands. At 13 months of age, the pituitary and thyroid glands of all mice except the control reached the maximum weight (Table 1). Body weights at sacrifice in groups 2～5 and 7～10 were significantly lower than those of the respective controls in both sexes. Pituitary weights in groups 2～5 and 7～10 were conversely higher than those of the respective controls by an average increase of 3.3 to 7.8 times due to IDD and additional E_2 treat-

Table 1. Experimental groups, body, pituitary and thyroid weights in BCF_1 mice given IDD, E_2 and ^{131}I[a]

Group	Treatment	Sex	No. examined	B.W. (g ± SD)	Pituitary	Thyroid
1	Control	♂	16	40.7 ± 3.9[b]	3.1 ± 0.7	20.8 ± 3.1
2	IDD	♂	15	26.1 ± 1.6[c]	11.0 ± 3.1[d]	133.7 ± 30.8[e]***
3	IDD+E_2	♂	14	22.3 ± 5.4[c]	24.2 ± 5.8[d]	113.6 ± 13.0[e]
4	IDD+^{131}I	♂	15	25.5 ± 2.8[c]	10.2 ± 7.5[d]**	ND
5	IDD+^{131}I+E_2	♂	16	25.4 ± 2.5[c]	23.8 ± 8.2[d]	ND
6	Control	♀	17	33.3 ± 4.1	2.9 ± 0.2	21.6 ± 3.5
7	IDD	♀	16	25.2 ± 2.7[c]	9.9 ± 2.4[d]	89.5 ± 32.0[f]
8	IDD+E_2	♀	16	21.0 ± 4.8[c]	15.4 ± 6.4[d]	75.2 ± 25.1[f]
9	IDD+^{131}I	♀	16	24.7 ± 2.8[c]	9.8 ± 2.8[d]	ND
10	IDD+^{131}I+E_2	♀	15	24.7 ± 2.1[c]	14.7 ± 4.7[d]	ND

[a]All mice were observed for 12 months after starting IDD, 0.25 mg of E_2 pellet or 60 μ Ci of ^{131}I treatments.
[b]Mean ± SD; [c]Significantly decreased from respective control by $p<0.01$ (t-test).
[d,e,f]Significantly increased from respective control by $p<0.01$ (t-test); ND—Not detectable.
**One of the pituitary tumor (PT) was grafted into hormonally conditioned mice shown in Table 2.
***One piece of thyroid tumor (TT) was grafted into hormonally conditioned mice shown in Table 3.

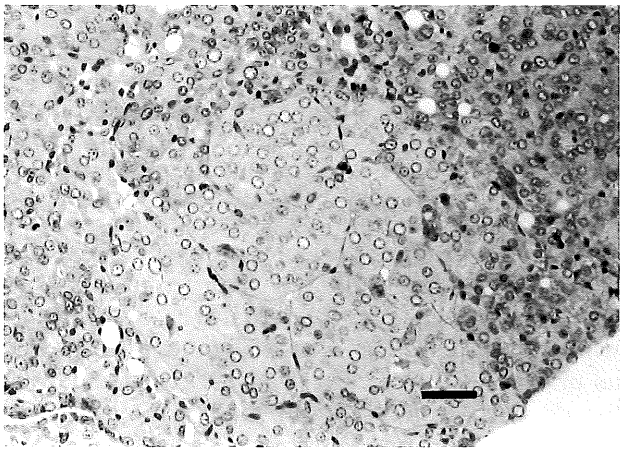

Photo. 1. An IDD induced pituitary tumor in a male mouse at 13 months of age.
The hyperplastic nodule is composed of triangular basophilic cells. H.E. stain.
Scale bar = 14.5 μm.

ment which further promoted the growth of the pituitary. Thyroid weights in groups 2, 3 and 7, 8 were significantly higher than those of the respective controls by an average increase of 3.5 to 6.4 times. In contrast to the pituitary, E_2 was rather inhibitory for thyroid growth. We could not detect thyroid tissues in groups 4, 5 and 9, 10 because they were completely destroyed by a flash injection of 60 μ Ci ^{131}I.

Transplanted pituitary tumor (Table 2) and thyroid tumor (Table 3): An enlarged pituitary tumor in group 2 of Table 1 was s.c. grafted in hormonally conditioned recipients. It was composed of a few focal hyperplasias of basophilic pituitary cells (Photo. 1) and some of them were stained with anti-TSH antibody. No malignant cells were observed in this tumor. Serum TSH levels were significantly increased in IDD mice and T_4 levels were significantly decreased from respective control values (Table 2). In the subsequent passages, the tumor became rather anaplastic without

Table 2. Transplanted pituitary tumor in various doses of ^{131}I and/or IDD treated mice

Treatment	Sex	No	Tumor positive (%)	Observation period (months)	Serum level			
					N	TSH (ng/ml)	N	T_4 (μ U/dl)
Control	♀	23	0 (0)	12	10	87 ± 4.3	8	12.2 ± 0.86
IDD	♀	17	17 (100)	12	5	968 ± 214	5	5.1 ± 0.29
IDD+ ^{131}I (μ Ci)								
10	♀	21	0 (0)	12		ND[a]	7	4.02 ± 0.38
30	♀	18	0 (0)	12		ND	6	2.82 ± 0.54
60	♀	21	15 (71.5)	12		ND	8	1.30 ± 0.63

[a]Not determined

Table 3. Influence of E_2, T_4 or T_3[a] for the growth of grafted thyroid tumors (TT)

Group	Treatment	Sex	No. examined	B.W (g±SD)	Pituitary (mg)	Thyroid (mg)	Transplanted TT		
							Latency (weeks)	Incidence (%)	Weight (mg)
1	Control	♂	16	41.6±4.0	3.2±0.8	19.7±2.8	58	0	ND
2	IDD	♂	10	35.9±3.8	12.9±3.2	130.9±29.1*	42	10 (100)	145.5±36.3
3	IDD+E_2	♂	10	33.9±3.7	26.8±6.1	102.6±23.4*	53	5 (50)	94.9±41.6
4	IDD+T_4(L)	♂	14	39.4±5.9	3.0±0.4	20.6±2.1	58	0	ND
5	IDD+T_4(H)	♂	15	39.5±3.2	3.2±0.5	19.8±3.9	58	0	ND
6	IDD+T_3(L)	♂	14	40.3±3.6	2.9±0.3	19.4±1.2	58	0	ND
7	IDD+T_3(H)	♂	15	39.6±1.8	2.9±0.3	19.9±4.3	58	0	ND
8	Control	♀	16	33.1±4.1	2.4±0.2	18.8±2.9	58	0	ND
9	IDD	♀	17	29.5±3.0	9.7±2.2	90.9±33.1*	46	17 (100)	157.6±123.5
10	IDD+E_2	♀	27	28.7±5.3	12.9±5.4*	70.6±28.1*	50	13 (48)	48.4±58.4
11	IDD+T_4(L)	♀	15	34.4±3.2	2.4±0.3	18.5±2.5	58	0	ND
12	IDD+T_4(H)	♀	14	34.5±2.5	2.3±0.3	18.4±3.3	58	0	ND
13	IDD+T_3(L)	♀	15	32.8±3.0	2.3±0.3	18.6±2.6	58	0	ND
14	IDD+T_3(H)	♀	15	33.9±2.8	2.4±0.3	18.6±2.4	58	0	ND

[a]T_3 and T_4 were given orally in drinking water; T_4(L):2.5 mg/lit. DW; T_4(H):10 mg/lit. DW; T_3(L):1 mg/lit. DW; T_3(H):5 mg/lit. DW.
*Significantly increased from respective control by $p<0.01$(t-test).

showing any original pituitary architecture (Photo. 2). The incidence of transplanted pituitary tumor was 100% in IDD mice and 71.4% in mice with an abrogation of thyroid tissue due to high doses of ^{131}I. Neither IDD plus small doses of 10~30 μ Ci of ^{131}I nor the control diet was conductive to the growth of pituitary tumors. In Table 3, the weights of both *in situ* thyroid glands and grafted thyroid tumors (TT) are significantly higher compared with groups 2, 3 in the male and 9, 10 in the female. E_2 had a somewhat inhibitory effect on transplanted TT as well as primary TT, and may be too high a dose of E_2 for *de novo* thyroid tissue. The grafted thyroid

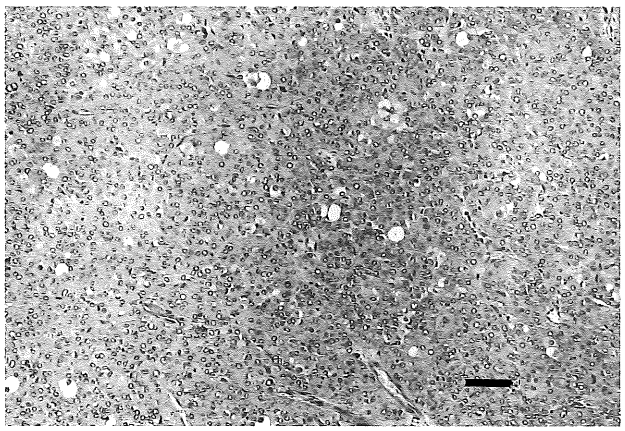

Photo. 2. Transplanted pituitary tumor in a mouse fed an IDD.
The tumor is monotonous with no special architecture. Some mitotic cells and microphages are seen. H.E. stain.
Scale bar = 10.5 μm.

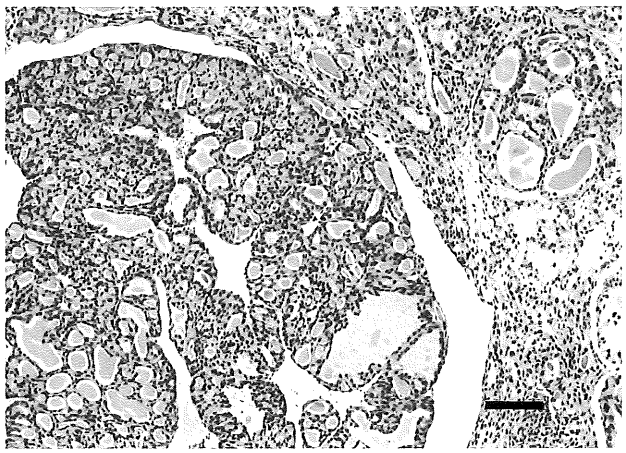

Photo. 3. An IDD induced thyroid tumor in a male mouse at 13 months of age.
There is a focal adenomatous nodule among diffuse hyperplasia. H.E. stain.
Scale bar = 14.5 μm.

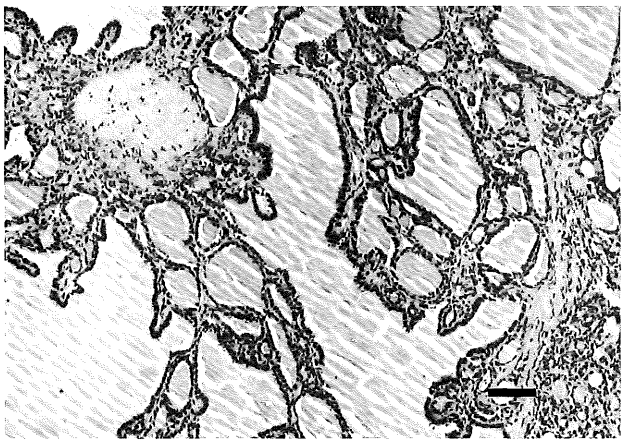

Photo. 4. A Transplanted TT grown in an IDD mouse. It is characterized by papillary adenocarcinoma with abundant thyroid follicles. H.E. stain. Scale bar = 14.5 μm.

tumor was composed of follicular hyperplasia and ademonas characterized by hyperplastic follicular cells with poor or absent colloidal follicles, and these pathological characteristics were maintained in the transplanted tumors (Photos. 3 & 4). Grafted TT was 50% inhibited in E_2-treated mice in both sexes, whereas it did not grow either in intact mice or in mice supplemented with T_4 or T_3 in both sexes.

The effect of RA or TAM on transplanted TT growth: In a separate study, transplanted TT was examined for its growth in mice fed with IDD plus various doses of RA (Table 4). The growth of transplanted TT in the male was 100% in the RA-0, RA-50 and RA-100 groups and 50% in the RA-25 group. In the female, RA-0 was only 12.5%, but the RA-25, 50 and 100 groups showed 100% and tranplanted TT was significantly increased in growth by RA in the female, but inconsistent in the male.

TAM was similarly examined for the growth of *in situ* as well as grafted TT (Table 5). It was significantly inhibitory for the growth in body weight in the female, but not in the male. *De novo* thyroid tissues and transplanted TT in the male were both significantly inhibited in their growth at a high dose of TAM, whereas transplantability in the various groups was inconsistent in the female.

BrdU incorporation in TT by treatment with RA and TAM (Table 6): BrdU labelled cells in *in situ* and grafted thyroid tumors were numerated at 30 weeks old in mice treated with RA and TAM. In RA treated female mice, BrdU uptake in *in situ* thyroid tissues was negative in all cases. In grafted thyroid tumors, it was higher in RA treated females than in the respective controls, whereas it was the same in all groups in the male. In TAM-treated male mice, BrdU uptake in *in situ* thyroid cells was low, but it was increased in grafted TT without any differences among experimental and control groups.

DISCUSSION

The present study was undertaken first to establish hormone dependent transplantable pituitary and thyroid tumors in BCF_1 mice, because no such hormone dependent tumor cell lines are available in this mouse strain. In addition, animal models of hormone dependent tumors are

Table 4. Influence of RA for the growth of thyroid gland and transplanted TT[a] in IDD mice

RA (mg)	No. of mice examined	B.W. (g)	Transplanted TT take	*In situ* thyroid (mg)	Transplanted TT (mg)
Male					
0	8	34.4±2.19	100%	94.1±4.6	218.4±144.4
25	8	28.5±2.48[b]	50%	133.7±15.4[c]	60.0±83.2[d]
50	8	28.6±3.80[b]	100%	141.5±12.0[c]	146.3±11.8
100	8	30.6±4.66	100%	137.8±4.4[c]	151.7±5.0
Female					
0	8	32.3±4.47	12.5%	29.9±7.22	1.3±3.75
25	8	25.2±1.61[b]	100%	109.8±19.6[c]	178.3±156.0[c]
50	8	26.3±1.40[b]	100%	145.9±10.6[c]	126.7±101.4[c]
100	8	24.2±2.24	100%	135.1±27.1[c]	205.7±206.3[c]

[a]Grafted TT was obtained from group 2 of Table 3 and recipient mice were observed for 26 weeks after the graft of TT
[b]Significantly decreased from respective control by $p<0.01$ (t-test).
[c]Significantly increased from respective control by $p<0.01$ (t-test).
[d]Significantly decreased from respective control by $p<0.05$ (t-test).

Table 5. Influence of tamoxifen for the growth of thyroid gland and transplanted TT[a] in IDD mice

TAM (mg)	No. of animal examined	B.W. (g)	Transplanted TT take	*In situ* (mg)	Transplanted TT (mg)
Male					
0	8	29.1±1.80	100%	97.9±19.4	212.7±97.5
1	8	30.1±0.51[b]	100%	94.8±6.4	201.2±156.4
5	8	31.3±1.14	100%	81.2±13.2	149.0±111.6
25	8	27.6±1.31	62.5%	65.3±10.41[b]	74.4±96.0[d]
Female					
0	8	31.4±3.07	0	34.0±16.52	0
1	8	25.6±1.62[b]	50%	51.6±11.56[c]	13.6±27.7
5	8	26.1±1.45[b]	0	26.4±7.10	0
25	8	26.5±2.14[b]	50%	36.4±12.42	10.3±22.9

[a]Grafted TT was obtained from group 2 in Table 3 and they were observed for 26 weeks after TT grafting.
[b]Significantly decreased from respective control $p<0.01$ (t-test).
[c]Significantly increased from respective control $p<0.05$ (t-test).
[d]Significantly decreased from respective control $p<0.05$ (t-test).

Table 6. BrdU incorporated cells in each piece[a] of de nova and transplanted thyroid tumors treated with RA and TAM

Treatment (mg)	Sex	Thyroid		Treatment (mg)	Sex	Thyroid	
		In situ	Transplanted tumor			*In situ*	Transplanted
RA-0	M	−	+	RA-0	M	−	++
RA-25	M	−	+	RA-25	M	−	++
RA-50	M	−	+	RA-50	M	+	+++
RA-100	M	−	+	RA-100	M	+	++
RA-0	F	−	+	RA-0	F	−	*
RA-25	F	−	++	RA-25	F	−	+
RA-50	F	−	++	RA-50	F	−	*
RA-100	F	−	++	RA-100	F	−	+

[a]We have counted BrdU incorporated cells from 10^3 square μm area of each piece of tumor.
*Tumor was not grown. '−', no BrdU incorporated cell. '+', ≦5 BrdU incorporated cells.
'++', 6~10 BrdU incorporated cells. '++', 11~15 BrdU incorporated cells.

necessary because of public demand for a risk assessment to human health of numerous new chemicals and medicines. TAM are representative compounds to be assessed for chemoprevention upon various types of clinical tumors.

IDD treatment significantly reduced body weight, but significantly increased the pituitary and thyroid weights in the male. Additional treatment of E_2 further increased pituitary weight, but thyroid weight was adversely decreased, which was a rather contradictory finding compared to rat thyroid tumorigenesis[20]. With a combination of IDD and ^{131}I, pituitary weight did not differ from IDD alone, but the thyroid tissue was completely abolished. An induced pituitary tumor and a thyroid tumor under IDD were propagated into variously conditioned recipients, and their results were tabulated on pituitary tumor in Table 2 and on thyroid tumor in Table 3. Transplanted pituitary tumor was grown either in IDD mice or in 60 μ Ci-treated mice with a 12 months observation period. Thus, the existence and function of the thyroid is not necessary for the development of the pituitary tumor.

Transplanted TT grew only in mice with IDD alone or with IDD plus E_2, but it did not grow in mice given either T_3 or T_4 in either sex. This indicates that the key hormone for the growth of TT may be an elevated level of serum TSH caused by IDD. Additional treatment of E_2 was

rather inhibitory for the growth of TT, but it did not inhibit completely like T_3 or T_4. Concerning TT transplantability, *de novo* pituitary and thyroid glands showed similar changes to grafted TT, in which IDD significantly increased the growth of both the pituitary and thyroid gland[21,26,27] and E_2 promoted the growth of pituitary, but inhibited the growth of thyroid in both sexes. It is concluded that IDD promoted the growth of both *de novo* and grafted tumors in the pituitary and thyroid glands, and that E_2 worked promoted the growth of pituitary tumors, but inhibited thyroid tumors.

The influence of RA on transplanted TT was assessed in IDD mice. The tumor transplantability and weight of TT were unstable in the male, but increased dose dependently in the female. This can be best explained as follows: RA may have an influence on the estrogen receptor and help the growth of estrogen dependent tumor. RA influenced the promotion of papillary carcinoma in transplanted TTs.

The influence of TAM[4,18,22] was also studied in both IDD treated male and female mice. In the male, the highest dose of 25 mg of TAM was significantly inhibitory for the weight of TT. In the female, the effect of TAM was not clear, since the tumor transplantability of TT was inconsistent in the various dose groups. This may be due to the fact that E_2 may be inhibitory for the growth of TT. In this treatment, all transplanted TT were follicular carcinomas in histology. Both pituitary and thyroid tumors studied in the present experiment were induced in BCF_1 mice who were treated with IDD for a prolonged period. In tumor transplantation studies, both tumors also grew well only in IDD treated mice, and TT could not grow after addition of T_3 and T_4. The mechanism of *in vivo* growth of TT has not been clearly identified yet, but the present findings clearly indicate that the elevated level of serum TSH caused by IDD may be the key factor.

A previous study in our laboratory showed that E_2 may be important for the growth of thyroid tumorigenesis in rats[6,20], but the present study in mice showed that E_2 was rather inhibitory for the growth of thyroid tumor. This contradictory effect may be due to the species difference.

In summary, we successfully established IDD dependent transplanted TT in BCF_1 mice in the present study. The TT grew well in IDD mice, but not in normal or IDD plus T_3 or T_4 supplemented mice. The increase of serum TSH levels may be essential for the growth and maintenance of TT. We also examined the effect of E_2 and classified it as inhibitory for TT growth, which is contradictory to our previous study in rat thyroid tumorigenesis.

TT growth was promoted by RA in the female and inhibited by TAM in the male. The underlying mechanism of the IDD dependent growth of TT should be further explored by introducing molecular mechanisms.

ACKNOWLEDGMENTS

The authors are greatly indebted to K. Ishimaru and Y. Sakai for their technical assistance. A part of this work was supported by a grant-in-aid from the Ministry of Health and Welfare, Japan.

(Received September 16, 1997)
(Accepted December 4, 1997)

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