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Theophylline and Melanocyte-Stimulating Hormone Effects on Gamma-Glutamyl Transpeptidase and DOPA Reactions in Cultured Melanoma Cells

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Gamma-glutamyl transpeptidase (GGT), an enzyme of the γ-glutamyl cycle, was demonstrated in 3 of 6 cell lines derived from a single B16 murine melanoma. Its activity in these cells varied a great deal, appeared to be correlated with the developmental cycle of the cells, and was greatest in young, actively melanogenic cells. Generally, the activity seemed parallel to that of tyrosinase, an enzyme specific for melanin synthesis. The levels of both enzymes tended to decline with prolonged *in vitro* cultivation, but could be readily renewed after one animal passage. The 3 cell lines that were GGT-negative were nonpigmented and DOPA-negative; so was a non-

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Abbreviations:
BNF: buffered neutral formalin
cAMP: cyclic AMP
dopa: L-β-3,4-dihydroxyphenylalanine
DOPA: dopa oxidase, tyrosinase
GGT: gamma-glutamyl transpeptidase
MSH: melanocyte-stimulating hormone

melanogenic and nonmelanocytic rhesus cell line. Melanocyte-stimulating hormone (MSH) and theophylline both enhanced pigmentation in murine melanoma cells. The mechanisms of their action apparently differed. We found that theophylline increased both DOPA- and GGT-reactive cells, whereas MSH only increased DOPA-reactive cells. All 3 GGT-positive lines were tumorigenic, and 2 GGT-negative lines were not tumorigenic. Our observations suggest that GGT plays a role in the melanin biosynthetic pathway and that its activity is greater in melanoma cells that are tumorigenic.

Gamma-glutamyl transpeptidase (GGT), one of the enzymes of the γ-glutamyl cycle, has been found to be active in cells during early differentiation and malignant transformation. Its function is believed to involve transmembrane amino acid transport [1] and support of protein synthesis.

Human and rat lymphocytes undergoing blast transformation increase their transpeptidase content [2]. It has been suggested that GGT is an important surface marker enzyme reflecting differences between normal and neoplastic cells [2]. However, Takahashi, Seifter, and Ritas [3] compared GGT activities in human diploid fibroblasts and other mammalian cells in culture and found that transformed cells (VA, HeLa S_3, and CHO) had the least activity, followed in increasing order by fibroblasts, human and bovine epithelial cells, and monkey kidney epithelial cells. Gamma-glutamyl transpeptidase activity also has been reported to increase in aging fibroblasts (WI38) as they approached "phase-out" [4]. Takahashi, Seifter, and Ritas thought that the large increase in cell size at this stage could lead to changes in the membranes where the enzyme is located. Growth in soft agar and cytochemical assay of GGT activity were suggested as reliable markers for transformation of adult rat liver epithelial cell cultures [5,6]. We have found that not only melanoma cells are GGT-positive; normal melanocytes at certain stages of development also have GGT activities [7,8]. The latter activity appears to be related to the active growth phase of the cells. Generally, we have found that malignant melanoma cells have greater activity than normal and nontumorigenic melanocytes. Thus, a correlation between tumorigenicity and GGT activity appears to exist.

The GGT activity in cultured melanoma cells varies a great deal. It is great in melanocytes during their melanogenic stage, but absent from cells not engaging in melanin synthesis [7,8]. Its absence from nonmelanogenic melanocytes and high level in melanin-synthesizing cells suggest a relationship between this enzyme and melanin production. Melanocyte-stimulating hormone (MSH) and theophylline have been shown to enhance pigmentation in pigment cells [9–12]. In the study described here, we investigated the effects of theophylline and MSH on GGT activity in cultured murine melanoma cells.

MATERIALS AND METHODS

Cell Lines

Established murine B16 melanoma cell lines [13–15], designated as HFH18-(15)-F, HFH-(15)-C, HFH18-(94), NP133, NP135, and P51 [14–15], as well as primary monolayer cell cultures of mouse tumors resulting from injection of the above-mentioned established cell lines,* designated as HFH18-(15)-FT, HFH18-(15)-CT, HFH18-(94)-T, NP133-T, and P51-T, were used. Because NP135 is not tumorigenic, only the original cell line was used. All lines were established at different times from cell cultures (HFH18) originated in 1963 from a B16 melanoma carried in C57BL/6 mice in my laboratory [13]. The primary cultures were carried *in vitro* for 22 passages, and frozen in 1963 and thawed in 1975. All 3 HFH18 sublines—HFH18-(15)-C, HFH18-(15)-F and HFH18-(94)—were derived from these frozen-thawed cells. P51 line was established from pigmented tumors after injections of HFH18 cells into C57BL/6 mice in 1964 [15]. NP lines were derived from a nonpigmented tumor established in 1964 by both *in vitro* and *in vivo* selection of nonpigmented HFH18 cells. NP133 cells underwent passage 133 times in mice before they were put into cultures in 1972. They underwent subculture 16 times and one freezing-thawing between 1972 and 1973; then they underwent passage 35 times *in vivo* until 1975, when primary cultures were made from one of these nonpigmented tumors. NP135 cells, unlike NP133 cells, had 2 additional passages in mice before they were put into culture in 1972. Subsequent passage histories of all lines used in this study are summarized in Table I. Line (15)-C is more pigmented than line (94), which has only a few pigmented cells. Line P51 has even less pigmented cells than line (94). Line (15)-F is the most pigmented. Both NP lines completely lack pigment.

All melanoma lines grew well in culture. Morphologically, NP cells, particularly NP135 cells, were different from pigmented cells, i.e., they were larger and flatter and did not pile up as much. In addition, RF/6A, a cell line derived from the eyes of a 64-day-old rhesus fetus, was included as a nontumorigenic and nonmelanogenic control [16].

Histochemical Reactions

1. Gamma-glutamyl transpeptidase reaction: For histochemical demonstration of GGT activity, monolayer cell cultures were fixed with 10% buffered neutral formalin (BNF) (pH 7.0) for 10 min and were assayed with a technique modified by Rutenburg et al [17]. Two basic stock solutions were prepared. The first contained 5 mg of γ-glutamyl-4-methoxynaphthylamide (Vega-Fox Chemicals, Tucson, Arizona), 0.1 ml of dimethylsulfoxide, 0.1 ml of 1 N NaOH, and 1.8 ml of distilled water; the second contained 20 mg of glycylglycine (Sigma) in 10 ml of 0.1 M tris(hydroxymethyl)aminomethane (pH 7.4) and 28 ml of 0.85% NaCl. Solutions 1 (2 ml) and 2 (38 ml) were combined, 20 mg of fast blue BBN (Sigma) were added, and the resulting medium was immediately filtered and used. The fixed cells were placed in this solution for 1 hr at 37°C. After this incubation period, they were rinsed serially in 0.85% NaCl, 0.1 M $CuSO_4$, 0.85% NaCl, and distilled water and then were mounted in glycerol-gelatin.

2. DOPA reaction: For histochemical demonstration of tyrosinase (DOPA) activity, we adapted the DOPA reaction procedure described by Laidlaw and Blackberg [18] to cultured monolayer cells. Briefly, the cells were fixed in 10% BNF for 10 min, rinsed twice with 0.055 M phosphate buffer, and incubated in 0.1% L-β-3,4-dihydroxyphenylalanine (dopa) in phosphate buffer at 37°C for 4 hr. Then the cells were rinsed twice with phosphate buffer, rinsed with distilled water, and mounted with glycerol-gelatin.

3. Combined DOPA and GGT reaction: Monolayer cell cultures were fixed with 10% BNF for 10 min and were incubated in a 0.1% dopa solution for 4 hr (as described for the regular DOPA procedure). At the end of 4 hr, the cells were briefly rinsed 2 times with physiological saline; and then were incubated in the GGT substrate for 1 hr at 37°C (as described for GGT procedure above).

4. Other histochemical reactions: The following combinations were also used: GGT substrate plus cysteine; GGT substrate plus glutathione; dopa plus cysteine; and dopa plus glutathione.

For the above, 40 mg of L-cysteine/100 ml or 30 mg of glutathione/100 ml (25 mM) were added to the GGT or DOPA substrate and incubated as described for regular GGT or DOPA procedure. In addition, cysteine or glutathione was added to the dopa in the first part of the combined DOPA and GGT procedure. The procedure otherwise was the same as the regular combined substrate procedure.

All slides were examined and compared for the number of pigmented cells, the degree of pigmentation, the number of GGT- or DOPA-positive cells, and the intensity of the reaction. In addition, the amounts

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* All melanoma cell lines—HFH18-(15)-C, HFH18-(15)-F, HFH18-(94), P51, NP133, and NP135—were examined for their tumorigenicity by intramuscular injection of 10^6 cells suspended in Hanks' balanced salt solution into both hind legs of 6-week-old C57BL/6 mice. Tumors usually developed in 3 to 4 weeks. When they were 1 cm in diameter, the tumors were excised and used to set up primary cell cultures.

Line HFH18-(15)-CT cells; theophylline 1.5 mM; the combined DOPA-GGT histochemical method (× 160). A mixture of DOPA-positive, GGT-positive, and nonreactive cells. Note some DOPA-positive cells have both juxtanuclear and peripheral reactions (type B). Some (the very dark cells) have only a peripheral reaction (type C). Most of the GGT-reactive cells also show a positive DOPA reaction (type A or B). Not all GGT-positive cells react to the same degree. The strongly positive cells are bright orange-red, the questionably reactive or nonreactive cells have only a slight tint of brownish-orange, and the weakly and moderately reactive cells are intermediate in color.

of growth and the shapes and sizes of the cells in each condition were compared.

Theophylline and Melanocyte-Stimulating Hormone Treatment

Cells grown on coverglasses or in culture dishes were treated 2 or 3 days after they had been seeded and had grown into large aggregates or had become semiconfluent. For treatments with theophylline (1, 3 dimethylxanthine, Sigma) we used 3 dosages ranging from 0.125, 0.5 to 1.5 mM for 3 time periods of 24, 48, and 72 hr. With MSH (α-MSH [BA33761] was generously supplied by Ciba Pharmaceutical) we used a fixed time-dosage schedule of 0.02 μg/ml for 48 hr. Afterwards the cultures were fixed and prepared for microscopic examinations as described above.

RESULTS

Table II summarizes the DOPA and GGT reactions of 6 melanoma cell lines and one rhesus cell line incubated in different substrates.

In general, both DOPA and GGT reactions were demonstrated in descending order in the pigmented melanoma cells, i.e., in HFH18-(15)-F, HFH18-(15)-C, and HFH18-(94) cells. A small number of P51 and NP133 cells had orange-red GGT-positive granules sparsely distributed in the cytoplasm. The NP135 and RF/6A cells had none. All tumors—from injections of HFH18-(15)-F, HFH18-(15)-C, HFH18-(94), P51, and NP133—showed more striking GGT reactions (i.e., more reactive cells and stronger reactions) than the respective original cell lines that produced the tumors; all tumors from injections of HFH18 cells were darkly pigmented, more so than the original cell lines. For example, both HFH18-(15)-C and HFH18-(94) cell lines (especially the latter) had only small numbers of pigmented cells. When cell suspensions were centrifuged, the pellet formed from HFH18-(94) was grayish rather than black as in the case of HFH18-(15)-F. The HFH18-(15)-C was darker than the HFH18-(94), but less so than the HFH18-(15)-F. All tumors, however, from these 3 cell lines were black (Table I). Cells in primary cultures made from HFH18 tumors [HFH18-(15)-FT, HFH18-(15)-CT, HFH18-(94)-T] were more pigmented, and were more DOPA- and GGT-positive than their respective cell lines. Therefore, a passage *in vivo* increased both pigmentation and GGT activity.

In all the melanogenic melanoma lines we examined—HFH18-(15)-F, HFH18-(15)-C, and HFH18-(94)—certain percentages of cells showed a GGT reaction. But the number of positive cells and the degree of reactivity varied a great deal among the lines and among cells in a given culture. Reactivity varied from negative or weakly positive (faint brown-orange tint) to strongly positive (bright orange-red). The variation occurred in both pigmented and nonpigmented cell populations. Generally, GGT activity was more prevalent in small ovoid cells than in large cells; it was absent from darkly pigmented cells, but frequently present in lightly pigmented small cells (Figure).

Similarly, all 3 melanogenic melanoma cell lines had cells showing a positive DOPA reaction. White, Hanson, and Hu [9] have described 3 types of positive L-DOPA reaction observed by light microscopy. Type A cells display a distinctive reticular pattern of tyrosinase activity in a juxtanuclear position; the more peripheral granular pattern characteristic of pigmented melanocytes is absent. Type B have peripheral granular-associated activity in addition to the juxtanuclear label present in type A. Type C cells have the granular label, but lack any

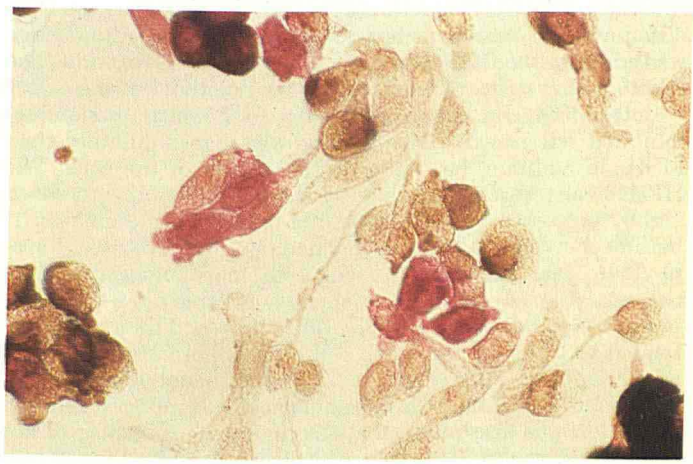

TABLE I. *Passage history of melanoma cell lines used for mouse injections*

Cell lines	History	Tumorigenicity	Tumor color
HFH18-(15)-F	62 *in vitro* passages with 3 freezings/thawings between 1975 and the time of injection (3/26/80)	Yes	Black
HFH18-(15)-C	79 passages with 2 freezings/thawings between 1975 and the time of injection (5/7/80); *in vitro* continuously since 1979	Yes	Black
HFH18-(94)	160 *in vitro* passages since 1975 with 2 freezings/thawings between 1978 and 1979; injection on 3/26/80	Yes	Black
P51	51 passages *in vivo* between 1964 and 1968; 631 *in vitro* passages since 1968 with 1 freezing/thawing between 1968 and the time of injection (7/28/80)	Yes	Grayish-black
NP133	35 passages *in vivo* between 1973 and 1975; 2 *in vitro* passages and 1 freezing/thawing between 1975 and the time of injection (4/9/80)	Yes	Nonpigmented
NP135	42 *in vitro* passages with 3 freezings/thawings between 1972 and the time of injection (4/9/80)	No	Not applicable

TABLE II. *Gamma-glutamyl transpeptidase and DOPA reactions in different cell lines with various substrates*

Cell line	Dopa	Dopa + cysteine	Dopa + glutathione	GGT	GGT + cysteine	GGT + glutathione
HFH18-(15)-F	+++	± or +	± or +	+++	+++	−
HFH18-(15)-C	++	±	±	++	++	−
HFH18-(94)	+	± or −	± or −	+	+	−
P51	±	ND	ND	± or +	ND	ND
NP133	−	ND	ND	± or +	ND	ND
NP135	−	ND	ND	−	ND	ND
RF/6A	−	ND	ND	−	ND	ND

Abbreviations: dopa, L-β-3,4-dihydroxyphenylalanine; GGT, substrate for gamma-glutamyl transpeptidase; ND, not done.

Symbols: +++, approximately 25% of the cells are reactive; ++, approximately 15% of the cells are reactive; +, scattered small aggregates of reactive cells, approximately 5% or less; ± or +, a few scattered reactive cells plus some weakly or questionably reactive cells; ±, weakly or questionably reactive cells; −, nonreactive cells.

discernible juxtanuclear concentration of reaction product. In our study, all the melanogenic lines had cells showing these 3 types of DOPA reaction. The reactions, however, varied from negative to strongly positive. The 2 NP lines did not react at all.

In culture, except a few rare pigmented cells, P51 cells were predominantly not pigmented and DOPA-negative. Injections of P51 cells into mice produced tumors that were dark, but not jet black; by contrast injections of all three HFH18 cell lines produced jet black tumors. In spite of the great variations, the differences between cell lines and changes in cells incubated in different substrates are consistent and reproducible. The data summarized in Table II are the results of experiments repeated at least 3, and sometimes as many as 10, times. The combined substrate histochemical method made it possible to demonstrate both DOPA and GGT reactions in the same cells. With this technique we found many of the lightly pigmented small ovoid or bipolar cells were both DOPA- and GGT-reactive. The HFH18-(15) cells—after their incubation in dopa reagent—could still react with the GGT substrate (Figure). When these cells were incubated in a solution of dopa and cysteine or dopa and glutathione, they showed some decrease in DOPA reactivity; but when they were subsequently incubated in the GGT substrate, the GGT reaction appeared to be enhanced. The difference was consistent but not dramatic. When glutathione was added to the GGT substrate, it abolished the reaction. The addition of cysteine did not change the reactivity of the cells.

Both MSH and theophylline (0.5 to 1.5 mM) moderately inhibited cell growth. Theophylline was more inhibitory than MSH. In addition, both altered the shapes of the cells. The HFH18 cells were predominantly small and ovoid, with short, stubby processes. Cells incubated in medium containing MSH became more spindle-shaped and had tapering dendrites. Theophylline treatment also changed cells morphologically. At a concentration of 0.5 to 1.5 mM, the cells became more dendritic, but not as spindle-shaped as the MSH cells, and their processes tended to come off the small cell bodies more abruptly. This effect was dose-dependent; at 0.125 mM theophylline did not significantly alter the cells morphologically. Its effect on growth inhibition was also minimal. Cells under the influence of low dosages resembled MSH-treated cells, i.e., spindle-shaped cells with tapering dendrites.

Table III compares the effects of MSH and theophylline on the GGT reaction and pigmentation in HFH18-(15) cells. It shows that theophylline increased the percentage of GGT-positive cells (35% compared to 12% for controls) as well as that of pigmented cells (60% versus 36%). On the other hand, MSH did not significantly increase the percentage of GGT-positive cells (15% compared to 12% for controls), but did cause an increase in pigmented cells (55% versus 36%). All HFH18 lines showed significant responses to theophylline treatment; there were increases in the numbers of pigmented cells, DOPA-positive cells, and GGT-positive cells, as well as increases in the intensities of these reactions (Table III). These phenomena occurred both before and after one animal passage. Theophylline had no effect on pigmentation in the NP lines. Line NP133 had a small number of cells with cytoplasm containing small orange-red granular deposits similar to those seen in GGT-reactive cells. Their number somewhat increased after treatment with 1.5 mM theophylline *in vitro* or after one *in vivo* passage. The reaction was mostly weak, i.e., the color was never as bright as the orange-red seen in the highly reactive HFH18 cells. Line NP135 was completely unresponsive.

The behavior of line P51 resembled that of NP133. Unlike NP133, line P51 did have occasional pigmented cells whose number increased a little (i.e., from 0–1 to 10–15 cells per coverglass) after theophylline treatment.

The α-MSH also increased the number of pigmented cells and degree of pigmentation and DOPA reactivity in all 3 HFH18 lines, but it had little or no effect on GGT activity.

DISCUSSION

Because melanocytes during development, as well as in culture, change from small ovoid to bipolar, dendritic, and finally large epithelioid or platelike cells, possibly the variation in GGT reactivity is related to the developmental cycle of the cell. Its absence in darkly pigmented large cells suggests that only younger, not terminally differentiated, cells exhibit such a reaction. This idea is supported by autoradiographic evidence (unpublished data): thymidine uptake occurred predominantly in small ovoid and bipolar cells, but rarely in large epithelioid or darkly pigmented cells. Labeling was not observed in strongly GGT-reactive small ovoid cells that were classified by their size and shape as young melanocytes. This finding seems in conflict with the statement that thymidine uptake occurred predominantly in the smaller younger cells. This contradiction may be explained by a generally well-recognized phenomenon, i.e., DNA synthesis and mitotic activity are usually curtailed in cells actively engaged in synthesizing a differentiation product (melanin in this particular case). Strongly GGT-positive small cells may or may not contain visible melanin, but they are potentially melanogenic. In the combined histochemical reactions these small cells were often positive for both DOPA and GGT. The other small cells that actively took up thymidine were destined for division. Their enzyme activities were usually weak or nondetectable. The GGT reactions were observed in both pigmented and nonpigmented cells in the melanogenic lines (HFH18 cells) and iridial stromal melanocytes [7,8], but were absent from nonmelanogenic lines (NP133, NP135, and RF/6A) and from postnatal choroidal melanocytes and retinal pigment epithelium [7,8]. The lack of GGT reactivity in nonmelanogenic cell lines suggests strongly that GGT plays a role in melanin synthesis. Nonmelanotic cells grow well in culture. They do not behave differently from their melanogenic counterparts (all HFH18 lines) except in their ability to synthesize melanin. Prota, Rorsman, and their colleagues [19–21] believe that GGT converts glutathionedopa to cysteinyldopa, which is an intermediate in the pheomelanin biosynthetic pathway. The GGT reaction in melanogenic melanocytes appears to be enhanced by preincubation with dopa together with glutathione or cys-

TABLE III. *Effects of theophylline and melanocyte-stimulating hormone on pigmentation and distribution of gamma-glutamyl-transpeptidase-positive cells in the HFH18-(15) line*

Cells	Percentage of GGT-positive cells[a]				Percentage of pigmented cells[a]				
	0	±	+	++	0	±	+	++	+++
Control	82	6	11	0.7	9	55	32	3.5	0.13
	(1357)	(100)	(185)	(12)	(142)	(838)	(483)	(54)	(2)
MSH	75	9	15	0	15	28	32	21	2
	(1262)	(154)	(258)	(0)	(253)	(434)	(501)	(335)	(40)
Theo	50	14	33	2	20	19	38	22	0
	(897)	(252)	(595)	(34)	(372)	(344)	(701)	(410)	(0)

Abbreviations: GGT, γ-glutamyl transpeptidase; MSH, melanocyte-stimulating hormone; Theo, theophylline.

Symbols: 0, GGT-negative or not pigmented; ±, questionable; +, weakly but definitely reactive or lightly pigmented; ++, moderately GGT-positive or moderately pigmented; +++, darkly pigmented.

[a] Number in parentheses is the number of cells counted.

teine, which keeps the reaction from proceeding from dopaquinone to eumelanin formation. This phenomenon is in keeping with Prota's proposed pheomelanin pathway, in that both cysteinyldopa and glutathionedopa in the presence of GGT can proceed toward the synthesis of pheomelanin.

It has been shown that glutathione inhibits the reaction from tyrosine or dopa to melanin [22]. Glutathione, but not cysteine, competes for the enzyme in the GGT reaction, and so when glutathione is added to the incubation substrate for GGT it abolishes the reaction [17]. The GGT activity appears to coincide with the growth and early differentiation of melanocytes; it declines with maturation and is finally lost at terminal differentiation [7,8]. The activity of tyrosinase somewhat parallels that of GGT; it begins to appear during early development, reaches its peak at maturity, and declines when the cells grow old. Both enzymes exist in the growth and development phase of melanocytes, when the cells are active in melanogenesis. The activities of the 2 enzymes, however, are expressed without interference from each other. One can perform cytochemical assays for DOPA and GGT in succession without altering their respective activities.

San et al have used GGT activity as a cell marker to identify neoplastic transformation of liver epithelial cells in culture [6]. Similarly, we found that the tumorigenic melanoma cell lines HFH18-(15)-C, HFH18-(15)-F, and HFH18-(94) had greater GGT activity than the nontumorigenic line NP135. In addition, we observed that one *in vivo* passage in syngeneic hosts increased both the DOPA and the GGT activity in tumors and in primary cell cultures made from these tumors. The HFH18-(15)-F was more pigmented and more GGT-positive than its sister cell lines HFH18-(15)-C and HFH18-(94), which had undergone many more passages in culture (Table I). Generally, the degree of pigmentation and the GGT activity appeared to be in reverse proportion to the number of passages. The activity of these 2 enzymes decreased as the time in culture increased.

The NP cell lines were genetically nonmelanogenic owing to the lack of tyrosinase; a passage *in vivo* did not correct this defect. The cells remained DOPA-negative. Line NP135 was GGT-negative as well as nontumorigenic. Lines NP133 and P51 were tumorigenic; both were found to have a small number of cells containing GGT-reactive orange-red granules in their cytoplasm, which tended to increase in amount when the cells underwent passage *in vivo* or were treated with theophylline. The tumorigenicity of NP133 cells can be easily explained: they were cultured from a nonpigmented tumor and had undergone only 2 passages *in vitro* (Table I). Line P51, however, was carried continuously for 634 subcultures and was interrupted by only one freezing and thawing in a period of 12 yr. During this interval the number of pigmented cells decreased progressively, and now the line has become practically free of such cells. However, these cells did produce dark tumors when injected into C57BL/6 mice.

The growth and pigmentation responses of two melanoma cell lines (HFH18 and P/140) to theophylline and MSH have been shown by White, Hanson, and Hu [9] to be quite different. Theophylline-induced changes in pigmentation do not require the participation of higher intracellular cyclic AMP (cAMP) levels. This effect is in direct contrast to the effects of α-MSH, which are mediated through cAMP. Caffeine and theophylline inhibit postreplication repair in ultraviolet-light-irradiated cells in culture. This inhibition also does not seem to be mediated by cAMP, but is probably the result of a direct action of theophylline on DNA [23]. The effect of theophylline on GGT activity appears to resemble that on tyrosinase activity, i.e., theophylline stimulates both. However, MSH does not induce a significant increase in GGT-positive cells, although it is effective, as is theophylline, in enhancing pigmentation in melanogenic melanoma cell lines. Neither GGT nor tyrosinase activity has been demonstrated in the amelanotic melanoma cell line (NP135) with or without theophylline or MSH treatment. The nonmelanocytic cell line RF/6A is also not reactive. In theophylline-stimulated melanocytes there is a distinct increase in Golgi-related vesicles and cisternae, which are filled with DOPA reaction products [9]. The DOPA reaction is always negative in the nonmelanogenic cell lines (NP and RF/6A).

Gamma-glutamyl transpeptidase is known to catalyze the initial step in the degradative metabolism of glutathione. Halprin and Ohkawara [22] have suggested that reduced glutathione helps control pigmentation by inhibiting melanin formation from tyrosine and dopa. These investigators have demonstrated that glutathione reductase activity and reduced glutathione are less in Negro skin than in Caucasian, and hyperpigmentation after exposure to ultraviolet light is preceded by a drop in the levels of glutathione reductase and reduced glutathione.

Glutathione reacts with dopaquinone to form glutathionedopa, which, through the enzyme GGT, is converted to cysteinyldopa [19–21]. Cysteinyldopa can also be formed directly through the combination of dopaquinone and cysteine without the intervention of an enzyme. According to Prota's pheomelanin biosynthetic pathway, cysteinyldopa is the principal intermediate that leads to pheomelanin formation [19].

Our findings that GGT is present only in melanogenic melanocytes and that in vitro theophylline enhances pigmentation and GGT activity in melanogenic melanoma cells suggest active participation of GGT in the melanin biosynthetic process.

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ANNOUNCEMENTS

The joint meeting of the Society for Investigative Dermatology, Inc. and the European Society for Dermatological Research will be held at the Capitol Hilton Hotel in Washington, D.C., U.S.A., April 27–May 1, 1983.

All abstracts must be submitted to Dr. Kirk Wuepper, Secretary/Treasurer, The Society for Investigative Dermatology, Inc., 14435 S.W. Uplands Drive, Lake Oswego, OR 97034. Abstracts must be received on or before December 6, 1981, and postmarked on or before December 1, 1981. Forms may be obtained from Dr. Herbert Honigsmann, Secretary, ESDR, Department of Dermatology, I, University of Vienna, Alserstrasse 4, A-1080 Vienna, Austria, or Dr. Kirk D. Wuepper.

The International Symposium on Paediatric Dermatology, Mazara del Vallo (Trapani), will be held September 22–25, 1982. It is promoted by the Foundation for Research in Dermatology under the auspices of the European Society for Dermatological Research, Italian Society of Dermatology, Italian Dermatological Association, and the Italian Society of Paediatrics. President: F. Serri, Professor and Chairman, Department of Dermatology Catholic University, Rome; Scientific Secretariat: G. Fabrizi, S. Gatti, Department of Dermatology, Catholic University, 00168 Rome (Tel: 06-33054227/33054211); Scientific Organization Secretariat: E. Shargool, M.El Hachem, Foundation for Research in Dermatology, Largo Gemelli 8, 00168 Rome (Tel: 06-3385451).

The United Scleroderma Foundation awarded $3,000 to Yasuoki Moroi, Carol Peebles, Marvin Fritzler, J. Steigerwald, and Eng Tan from the University of Colorado Medical Center for their paper "Autoantibody to centromere (kinetochore) in scleroderma sera." (*Proc Natl Acad Sci* 77:1627–1631, 1980.) The United Scleroderma Foundation again plans to award $3,000 for the best research article on scleroderma published in 1982. Names of applicants for the award, together with supporting data, should be submitted by December 30, 1982. Results of the research must have been presented at a scientific medical meeting or published in a recognized medical journal. The award recipient will be determined by a committee. Address all communications to Denny L. Tuffanelli, M.D., Department of Dermatology, University of California School of Medicine, San Francisco, CA 94143.

The Westwood Pre-Board Slide Seminar will take place September 10–12, 1982 at the O'Hare/Kennedy Holiday Inn in Rosemont (Chicago), Illinois. For information and registration, contact Frank Brisben or Kris Eimiller at Westwood Pharmaceuticals, 468 Dewitt Street, Buffalo, NY 14213, or call 716-887-3702.

ACKNOWLEDGMENT

In order for The Society for Investigative Dermatology to generate additional funds and further expand its activities in the field of Dermatology, a new class of membership, Corporate Sustaining Membership, has been established. The Society wishes to acknowledge the support of the following companies, who are Corporate Sustaining Members

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