Dexamethasone, Prostaglandin A, and Retinoic Acid Modulation of Murine and Human Melanoma Cells Grown in Soft Agar1,2,3

Marvin D. Bregman, 4 Elizabeth Peters, 4 Diane Sander, 4 and Frank L. Meyskens, Jr. 4,5

ABSTRACT—The cloning efficiencies of a murine melanoma cell line (S91 CCL 53.1) and a human melanoma cell strain (C8146c) were inhibited by dexamethasone (DEX), prostaglandin A1 (PGA1), and β-all-trans-retinoic acid (RA) in a dose-dependent manner. Murine melanoma tumor colony-forming units (MTCFU) were inhibited more than 99% by DEX (1×10⁻⁷ M) and RA (1×10⁻⁷ M) with a concentration needed to produce a 50% reduction in colony formation for both hormones of 5×10⁻⁹ M. Combinations of DEX and RA affected a synergistic inhibition on colony formation, which was reflected by a 1½ log reduction in the hormone concentration needed to produce a greater than 99% inhibition of colony formation. When PGA1 was added to DEX and RA, a greater than additive reduction in colony formation was observed. Human MTCFU from cell strain C8146c were inhibited more than 85% at an RA concentration of 1×10⁻⁷ M, but they were reduced only to 40% of control at a DEX concentration of 1×10⁻⁶ M. DEX-RA produced an additive inhibition of colony formation. Addition of submaximal amounts of PGA1 to DEX-RA combinations or to either hormone alone resulted in synergistic reduction of human MTCFU. These results demonstrated that the proliferative potential of human and murine melanomas can be simultaneously regulated by DEX, PGA1, and RA. —JNCI 1983; 71:927–932.

Several hormones including steroids, retinoids, and prostaglandins inhibit melanoma cell proliferation and/or induce differentiation (1–8). Glucocorticoids inhibit the proliferation of hamster melanoma cells (4) and induce differentiation in murine melanoma cells as measured by changes in tyrosinase activity (3). The inability of glucocorticoids to produce complete regressions of melanomas in vivo may be due to resistant mutants, as suggested by a recent study in which nonresponisive clones were isolated from a glucocorticoid-sensitive melanoma cell line (4). The high incidence of glucocorticoid receptors in cells from biopsy samples obtained from patients with melanoma suggests that human melanoma cells may also be responsive to this hormone (9).

Retinoids inhibit the proliferation and induce differentiation of murine melanoma cells in vitro (1, 10). Some human melanoma cells in culture are inhibited by RA, but the response is not uniform, with the growth of some cell lines being either insensitive to or even stimulated by RA (11). Even clones selected from individual human (12) and murine (13) melanoma cell lines displayed differential sensitivities to RA. We have found that cells from biopsy specimens of melanoma tissue grown in agar also demonstrated marked heterogeneity in response from patient to patient, ranging from a substantial inhibition of melanoma colony formation to no effect (14).

Prostaglandins also inhibit the growth of murine melanoma tumors in vivo (15, 16). PGA1 is a strong inhibitor of DNA synthesis (6, 17), and we have found the growth of murine melanoma cells in soft agar to be strongly inhibited by PGA1 (7). Turner et al. (18) recently reported that irreversible inhibition of murine melanoma growth occurs only if the cells are exposed continuously to PGA1 for more than 72 hours. Our laboratory has also found that PGA1 is a potent inhibitor of human melanoma colony formation and that prostaglandins B1, F1α, and F2α were inactive (19).

Previous studies have measured the activity of a single hormone such as DEX or retinoids on melanoma cell growth. The use of antiproliferative hormones in combination may lead to an enhanced biological response. In this communication we measured the combined effects of DEX, RA, and PGA1 on human and murine clonogenic tumor cells in vitro. The reduction of MTCFU in soft agar was enhanced by continuous exposure to the combined hormones.

MATERIALS AND METHODS

Chemicals.—DEX and RA were purchased from Sigma Chemical Co., St. Louis, Mo. Ham's F-10 medium was obtained from GIBCO, Santa Clara, Calif. FCS and HS were from KC Biological, Lenexa, Kans. PGA1 was obtained from The Upjohn Co., Kalamazoo, Mich.

Cell cultures.—The Cloudman S91 murine melanoma clone CCL 53.1 was obtained from the American Type Culture Collection, Rockville, Md., and was maintained by serial transplantation in DBA/2J mice. The tumors were harvested, and single-cell suspensions were obtained as previously described (7). The cells were added to a flask containing F-10 media with 10% HS and 2% FCS. CCL 53.1 cells readily formed a monolayer and were subsequently subcultured. All experiments were performed on cells that had been subcultured no more than 10 times after isolation from mouse melanomas.

ABBREVIATIONS USED: DEX-dexamethasone; FCS=fetal calf serum (heat-inactivated); HS=horse serum; ID50=concentration needed to produce a 50% reduction in melanoma colony formation; MTCFU=melanoma tumor colony-forming units; PGA1=prostaglandin A1; RA=β-all-trans-retinoic acid.

1Received January 18, 1983; accepted June 21, 1983.
2Supported in part by grant PDT 184 from the American Cancer Society and by Public Health Service grants CA-27502 and CA-47094 from the National Cancer Institute.
3Research procedures were according to the ethical standards of the Human Studies Committee, University of Arizona.
4Department of Internal Medicine and Cancer Center, Arizona Health Sciences Center, University of Arizona, 1501 North Campbell Ave., Tucson, Ariz. 85724.
5We thank R. Markmann and L. Kimball for preparation of the manuscript and the graphics.
The human melanoma cell strain C8146c was developed in our laboratory from cells obtained from biopsies of subcutaneous nodules and is described in detail elsewhere (19). Early subcultures of C8146c were stored in the vapor phase of liquid nitrogen. C8146c was grown in Ham's F-10 medium containing 10% FCS and was not used for more than 10 subculturings. C8146c has tyrosinase activity (19), and the cells grown in agar contain melanosomes (Hendrix M: Personal communication). In culture the doubling time of C8146c was 51 hours. These human melanoma cells when implanted in nude mice generated tumors within 6 weeks.

Clonogenic assay.—The assay system is a simplification of the bilayer agar assay developed by Salmon and coworkers (20, 21), as we have previously described (22). A bilayer of agar in medium without additional additives was constructed in 35-mm-diameter petri dishes. For human melanoma cell lines the medium used in both agar layers was Ham's F-10 with 10% FCS, and 10,000–20,000 cells were plated per dish. The murine cell line was plated at a concentration of 5,000 cells/ml in Ham's F-10 medium containing 10% HS and 2% FCS. The petri dishes were incubated at 37°C in a humidified atmosphere containing 6% CO₂ for 10–14 days. The colonies were counted and grouped into size classes based on colony diameter with the use of an optical image analyzer (Omnicon FAS II; Bausch & Lomb, Rochester, N.Y.) (23). The colony size cutoff was arbitrarily chosen as 60 μm, which corresponded to 28 cells for CCL 53.1 on day 10 and 10 cells for C8146c on day 14.⁸�PGA₁, DEX, and RA were added at various concentrations with the cells on day 0. The experiments were done in the dark, and all stock solutions were protected from light with a foil wrap.

Data analysis.—Cloning efficiencies were calculated from the total number of cells plated. Visual inspection of the dishes through a microscope on day 0 found the top agar layer contained only single cells. The in vitro hormone effects in the combination studies were quantitated according to the methods of Valeriote and Lin (24) and Drewinko et al. (25). The surviving fraction (SF) of MTCFU resulting from each hormone individually and the surviving fraction of the hormone combination were determined experimentally. If the SFₐ×ₜ is equal to (SFₐ)·(SFₜ), the combined drug effects were additive. If the SFₐ×ₜ is less than (SFₐ)·(SFₜ), the combined effect was defined as greater than additive or synergistic. If the SFₐ×ₜ is greater than (SFₐ)·(SFₜ) but less than (SFₐ) and (SFₜ), the combination interaction was defined as subadditive. If the SFₐ×ₜ is greater than (SFₐ)·(SFₜ) and greater than or equal to the lowest value SFₐ or SFₜ, the combination interaction was defined as antagonistic (26). Statistical comparisons between the experimental and calculated data were done for each of the hormones.

For each combination of hormones, the difference for

⁸The number of cells per colony size was derived either by computation or exact measurement (Meyskens FL Jr, Thomson SP, Moon TE: Unpublished observations).

the observed minus expected percentage survivals of MTCFU was calculated for each experiment. The z-statistic calculated for the hormone combination is the difference (observed minus expected) of MTCFU survival divided by the square root of the observed variance of the surviving fraction for the hormone combination, yielding a standard normal z-statistic with corresponding P-values according to the method of Drewinko et al. (25). With the use of this approach, drugs that produce a perfectly additive response give a z-value of 0. We have designated a P-value of .05 or less as indicating an antagonistic or synergistic effect of the combination (depending on the direction of the z-value) and a P-value of greater than .05 but less than .20 as indicating a subadditive or subsynergistic response.

RESULTS

Effect of DEX and RA on Melanoma Colony Formation

CCL 53.1 murine melanoma cell growth in soft agar was sensitive to RA in a dose-dependent fashion (text-fig. 1). The ID₉₀ of RA was 5×10⁻⁹ M (table 1). A greater than 95% reduction in colony formation was achieved at an RA concentration of 1×10⁻⁷ M. This murine cell line responded in a like manner to DEX with an ID₉₀ occurring at 5×10⁻⁹ M. The inhibition of colony formation by DEX was dose-dependent with more than 95% inhibition occurring at a concentration of 1×10⁻⁷ M.

The sensitivity of human melanoma cells of strain C8146c to RA (text-fig. 1) was comparable to the response obtained with the murine melanoma cell line. An RA concentration of 5×10⁻⁹ M produced a 50% reduction in MTCFU, and a greater than 85% inhibition was obtained at a concentration of 1×10⁻⁷ M (table 1). When the treated cells were grown for an additional 5 days, the remaining colonies were larger, but no additional colonies appeared. Thus reduction in colony number effected by RA was not due to an increase in doubling time but due to a total inhibition of growth. The human melanoma cells were not as sensitive to DEX as the murine cells. A 50% reduction in MTCFU was achieved with a DEX concentration of 1×10⁻⁸ M. The inhibition of colony formation by DEX was dose-dependent with more than 95% inhibition occurring at a concentration of 1×10⁻⁷ M.

When both RA and DEX were added to murine melanoma cells at nanomolar concentrations, there was a synergistic inhibition of the number of colonies formed (table 2). For example, at a concentration of 1×10⁻⁸ M, DEX and RA reduced MTCFU 8 and
text-figure 1.—Effect of increasing concentrations of DEX (△—△) and RA (○—○) on the anchorage-independent growth of CCL 53.1 (panel A) and increasing concentrations of DEX (△—△) and RA (○—○) on C8146c (panel B). Control plates were seeded with 5,000 CCL 53.1 and 15,000 C8146c cells and contained 2,750±56 and 2,676±86 colonies, respectively.

The experiments were repeated two more times, and similar results were obtained.

18%, respectively. The expected inhibition was 25%; however, the observed reduction in MTCFU was much greater than additive (table 2). This synergism occurred in a dose-dependent manner between the concentration range of 1–10 nM. When RA and DEX were used together, the ID_{50} was slightly greater than 1×10^{-9} M. This was a half-log decrease over the concentration needed when either DEX or RA was added alone. Also, when RA and DEX were added simultaneously, there was a decrease of 1–1.5 logs over the concentration needed for each hormone alone to elicit greater than 98% inhibition (table 2).

The data in table 2 show that DEX and RA can act together to inhibit the MTCFU from the human melanoma cell strain C8146c. An additive reduction in MTCFU was obtained with all concentrations up to 1×10^{-8} M. The sensitivity of these human melanoma cells to the combined action of DEX–RA was not as dramatic as those inhibitions seen with the murine cell line. DEX–RA at 5×10^{-9} M effected a significant 70% reduction in colony number. Only 60% of the MTCFU in this human melanoma cell line were sensitive to DEX (text-fig. 1). Yet when DEX was added with RA at 5×10^{-9} M, a further 40% reduction in colony number was observed over that obtained with RA alone.

**Effect of PGA on DEX and RA Inhibition of MTCFU**

We have previously demonstrated that PGA_{1} irreversibly inhibited the anchorage-independent growth of murine melanoma (19), but the concentration needed to produce complete inhibition was very large (7 μM). Because PGA_{1} may be inhibiting melanoma cell growth through an as yet undefined biological mechanism, the effect of lower concentrations in combination with other hormones was tested. As shown in text-figure 2, submaximal concentrations of PGA_{1} resulted in a dramatic decrease in murine melanoma colony formation in the presence of RA and DEX. The observed inhibition curve was greater than the calculated additive effects (text-fig. 2). DEX–RA used at a low concentration (1×10^{-9} M) or PGA_{1} at a low concentration (100 ng/ml) produced only a minor reduction in MTCFU. The addition of PGA_{1} to

---

**Table 1.** Hormone sensitivity of established murine (CCL 53.1) and human (C8146c) melanoma cells in soft agar

| Cell line | Treatment | ID_{50}, M | Maximum inhibition of MTCFU |
|-----------|-----------|------------|-----------------------------|
| CCL 53.1a | DEX       | 5×10^{-9}  | 3 1×10^{-7}                |
|           | PGA_{1}   | 2×10^{-6}  | 0 7×10^{-9}                |
|           | RA        | 5×10^{-9}  | 4 1×10^{-9}                |
| C8146c   | DEX       | 5×10^{-6}  | 40 1×10^{-4}               |
|           | PGA_{1}   | 2×10^{-6}  | 0 7×10^{-4}                |
|           | RA        | 5×10^{-9}  | 14 1×10^{-7}               |

*Single-cell suspensions of CCL 53.1 were plated in quadruplicate as described in "Materials and Methods." Control plates yielded 3,056±52 colonies >60 μm on day 10. Cloning efficiency was 61%.

*Single-cell suspensions of C8146c were plated in quadruplicate as described in "Materials and Methods." Control plates yielded 1,640±56 colonies >60 μm on day 14. Cloning efficiency was 16%.
**Table 2.** Effect of DEX-RA combination on melanoma colony formation

| Cell line   | Treatment (concentration)                      | Percent inhibition | Statistical evaluation |
|-------------|-----------------------------------------------|--------------------|-----------------------|
|             |                                               | Expected ±SE       |                       |
|             |                                               | z                  | p                     |
| CCL 53.1    | RA-DEX (both 1×10⁻⁹ M)                        | 25                 | 46±1 S               | -10.5 | .0001 |
|             | RA (1×10⁻⁹ M) + DEX (5×10⁻⁹ M)                | 44                 | 84±1 S               | -20.0 | .0001 |
|             | RA (1×10⁻⁸ M) + DEX (5×10⁻⁹ M)                | 91                 | 98±0.5 S             | -7.00 | .0001 |
|             | RA-DEX (both 1×10⁻⁸ M)                        | 93                 | 100 S                | 0      |       |
|             | RA-DEX (both 2.5×10⁻⁹ M)                      | 33                 | 32±1 A               | 0.50   | .61   |
|             | RA-DEX (both 5×10⁻⁹ M)                        | 70                 | 70±2 A               | 0      | 1     |
|             | RA (1×10⁻⁸ M) + DEX (5×10⁻⁹ M)                | 75                 | 75±0.5 A             | 0      | 1     |
|             | RA-DEX (both 1×10⁻⁹ M)                        | 78                 | 79±1.5 A             | -0.33  | .73   |

*The effect of the expected and observed inhibition was calculated as described in "Materials and Methods."*

*Results are expressed as standard errors relative to mean of control. A z-value of 0 indicates that the expected and observed survival percentages of the combination were equal. The larger a positive value, the greater the chance the combination was antagonistic (A). The larger a negative value, the greater the chance the combination was synergistic (S).*

**Text-figure 2.** Effect of 1×10⁻⁹ M DEX and 1×10⁻⁹ M RA in combination with increasing concentrations of PGA₁ on MTCFU of murine melanoma CCL 53.1 in soft agar. CCL 53.1 melanoma cells were plated in quadruplicate as described in "Materials and Methods," and the hormone(s) were placed in the culture dish. Control plates had 3,160±62 colonies >60 μm. Colonies >60 μm contained at least 28 cells, 54% of the colonies were >10⁴ μm and contained at least 105 cells, and 4% of the colonies were >149 μm on day 10 and contained at least 247 cells. PGA₁ alone; □=expected additive result of all three hormones; △=observed effect of RA plus DEX plus the calculated effect of PGA₁; ▲=DEX and RA plus an increasing concentration of PGA₁. Point A (—) shows the reduction in colony formation elicited by the DEX-RA combination. This experiment was done two times, and similar results were obtained.

DEX–RA produced an additional 50% reduction of murine MTCFU.

A similar result was also obtained with the human melanoma cell line (table 3). The addition of PGA₁ synergistically enhanced the inhibition of MTCFU in the presence of DEX and RA. A concentration of 100 ng PGA₁/ml was very effective in potentiating the inhibitory effect of 2.5-nM concentrations of DEX and RA. The addition of PGA₁ to DEX–RA increased the observed inhibition in this experiment (table 3) by more than 50%, which is more than the 11% one would expect for an additive result. Note that PGA₁ did not enhance the inhibition in the presence of DEX–RA at 1×10⁻⁹ M. However, this low dose of DEX–RA did interact synergistically with higher PGA₁ concentrations. The combination of PGA₁ and DEX or RA also resulted in a

**Table 3.** PGA₁ effect on human MTCFU inhibition by DEX and RA

| Treatment (concentration) | Total colony percent inhibition ± SE | Effect | Statistical evaluation | Percent inhibition of colonies, 104 μm² |
|---------------------------|--------------------------------------|--------|-----------------------|---------------------------------------|
| PGA₁ (100 ng/ml)          | 11±0.5                               | A      | 7.00                  | .0001                                 |
| Plus DEX–RA (both 1×10⁻⁹ M) | 35±1                                 | A      | -8.94                 | .0001                                 |
| Plus DEX (1×10⁻⁹ M)       | 61±1.0                               | S      | -16.00                | .0001                                 |
| Plus RA (1×10⁻⁹ M)        | 81±0.5                               | S      | -14.00                | .0001                                 |
| Plus PGA₁ (500 ng/ml)     | 45±1.0                               | A      | 7.00                  | .0001                                 |
| Plus DEX–RA (both 1×10⁻⁹ M) | 79±0.5                             | S      | -8.75                 | .0001                                 |
| Plus DEX (1×10⁻⁹ M)       | 92±0.5                               | S      | -4.38                 | .0001                                 |
| Plus RA (1×10⁻⁹ M)        | 94±0.5                               | S      | -7.14                 | .0001                                 |

*Single-cell suspensions of C8146c were plated in soft agar in continuous contact with the compounds as described previously (19).*

*A=antagonistic; S=synergistic.*

Total colonies >60 μm, 2,622, contained at least 10 cells, and 804 colonies were >104 μm on day 14 and contained at least 36 cells.
significant increase in the observed inhibition of colony formation and demonstrated that DEX and RA need not both be present for PGA1 to express its synergistic interactions (table 3). For example, the maximal inhibition effected by DEX at 0.1 \( \mu \)M or higher concentrations was 60%. The addition of PGA1, which alone produced a 75% inhibition (table 3), resulted in synergistic interaction, with 94% of the MTCFU being inhibited. Visual inspection revealed that most of the cells had failed to divide. There was not a large amount of clusters less than 60 \( \mu \)M, which one would expect if the hormone combination was retarding only the growth rate.

**DISCUSSION**

The growth of melanoma cells from established murine and human cultures can be strongly inhibited both in vitro and in vivo by DEX, PGA1, and RA (1, 2, 4–7, 14, 16). However, some melanoma cells are insensitive to either DEX or RA (4, 11, 13).

The murine melanoma cell line used in this study represents an ideal case. More than 96% of the colony-forming cells were totally responsive to single-agent treatments by DEX or RA. These results are comparable to those of a previous report in which the dose-dependent effects of RA on the monolayer growth of S91 melanoma were demonstrated (1). The human melanoma cell strain represents a more complicated situation. The colony-forming units were highly responsive to RA, but a large portion of these cells was resistant to DEX.

The combined use of DEX–RA in murine melanoma cells resulted in a dramatic reduction of MTCFU expression. The observed inhibition was much larger than that predicted for an additive response. The data in table 2 clearly demonstrated that nanomolar combinations of DEX–RA produced a synergistic inhibition of MTCFU expression. The enhanced reduction of colony formation may be the result of two processes. The second hormone could generate an enhanced response by acting on melanoma cells that are resistant to the first hormone, or it could act in concert with the first hormone at the cellular level. For example, at a concentration of 1 nM most of the murine melanoma cells did not respond to either DEX or RA. Since all the MTCFU can be inhibited by either hormone alone at reasonable concentrations (table 1), we speculate that the second hormone is not acting on cells resistant to the first hormone. At 1x10^{-9} M a subthreshold response was generated in most cells by either DEX or RA. Yet when DEX and RA are added together, about half of the MTCFU were blocked from forming colonies. DEX and RA may independently inhibit the expression of the same cellular process and when DEX and RA are combined, the integrated subthreshold effects lead to a block in cellular division. It remains to be elucidated which processes are regulated either independently or concurrently by these hormones.

The interpretation of the combined DEX–RA effects on the human melanoma cell strain is more complicated, since a large proportion of melanoma colony-forming cells did not respond to DEX. If one assumes that DEX and RA do not work together on the MTCFU to inhibit colony formation, then the maximal inhibition that could be achieved would be an additive effect. Since only an additive effect was observed, one cannot know whether RA and DEX were acting independently on different cell populations or if the hormones interacted at the cellular level to comodulate the human melanoma cells. At least 60% of the melanoma colony-forming cells were responsive to both DEX and RA, so the lack of synergism suggests that the proliferation of human melanoma may not be as tightly regulated by these hormones in comparison to murine melanoma. Nevertheless, the addition of DEX does contribute to the further suppression of anchorage-independent growth. For example, at an RA concentration of 10 nM only 32% of the melanoma colony-forming cells formed colonies, and the addition of 10 nM DEX reduced this survival fraction by another 35% (table 2).

In this report we have demonstrated that DEX, RA, and PGA1 comodulate melanoma cell growth and produce a synergistic inhibition of MTCFU expression. For the combination DEX–RA, these effects were observed at readily achievable plasma concentrations (27, 28). The other beneficial consequence of using these two hormones in concert is that they lowered the effective inhibitory threshold concentration, which is very important for a solid tumor, inasmuch as the effective concentration of hormone to which the tumor is exposed may be much lower than measured plasma levels. The additive effects of the DEX–RA combination on the MTCFU of the human melanoma cell strain suggest that the hormones may be useful in vivo, particularly since a significant number of cells from fresh biopsy specimens yielded at least partial inhibitory responses to RA (14). These results suggest that a subpopulation of patients may exist with adequate sensitivity to DEX and RA to allow a significant response to these hormones.

Previous clonogenic assay studies (19) also showed that PGA1 can act as a single agent to irreversibly inhibit melanoma cells, but only at a very high concentration (7 \( \mu \)M). In the present study a PGA1 concentration 25-fold lower greatly enhanced the inhibitory effects of DEX and RA. Little is known about the pharmacokinetics of PGA1 in vivo. A preliminary study is under way in which PGA1 has been administered to breast cancer patients (29). Our data suggest the potential usefulness of PGA1 at this low concentration with DEX and RA. Hopefully, an analogue of PGA1 may be developed with a high therapeutic index for patient studies.

Our observations demonstrate that the clonogenic capacity of melanoma cells in soft agar is under the control of several hormones. None of these hormones individually elicited a major response at useful concentrations, but in combination and at low concentrations they substantially inhibited MTCFU. The results presented here provide a rationale for a multihormone regimen for advanced melanoma. The coupling of antiproliferative hormones may also be applicable to other tumor types.
REFERENCES

(1) LOTAN R, GIOTTA G, NORK E, NICOLSON G. Characterization of the inhibitory effects of retinoids on the in vitro growth of two malignant melanomas. J Natl Cancer Inst 1978; 60:1035–1041.

(2) LEVINE N, MEYSKENS FL Jr. Topical vitamin A acid therapy for cutaneous metastatic melanoma. Lancet 1980; 2:224–226.

(3) ABRAMOWITZ J, CHAVIN W. Glucocorticoid modulation of adrenocorticotropic-induced melanogenesis in the Cloudman S-91 melanoma in vitro. Exp Cell Biol 1978; 46:268–276.

(4) HUTCHENS TW, HAWKINS EF, MARKLAND FS Jr. Identification of transformed glucocorticoid receptor from dexamethasone resistant melanoma. J Steroid Biochem 1982; 16:705–711.

(5) SANTORO MG, PHILPOTT GW, JAFFE BM. Inhibition of tumour growth in vivo and in vitro by prostaglandin E. Nature 1976; 263:777–779.

(6) HONN KV, ROMESE M, SKOFF A. Prostaglandin analogs as inhibitors of tumor cell DNA synthesis. Proc Soc Exp Biol Med 1981; 166:562–567.

(7) BREGMAN MD, SANDER D, MEYSKENS FL Jr. Prostaglandin A1 and E2 inhibit the plating efficiency and proliferation of murine melanoma cells (Cloudman S-91) in soft agar. Biochem Biophys Res Commun 1982; 104:1080–1086.

(8) COLSTON K, COLSTON MJ, FELDMAN D. 1,25-Dihydroxyvitamin D3 and malignant melanoma: The presence of receptors and inhibition of cell growth in culture. Endocrinology 1981; 108:1083–1086.

(9) BHAKOO HS, MILHOLLAND RJ, LOPÉZ R, KARAKOUSIS C, ROSEN F. High incidence and characterization of glucocorticoid receptors in human malignant melanoma. JNCI 1981; 66:21–25.

(10) LOTAN R, LOTAN D. Enhancement of melanin expression in cultured mouse melanoma cells by retinoids. J Cell Physiol 1981; 106:179–189.

(11) LOTAN R. Difference in susceptibilities of human melanoma and breast carcinoma cell lines to retinoid-induced growth inhibition. Cancer Res 1979; 39:1014–1019.

(12) MEYSKENS FL Jr, FULLER BB. Characterization of the effects of different retinoids on the growth and differentiation of human melanoma cell lines and selected subclones. Cancer Res 1980; 40:2194–2196.

(13) LOTAN R, NICOLSON GL. Heterogeneity in growth inhibition by β-trans-retinoic acid of metastatic B16 melanoma clones and in vivo-selected cell variant lines. Cancer Res 1979; 39:4767–4771.

(14) MEYSKENS FL Jr, SALMON SE. Inhibition of human melanoma colony formation by retinoids. Cancer Res 1979; 39:4055–4057.

(15) SANTORO MG, PHILPOTT GW, JAFFE BM. Dose dependent inhibition of B-16 melanoma growth in vivo by a synthetic analogue of PGE2. Prostaglandins 1977; 14:645–651.

(16) FAVALLI C, GARACI F, SANTORO MG, SANTUCCI L, JAFFE BM. The effect of PGA1 on the immune response in B-16 melanoma-bearing mice. Prostaglandins 1980; 19:587–593.

(17) HONN KV, DUNN JR II, MORGAN JR, BIENKOWSKI M, MARNETT LJ. Inhibition of DNA synthesis in Harding-Passey melanoma cells by prostaglandin A1 and A2: Comparison with chemotherapeutic agents. Biochem Biophys Res Commun 1979; 87:795–801.

(18) TURNER WA, TAYLOR JD, HONN KV. Effects of prostaglandin “A” series on tumor cells in vitro. In: Powles TJ, Boekman RS, HONN KV, Ramwell P, eds. Prostaglandins and cancer. Vol 2. Prostaglandin and related lipids. New York: Liss, 1982:369–373.

(19) BREGMAN MD, MEYSKENS FL Jr. Inhibition of human malignant melanoma colony-forming cells in vitro by prostaglandin A1. Cancer Res 1985; 45:1642–1645.

(20) HAMBERGER AW, SALMON SE. Primary bioassay of human tumor stem cells. Science 1977; 197:461–463.

(21) SALMON SE, HAMBERGER AW, SOEHNLEN BJ, DURIE BG, ALBERT DS, MOON TF. Quantitation of differential sensitivity of human tumor stem cells to anticancer agents. N Engl J Med 1978; 298:1321–1327.

(22) MEYSKENS FL Jr, SOEHNLEN BJ, Saxe DF, CASEY WJ, SALMON SE. In vitro clonal assay for human metastatic melanoma cells. Stem Cells 1981; 1:61–72.

(23) KRESSNER BE, MORTON RA, MARTENS AE, SALMON SE, VON HOFF DD, SOEHNLEN B. Use of image analysis systems to count colonies in stem cell assay of human tumors. In: SALMON SE, ed. Cloning of human tumor stem cells. Vol 48. Progress in clinical and biological research. New York: Liss, 1980:179–193.

(24) VALERIOTE F, LIN H. Synergistic interaction of anticancer agents: A cellular perspective. Cancer Chemother Rep 1975; 59:895–900.

(25) DREWINKO B, LOO TL, BROWN B, GOTTLIEB JA, FREIREICH EJ. Combination chemotherapy in vitro with adriamycin. Observations of additive, antagonistic, and synergistic effects when used in two-drug combinations on cultured human lymphoma cells. Cancer Biochem Biophys 1976; 1:187–195.

(26) MOMPARLER RL. In vitro systems for evaluation of combination chemotherapy. Pharmacol Ther 1980; 8:21–35.

(27) MIKLE AW, LAGERQUIST LG, TYLER FH. A plasma dexamethasone radioimmuno assay. Steroids 1973; 22:193–202.

(28) GOODMAN GE, EINSPAHJG, ALBERT DS, et al. Pharmacokinetics of 13-cis retinoic acid in patients with advanced cancer. Cancer Res 1982; 42:2087–2092.

(29) POWLES TJ, COOMBES RC, DEPPELDE M, MUIMP J, POWLES R. Prostaglandin administration to patients with cancer. In: Powles TJ, Boekman RS, HONN KV, Ramwell P, eds. Prostaglandins and cancer. Vol 2. Prostaglandin and related lipids. New York: Liss, 1982:825–830.