Effects of Phorbol-12-myristate-13-acetate on the Phenotypic Program of Cultured Chondroblasts and Fibroblasts

Mark E. Lowe, Maurizio Pacifici, and H. Holtzer

Department of Anatomy, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104

ABSTRACT

Phorbol-12-myristate-13-acetate (PMA) has a prompt, differential, and partially reversible effect on cultured chick chondroblasts. Within 36 hr PMA transforms sessile, polygonal, epithelioid chondroblasts into motile, multilayered, fibroblastic cells. In PMA chick chondroblasts rapidly cease to synthesize two of their terminal luxury molecules, the type IV sulfated proteoglycan that characterizes the extracellular matrix and a glycosylated protein with an apparent molecular weight of 180,000. This glycosylated protein constitutes approximately 5% of the total protein in normal chondroblasts. If returned to normal medium after 4 days in PMA, virtually 100% of the cells reininitiate the synthesis of their type IV sulfated proteoglycan, of the 180,000-dalton protein, and reacquire their polygonal, epithelioid morphology. If returned to normal medium after 12 days in PMA, the cells fail to synthesize their two characteristic luxury molecules, and 100% of the cells remain fibroblastic. PMA alters the morphology of chick fibroblasts but does not block synthesis of their characteristic type III sulfated proteoglycan. PMA proves to be a mitogen for chondroblasts but not for fibroblasts, in spite of the phenotypic similarities of these two cell types.

INTRODUCTION

The notion that oncogenic agents act on those mechanisms that regulate differentiation is an old one (2, 3, 6, 7, 13, 24, 25). More recently, it has been suggested that at least 1 cocarcinogen, PMA,4 also affects normal differentiation (10). Chick myogenic cells grown in PMA (a) do not withdraw from the cell cycle at the same frequency as their normal counterparts, (b) do not initiate the synthesis of muscle-specific myosin heavy and light chains, (c) do not assemble interdigitating thick and thin filaments, and (d) do not acquire those unique cell surfaces that permit myocardial fiber-to-fiber fusion. PMA selectively and reversibly blocks terminal differentiation in several cell lines. The cocarcinogen inhibits Friend erythroleukemic cells from synthesizing hemoglobin (27, 35) and also delays the emergence of adipocytes in populations of 3T3 cells (11).

In this paper we describe the effects of PMA on chick chondroblasts and chick fibroblasts. The morphology of both types of cells rapidly changes in PMA. Chondroblasts cease to synthesize their cell-unique protein with an apparent molecular weight 180,000 which, along with actin (18), accounts for over 10% of the total protein synthesized by normal chondroblasts. This differential effect is reversible after 4 days, partially reversible after 8 days, but relatively irreversible after 12 days in PMA. PMA is a mitogen for chick chondroblasts but not for chick fibroblasts. PMA has no obvious effect on the synthesis of the major sulfated proteoglycans synthesized by normal fibroblasts. A preliminary account of some aspects of this work has appeared (22).

MATERIALS AND METHODS

Cell Cultures. Cultures consisting of over 99% functional chondroblasts were prepared from embryonic chick vertebral cartilages as described (8). The freshly liberated chondroblasts were plated at 1 x 10^6 cells/100-mm dish in 8 ml of Eagle's MEM with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). Primary "floaters" were collected on Day 4 (8, 14). These cells were centrifuged, washed in Eagle's MEM, resuspended in 0.25% trypsin for 5 min, and then plated at standard densities (5.0 x 10^5 cells/100-mm dish). The cloning experiments and assays for fusion.

PMA selectively and reversibly blocks terminal differentiation in several cell lines. The cocarcinogen inhibits Friend erythroleukemic cells from synthesizing hemoglobin (27, 35) and also delays the emergence of adipocytes in populations of 3T3 cells (11).

In this paper we describe the effects of PMA on chick chondroblasts and chick fibroblasts. The morphology of both types of cells rapidly changes in PMA. Chondroblasts cease to synthesize their cell-unique protein with an apparent molecular weight 180,000 which, along with actin (18), accounts for over 10% of the total protein synthesized by normal chondroblasts. This differential effect is reversible after 4 days, partially reversible after 8 days, but relatively irreversible after 12 days in PMA. PMA is a mitogen for chick chondroblasts but not for chick fibroblasts. PMA has no obvious effect on the synthesis of the major sulfated proteoglycans synthesized by normal fibroblasts. A preliminary account of some aspects of this work has appeared (22).

MATERIALS AND METHODS

Cell Cultures. Cultures consisting of over 99% functional chondroblasts were prepared from embryonic chick vertebral cartilages as described (8). The freshly liberated chondroblasts were plated at 1 x 10^6 cells/100-mm dish in 8 ml of Eagle's MEM with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). Primary "floaters" were collected on Day 4 (8, 14). These cells were centrifuged, washed in Eagle's MEM, resuspended in 0.25% trypsin (Grand Island Biological) for 20 min, and then subcultured at 5 x 10^6 cells/100-mm dish. PMA (5 x 10^{-8} M), or the nonpromotor 4α-phorbol-12,13-didecanoate (1 x 10^{-7} M) was added to the medium for the experimental series. Inasmuch as the nonpromotor had no obvious effect, it will not be discussed. In some experiments BrdUrd (10 µg/ml; Sigma Chemical Co., St. Louis, Mo.) was added to the medium. For subculturing, the cells were washed with a calcium-magnesium-free MEM, treated with 0.05% trypsin for 5 min, and then plated at standard densities (5.0 x 10^6 cells/100-mm dish) or low densities (1 x 10^6 cells/100-mm dish). The cloning experiments and staining experiments to determine the presence of metaphasic matrix were as described by Chacko et al. (8).

Cultures of fibroblasts were prepared from the subdermal connective tissue of 11-day chick embryos. The cells were subcultured 3 times and for individual experiments were used at densities of 3 x 10^4 cells/100-mm dish. The cloning experiments and staining experiments to determine the presence of metaphasic matrix were as described by Chacko et al. (8).

Proteoglycan Extraction and Characterization on Su-
crose Gradients. Cultures of chondroblasts or fibroblasts were labeled for 6 hr in fresh medium containing [35S]sulfate (20 μCi; New England Nuclear, Boston, Mass.). Radioactive medium was removed from the dishes, floating cells were removed by centrifugation, and then the medium was precipitated by 70% ethanol-1% potassium acetate at 4°. The precipitated material was collected and resuspended in GuHCl solution. Three to 5 hrs later the precipitated material was again extracted with ethanol-potassium acetate at 4° for 24 hr. This procedure was repeated a third time, and the final precipitate was extracted by shaking for 24 hr at 4° with GuHCl (20, 21).

Cells were homogenized in a minimum of 10 volumes of GuHCl solution. After standing overnight at 4°, the homogenate was precipitated with ethanol-potassium acetate. The precipitate was collected by centrifugation, washed twice with 70% ethanol, and then extracted with GuHCl solution by shaking for 24 hr at 4°.

Extracts from cells and medium were clarified by centrifugation at 16,000 rpm for 30 min. The supernatants contained more than 95% of the labeled material. Aliquots of the supernatants were mixed to obtain the original proportion of cell-bound and medium-released material, and the mixture was layered on linear 5 to 20% sucrose gradient in the GuHCl solution (21). Gradients were centrifuged for 24 hr in a SW 27.1 rotor at 27,000 rpm at 20°, and 0.5-ml fractions were collected from the bottom. Aliquots were counted in Aquasol (New England Nuclear) and corrected to dpm by the external standard.

Electrophoresis. Cultures were labeled by incubating for 24 hr in 3H-labeled amino acid mixture (1 μCi/ml; New England Nuclear). Unlabeled cultures were also used in some experiments. Labeled or unlabeled cultures were rinsed 4 times with phosphate-buffered saline (0.9% NaCl-0.01 M phosphate, pH 7.2), and the cells scraped from the substrate and pelleted by centrifugation. In a particular experiment chondroblasts were treated with either twice-crystallized trypsin (100 μg/ml) or pronase (10 μg/ml) for 10 min at room temperature before being harvested. They were then rinsed, scraped from the substrate, and pelleted. Pelleted cells were dissolved in 2% sodium dodecyl sulfate, 0.625 M Tris-HCl (pH 6.8), 10% glycerol, 0.001% bromophenol blue, and 5% mercaptoethanol. Samples were treated at 100° for 5 min, sonically disrupted, and re-heated for 15 min. Electrophoresis of the total cellular proteins was done in polyacrylamide gels, with the buffer system of Laemmli (19). After running, gels were fixed and stained in 0.1% Coomassie blue in acetic acid and methanol and destained in 10% acetic acid and 5% methanol. When used for 3H fluorography, gels were treated as described by Bonner and Laskey (5). DNA was determined by the fluorometric assay of Hinegardner (12).

RESULTS

Effect of PMA on Attachment, Morphology, and Replication of Chondroblasts. One pronounced effect of PMA on chondroblasts was observed within 24 hr. In control cultures approximately 40% of the cells adhered to the substrate and eventually gave rise to colonies of polygonal cells. The remaining 60% did not attach but persisted as floaters (8, 14, 22). In contrast, virtually 100% of the chondroblasts in the PMA-treated cultures attached to the substrate with 24 hr. Floaters never appeared in PMA-treated cultures, whereas they were always generated in control cultures. In time, however, these floaters generally adhered to the substrate and formed colonies of polygonal, functional chondroblasts. When control cultures were fed, the floaters were recovered by centrifugation and added back to the dishes containing the attached cells.

The second prompt effect of PMA was on the morphology of the chondrogenic cells (Figs. 1 and 2). By 24 hr most cells in PMA had lost their polygonal morphology, and by 48 hr 100% of the cells had lost the epithelioid arrangement of normal cultured chondroblasts. Frequently, these cells displayed long, fine, pseudopodial processes exceeding 50 μm in length (Figs. 3 and 4). Chondroblasts in PMA did not exhibit contact inhibition of migration, and at all densities there was much overlapping of their processes. PMA-treated cells became multilayered and achieved densities many times greater than those observed in controls (Figs. 3 and 4; Chart 1). Chondroblasts in PMA never exhibited metachromatic matrix after staining with toluidine blue. In contrast, after 4 or 5 days most of the polygonal cells in the control cultures displayed metachromatic extracellular matrix.

If after 4 days in PMA the altered or dedifferentiated chondroblasts (8, 15, 16) were replated in normal medium at high or low densities, virtually all of the cells reacquired the polygonal morphology of functional chondroblasts and again deposited metachromatic extracellular matrix. In contrast, when chondroblasts that had been in PMA for 8 or 12 days were shifted to normal medium, the majority did not reacquire the epithelioid morphology of functional chondroblasts when subcultured at high densities.

Single chondroblasts, surrounded by nonchondrogenic cells or by dedifferentiated or BrdUrd-suppressed chondroblasts, often do not display their characteristic polygonal morphology and hence are difficult to detect microscopically (9,16). The relative frequency of such "cryptic" chondroblasts can be determined, however, by cloning the total population under conditions selective for chondroblast replication and phenotypic expression. Accordingly, chondroblasts reared in PMA for 8 or 12 days were plated at clonal densities (5 × 10⁴ cells/100-mm dish). Three separate
M. E. Lowe et al.

Experiments were performed (a) with the 8-day PMA-treated cells and (b) with the 12-day PMA-treated cells. Appropriately aged untreated chondroblasts were plated at the same clonal densities. The dishes were scored for clones of polygonal, metachromatic cells after 12 days. Of the clones from normal chondroblasts, over 95% were positive. Of the clones from cells treated with PMA for 8 days, 25 to 30% were positive, the remainder being clones of dedifferentiated cells. Approximately 5% of the clones that were initiated with cells that had been in PMA for 12 days were positive. Clearly, the trend of chondroblasts to dedifferentiate in culture is greatly accelerated in PMA, and in this respect PMA simulates the effect of BrdUrd on cultured chondroblasts (8, 9, 14, 16).

The cumulative effect of PMA on chondroblast replication is shown in Chart 1. When cells that had been in PMA for 4 days were transferred to normal medium, they continued for some time to replicate at a higher rate than did the controls.

**Synthesis of Chondroblast-unique Molecules.** Cells in culture synthesize a variety of sulfated proteoglycans that arbitrarily have been designated types I, II, III, and IV, depending upon their location in a guanidine-sucrose gradient (17, 20, 21). Of many cell types tested, only definitive, polygonal chondroblasts synthesize the type IV sulfated proteoglycan. Type IV sulfated proteoglycan is distinguished from type III sulfated proteoglycan synthesized by (a) fibroblasts, (b) dedifferentiated or BrdUrd-suppressed chondroblasts, (c) presumptive chondroblasts from limb buds or somites, (d) embryonic nerve cells, and (e) myoblasts. Chondroblasts reared in PMA for 48 hr no longer synthesize detectable quantities of the chondroblast type IV sulfated proteoglycan (Chart 2, A and B). The treated chondroblasts, however, synthesize what may prove to be a constitutive type III sulfated proteoglycan. Work detailed elsewhere (23) suggests that the type III sulfated proteoglycan synthesized by PMA-treated chondroblasts and the type III molecules synthesized by limb bud presumptive chondroblasts and presumptive myoblasts are similar. This conclusion is based on (a) the size of their glycosaminoglycan chains determined on Sepharose 6B after digestion with papain, (b) their failure to interact specifically with hyaluronic acid to form macromolecular complexes large enough to be excluded from a Sepharose 2B column, and (c) that about 95% of the labeled type IV macromolecules were digestable with chondroitinase ABC (21).

The suppression of the synthesis of type IV sulfated proteoglycan by PMA is reversible, providing that the cells are in PMA no longer than 4 days. Chart 2D shows that chondroblasts grown in normal medium after 4 days in PMA reinitiate the synthesis of type I sulfated proteoglycan. These findings correlate well with the morphological condition of the chondroblasts in these cultures.

Chondroblasts reared in PMA for 8 days and then subcultured in normal medium did not reverse as readily as those in PMA for only 4 days. After 8 days in normal medium, these cultures consisted mostly of fibroblastic cells with only modest numbers of recognizable chondroblasts (approximately 20%). As shown in Chart 3, these cultures produced type IV and type III sulfated proteoglycans. Cells grown in PMA for 12 days and then transferred to normal medium for 8 days synthesized barely detectable quantities of type IV sulfated proteoglycan, and in these cultures no polygonal cells were observed.

In addition to synthesizing type IV sulfated proteoglycans, normal cultured chondroblasts synthesized what appears to be a chondroblast-unique protein with a molecular weight of approximately 180,000 (Fig. 5). This protein has not been detected in cultures of (a) fibroblasts, (b) pre-

---

**Chart 2.** Pattern of $^{35}$S-labeled proteoglycans on linear sucrose gradients synthesized by control and PMA chondroblasts. Chondroblasts were grown in PMA for 2 or 4 days, then subcultured, and grown in normal medium for 6 days. Control chondroblasts were grown according to the same schedule. A, control chondroblasts, Day 2 cultures; B, chondroblasts treated with PMA for 2 days (8) or 4 days (O); C, control chondroblasts grown for 4 days and then subcultured in normal medium for 4 days; D, chondroblasts treated with PMA for 4 days and then subcultured in normal medium for 4 days. As shown in B, the suppression of Peak IV sulfated proteoglycan is virtually complete after 2 days in PMA.

**Chart 3.** Pattern of $^{35}$S-labeled proteoglycans on linear sucrose gradient synthesized by chondroblasts treated for 8 days with PMA and then subcultured for 8 days in normal medium. Broad peaks consisting of mixtures of Peak IV and Peak III sulfated proteoglycans comparable to that illustrated here were also characteristic of cultures in the process of dedifferentiating or in cultures of somites or limb buds just beginning to condense, and of temperature-sensitive Rous sarcoma mutant-transformed chondroblasts shifted from permissive to nonpermissive temperature (22).

---

2352

CANCER RESEARCH VOL. 38
sumptive chondroblasts from limb buds or somites, (c) myoblasts, (d) blastodisc cells, (e) smooth muscle cells (M. E. Lowe, unpublished thesis). As shown in Figs. 5, 6, and 7, chondroblasts reared in PMA for 4 days or in BrdUrd for 6 days did not display detectable quantities of the 180,000-dalton protein.

To demonstrate that the 180,000-dalton protein was synthesized in sizeable quantities only by normal chondroblasts, the following experiments were performed. Cultures of (a) floaters, (b) attached polygonal cells, (c) chondroblasts treated with PMA for 4 days, and (d) fibroblasts were exposed for 24 hr to a mixture of 3H-labeled amino acids. As shown in Fig. 6, only floaters and polygonal chondroblasts synthesized the 180,000-dalton protein. Densitometric scanning of these gels suggests that the amount of this protein is comparable to that of actin, which in these cells constitutes over 5% of the total protein of the cell (18). In brief, the 180,000-dalton protein plus the 43,000-dalton protein constitute over 10% of the total protein in cultured chondroblasts.

The 180,000-dalton protein is probably weakly glycosylated, since it was lightly labeled with both [3H]glucosamine and [3H]fucose (data not shown). As a glycoprotein it may be a component of the type IV sulfated proteoglycan, a component of the cell membrane, or located intracellularly. Gentle digestion of chondroblasts with pronase or trypsin strips off their type IV sulfated proteoglycan (8, 16). As shown in Fig. 7, chondroblasts subjected to such digestions did not lose their 180,000-dalton protein. These results suggest that the protein either is an integral part of the cell membrane or is located intracellularly. In either case it was not simply absorbed from the medium.

**Effect of PMA on Fibroblasts.** Although the effect of PMA on the morphology of fibroblasts was prompt and consistent, it was relatively modest (Figs. 8 and 9). The cell processes of treated fibroblasts are longer and finer, and the area of surface adhering to the substrate less, than in controls. At the concentration of PMA (5 x 10^-8 M) which promoted cell replication of chondroblasts, the cocarcinogen was not mitogenic for fibroblasts. In 3 separate experiments the average ratio of total DMA of PMA-treated to untreated fibroblasts was 1.2. In 2 separate experiments, raising the concentration of PMA 2- and 4-fold did not affect total cell number. Clearly, although PMA acts as a mitogen for chick chondroblasts, it has no obvious mitogenic effect on chick fibroblasts.

The characteristic sulfated proteoglycan synthesized by fibroblasts are type III and type I (20-22). As shown in Chart 4, PMA had no detectable effect quantitatively or qualitatively on the synthesis of these 2 types of sulfated proteoglycans. Approximately 95% of the type III and approximately 85% of the type I molecules shown in Chart 4 are digested with chondroitinase ABC. Whether PMA affects the synthesis of hyaluronic acid or type I collagen chains has not yet been determined.

**DISCUSSION**

PMA has 2 pronounced effects on cultured myogenic cells. It promotes cell replication and acts directly on the cell surface. The latter observation was inferred by noting that PMA promptly blocked the alignment and fusion of myoblasts to form multinucleated myotubes (10). PMA promotes cell replication and has a rapid effect on the cell surface of chondroblasts.

It will be important to learn whether this mitogenic effect on chondroblasts involves all cells equally or whether it repeatedly stimulates a subset of cells. The latter possibility could account for the diminishing ability of chondroblasts to revert to their normal phenotype after longer periods in PMA. Cells that do not revert when transferred to normal medium may be the descendants of the subset of repeatedly replicating cells. Failure of the descendants of cultured chondroblasts to display their characteristic properties has long been known and has been referred to variously as dedifferentiation or alteration (14, 16). Whatever the molecular mechanisms for this important biological phenomenon, it is clear that PMA along with BrdUrd (8, 9, 14, 16), and the transforming factor of the Rous tumor virus (21) induce it rapidly.

That PMA acts on the cell membrane of chondroblasts was suggested by: (a) the rapid transformation from sessile, polygonal, epithelioid cells into mobile cells with long processes; (b) the multilayering both at low and high densities; and (c) the fact that chondroblasts in PMA rapidly attach to the substrate while in PMA never give rise to floaters. This persistent attachment to the substrate in PMA cannot be due to a generalized enhancement of the adhesive properties of the cell. Chondroblast-chondroblast adhesion in PMA was greatly reduced as compared to chondroblast-chondroblast adhesion in normal medium. Chondroblast-chondroblast adhesion is greatly reduced both in BrdUrd-suppressed and viral-transformed chondroblasts (14, 16, 21, 26).

PMA selectively and reversibly blocks terminal differentiation in several cell lines (11, 27, 35). Nevertheless cells replicating in PMA yield progeny that remain committed to their respective lineages. Although PMA blocks expression of the terminal phenotype of the cell, the cocarcinogen does not cancel those genetic mechanisms that ensure that the unique phenotypic options of the cell be transmitted to its progeny. PMA does not induce the cells to become "embryonic" or simplify them epigenetically.
Phenomenologically, the behavior of chondroblasts in PMA is similar to that of myogenic or chondrogenic cells suppressed with BrdUrd or to myogenic or chondrogenic cells transformed with temperature-sensitive Rous Sarcoma mutant (15, 17, 20, 21, 26). In these cases the affected cell replicates and transmits to its progeny its position in a given lineage, this despite the fact that PMA, BrdUrd, and the RSV-SSC gene product block accumulation of those luxury molecules characteristic of the terminally differentiated cells. These observations suggest that, irrespective of their site of action, agents that block the synthesis of terminal luxury molecules may indirectly result in a cell the response of which to replication and/or cell adhesion is less subject to normal regulatory influences.

It is not clear how the sequential activation of total RNA, protein, and DNA or the rapid rise in synthesis of phospholipid or ornithine decarboxylase (1, 6, 7, 37) induced by PMA in skin cells could block terminal differentiation in chondrogenic, myogenic, erythrogenic, or fat cells. PMA acts as a mitogen on stratum germinatum cells and on a subclass of lymphocytes (34). However, it temporarily blocks DNA synthesis in mouse embryo cells (13) and inhibits replication in growing HeLa and 3T3 cells. PMA has no effect on the postmitotic condition of nuclei in mononucleated myoblasts or in myotubes (10).

The differing mitogenic effects of PMA on chondroblasts and fibroblasts is of considerable theoretical interest from the point of view of cell differentiation and the molecular target of growth factors. Notwithstanding the close lineage relationship between chondroblasts and fibroblasts (both synthesize types of collagen chains and types of sulfated proteoglycans, and both are the descendents of mesenchyme cells (17)), PMA promoted the cycling of one but not that of the other. This differential response demonstrates how the activity of an exogenous molecule largely depends on the phenotypic activities of the responding cell. Although it is likely that PMA does not exert its effect as a cocarcinogen solely by being a mitogen (31–33), to date its suppression of differentiation has been demonstrated only on replicating cells. It will be interesting to determine what effects PMA might have on the differentiation of nondividing cells.

REFERENCES

Fig. 1. Phase micrograph of a 5-day control culture. Virtually 100% of the cells displayed the characteristic polygonal morphology of functional chondroblasts. The deposition of metachromatic matrix, particularly between cells, was conspicuous following staining with toluidine blue. Approximately 40% of the cells in this culture were floating in the medium (not shown). × 350.

Fig. 2. Phase micrograph of a 5-day-old culture treated with PMA for the last 24 hr. Note the irregular outlines of the cells, which will become more pronounced with time. There were no floaters in these cultures. × 350.

Fig. 3. Phase micrograph of a 10-day culture of control chondroblasts. The great majority of cells have retained their polygonal morphology, although an occasional cell is in an early state of dedifferentiating (i.e., losing its polygonal morphology). These cells stain metachromatically with toluidine blue. Floaters are no longer present in these older cultures. × 150.

Fig. 4. Phase micrograph of a 10-day culture of chondroblasts treated with PMA for 10 days. Note the considerable overlap between the fibroblastic cells. Many cells displayed fine, exceedingly long processes that extended over 50 μm. Observe the greater cell density in these PMA-treated cultures than in controls shown in Fig. 3. × 150.
Fig. 5. Electrophoretic pattern of total cell proteins in linear 7 to 16% acrylamide gels. 1, control floating chondroblasts; 2, substrate-bound control chondroblasts; 3, chondroblasts treated with PMA for 4 days; 4, control, normal fibroblasts. Arrow, position of the 180,000-dalton protein which, if not unique to this cell type, is nevertheless synthesized in most unusual quantities by functional chondroblasts. Molecular weights were determined by running parallel samples of known molecular sizes. Gels stained with Coomassie blue.

Fig. 6. Electrophoretic pattern of 3H-labeled total cell proteins on a linear 7 to 16% acrylamide gel. Cell proteins were labeled by incubating the respective cells in a mixture of labeled amino acids for 24 hr. 3H fluorographs were made as described in "Materials and Methods." Sample numbers same as in Fig. 5.

Fig. 7. Electrophoretic pattern of total cell proteins on 7% acrylamide gel. 1, control chondroblasts; 2, control chondroblasts treated with pronase; 3, control chondroblasts treated with trypsin; 4, chondroblasts treated with PMA for 4 days; 5, chondroblasts treated with BrdUrd for 6 days. Control chondroblasts were treated for 10 min with trypsin or pronase at concentrations of 100 and 10 μg/ml, respectively.

Fig. 8. Phase micrograph of control fibroblasts grown for 3 days. × 350.

Fig. 9. Phase micrograph of a Day 3 culture of fibroblasts grown in PMA for the last 2 days. Note the high frequency of bipolar cells and their extended processes. The overlapping of the PMA-treated fibroblasts equals that of the PMA-treated chondroblasts. In older cultures it is impossible under the phase microscope to distinguish PMA-treated fibroblasts from PMA-treated chondroblasts.
Effects of Phorbol-12-myristate-13-acetate on the Phenotypic Program of Cultured Chondroblasts and Fibroblasts

Mark E. Lowe, Maurizio Pacifici and H. Holtzer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/8/2350

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.