



US005352440A

# United States Patent [19]

[11] Patent Number: **5,352,440**

Gilchrest et al.

[45] Date of Patent: **Oct. 4, 1994**

[54] **METHODS FOR INCREASING MELANIN CONTENT IN MELANOCYTES USING DIACYLGLYCEROLS AND USES THEREOF**

[75] Inventors: **Barbara A. Gilchrest**, Brookline, Mass.; **Philip R. Gordon**, Philadelphia, Pa.

[73] Assignee: **Trustees of Boston University**, Boston, Mass.

[21] Appl. No.: **934,872**

[22] Filed: **Aug. 21, 1992**

### Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 635,405, Dec. 11, 1990, abandoned, Ser. No. 625,236, Dec. 10, 1990, abandoned, and Ser. No. 624,453, Dec. 10, 1990, abandoned, which is a continuation of Ser. No. 175,129, Mar. 30, 1988, abandoned.

[51] Int. Cl.<sup>5</sup> ..... **A61K 7/18; A61K 31/22; A61K 31/225**

[52] U.S. Cl. .... **424/59; 514/546; 514/542; 514/549**

[58] Field of Search ..... **514/546, 547, 549; 424/59**

### [56] References Cited

#### U.S. PATENT DOCUMENTS

4,443,546	4/1984	Sterman et al. ....	435/240
4,508,706	4/1985	Pawelek et al. ....	424/60
4,618,484	10/1986	Pawelek .....	424/1.1
4,695,449	9/1987	Pawelek .....	424/1.1
4,816,450	3/1989	Bell .....	514/25

#### FOREIGN PATENT DOCUMENTS

0255964	2/1988	European Pat. Off. .
WO91/07167	5/1991	PCT Int'l Appl. .
WO91/07168	5/1991	PCT Int'l Appl. .

#### OTHER PUBLICATIONS

Molleyres, L. P. and Rando, R. R., "Structural Studies on the Diglyceride-mediated Activation of Protein Kinase C", *J. Biol. Chem.* 263(29):14832-14838 (1988).  
Ganong, B. R. et al., "Specificity and Mechanism of Protein Kinase C Activation by sn-1,2-Diacyl-

glycerols", *Proc. Natl. Acad. Sci. USA* 83:1184-1188 (1986).

Mori, T. et al. "Specificity of the Fatty Acyl Moieties of Diacylglycerol for the Activation of Calcium-Activated, Phospholipid-Dependent Protein Kinase", *J. Biochem.* 91:427-431 (1982).

Sasakawa, N., et al., "Induction of Ornithine Decarboxylase Activity by 1-Oleoyl-2-Acetyl-Glycerol in Isolated Mouse . . .", *Biochemical & Biophys. Res. Comms.* 128(2): 913-920 (1985).

Smart, R. C., et al., "sn-1,2-Diacylglycerols mimic the effects of 12-0-tetradecanoylphorbol-13-acetate . . .", *Carcinogenesis*, 7(11): 1865-1870 (1986).

Wren, F., et al., "Ultraviolet-Mediated Melanogenesis in Cultured Human Melanocytes is not Modulated by Prostaglandins e1, E2 or TPA", *Abstr.* 29: vol, 91(4) (1988).

Gordon, P. R., et al., "Human Melanogenesis is Stimulated by Diacylglycerol", *Jour. Of. Invest. Derm.*, 93 (5): 700-702 (1989).

Korner, A., et al., "Activation of melanoma tyrosinase by a cyclic AMP-dependent protein kinase in a cell-free system", *Nature*, 267:444-447 (1977).

Nishizuka, Y., "Studies and Perspectives of Protein Kinase C", *Science*, 233: 305-312 (1986).

Friedmann, P. S., et al., "Ultraviolet Radiation Directly Induces Pigment Production by Cultured Human Melanocytes", *Journ. of Cell. Phys.*, 133:88-94 (1987).

Friedmann, P. S., et al., "Ultraviolet Stimulated Melanogenesis by Human Melanocytes is Augmented by Diacylglycerol but not TPA" *Journ. of Cell. Phys.* 142:334-341 (1990).

(List continued on next page.)

*Primary Examiner*—Gregory Hook

*Attorney, Agent, or Firm*—Hamilton, Brook, Smith & Reynolds

### [57] ABSTRACT

A method for inducing melanin synthesis in melanocytes, thereby increasing the melanin content of melanocytes and, thus, increasing pigmentation, melanocytes with increased melanin content produced by these methods, and uses thereof.

**6 Claims, 6 Drawing Sheets**