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LETTER TO THE EDITOR

Expression of PKC isoforms in human melanocytic cells in situ

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12-*O*-tetradecanoylphorbol-13-acetate

To the Editor,

Protein kinase C (PKC) is a multifunctional protein-serine/threonine kinase and exists as a family of multiple isoforms [1]. PKC is thought to play important roles in human melanocytic cells since 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent PKC stimulator, greatly affects the growth, differentiation, and metastatic ability of these cells [2]. It has been demonstrated that both the protein levels and the intracellular distribution of PKC isoforms in melanocytic cells are easily affected by TPA in vitro [2]. For example, the addition of TPA to cultured melanocytes not only stimulates their growth but also causes the loss of PKC protein in the cells [2], the so-called down-regulation of PKC [1]. In addition, TPA treatment of some melanoma cell lines changes their metastatic ability concomitant with down-regulation or membrane translocation of PKC [2]. It has not been investigated in detail, however, if down-regulation and membrane translocation of PKC occurs in melanocytic cells in situ, although few and insufficient data [3,4] exist concerning the expression of PKC isoforms in these cells in situ. In this study, in situ as well as in vitro expression of PKC isoforms in melanocytic cells was examined.

Expression of PKC isoforms in cultured human melanocytes and melanoma cell lines [5] was examined by immunoblot analysis as described previously [6] (Fig. 1a and b). PKCα, PKCβ, and PKCδ were detected in all cultured melanocytes. The level of expression of each isoform was similar in different donors. PKCα and PKCδ were expressed in all melanoma cell lines. PKCδ was detected as a doublet (phosphorylated and non-phosphorylated forms) as

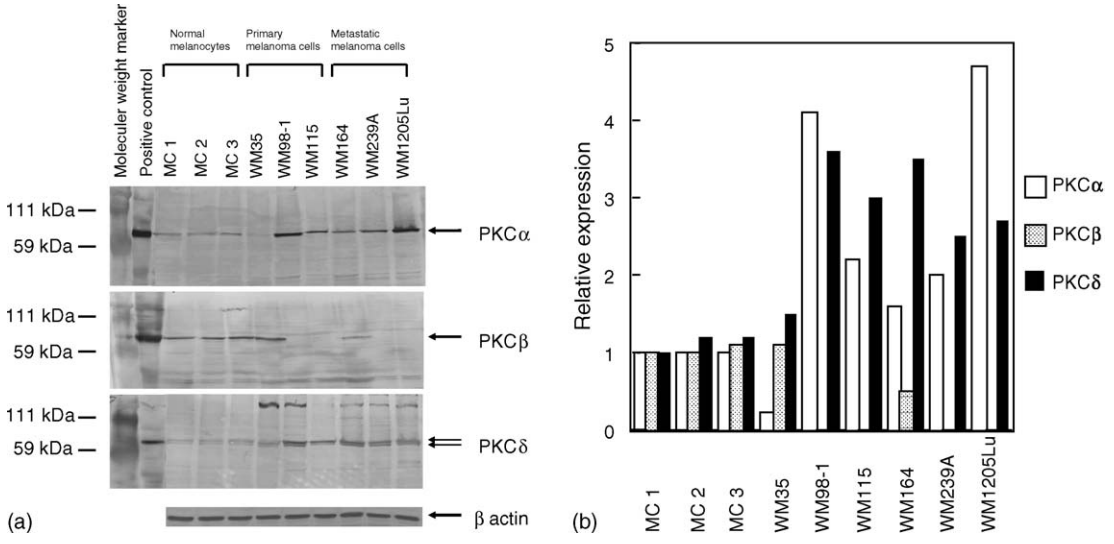

Fig. 1

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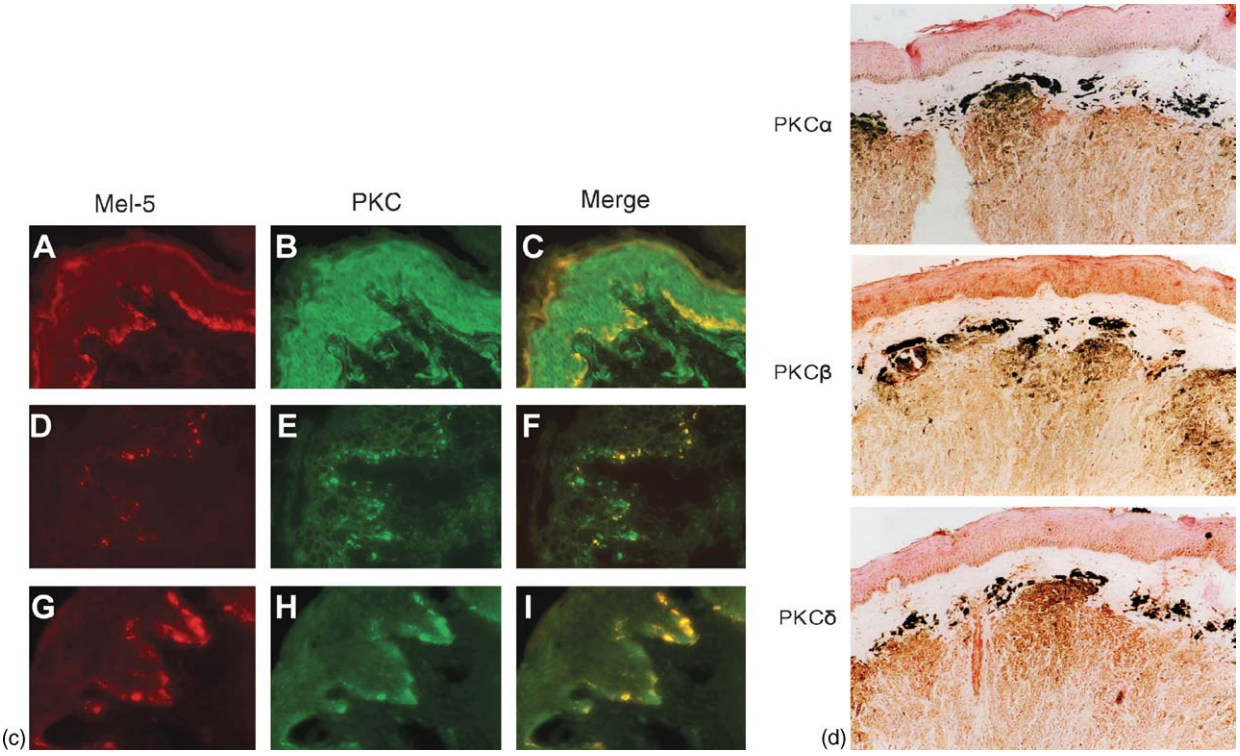

Fig. 1 (a) Expression of PKC isoforms in cultured human normal melanocytes and melanoma cell lines. Equal amounts (66 μg/lane) of the total cell lysate of cultured melanocytes from three different donors (MC1, MC2 and MC3) and six melanoma cell lines (three from primary and three from metastatic lesions) were subjected to immunoblot analysis using monoclonal antibodies against each PKC isoform. WM35, WM98-1, and WM115 were established from primary lesions of superficial spreading melanoma. WM164 and WM239A were established from the lymph node metastasis of nodular melanoma and superficial spreading melanoma, respectively. WM1205Lu was isolated from the lung metastasis of WM793 that was derived from superficial spreading melanoma, after subcutaneous injection into immunodeficient mice. The monoclonal antibody to PKCα was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and the monoclonal antibodies to PKCβ and PKCδ were purchased from Transduction Laboratories (Lexington, KY). Rat brain lysate was used as a positive control for PKCα, PKCδ, and PKCβ. (b) Quantification of immunoblots by secondary labeling with ^{125}I-labeled donkey anti-mouse IgG was performed. The ratio of each PKC band/each β-actin band was expressed as a ratio of that in MC1 (=1). (c) Double immunofluorescence of PKC isoforms and Mel-5 in melanocytes in situ. Cryostat sections of normal human neonatal foreskins were fixed with 3% paraformaldehyde for 12 min, and then incubated sequentially with Mel-5 (red fluorescence) (shown in A, D and G), Texas red-conjugated goat anti-mouse IgG, antibodies to PKCα (B), PKCβ (E), and PKCδ (H) (green fluorescence), biotinylated subtype specific goat anti-mouse IgG, and FITC-conjugated streptavidin. As a negative control, normal mouse serum was used instead of the primary antibody. A merged image is shown in the right panels (C, F and I). Immunohistochemistry of PKC isoforms in human melanocytic nevus (d) and melanoma (e) tissues. Four melanocytic nevus lesions, 14 primary (seven cases of acral lentiginous melanoma, five cases of superficial spreading melanoma, one case of lentigo maligna melanoma, one case of mucous melanoma) melanoma lesions and nine metastatic melanoma lesions were obtained from patients who underwent surgery in the Department of Dermatology at Kumamoto University School of Medicine, Kumamoto, Japan. Immunohistochemical staining for PKC isoforms was carried out as described previously [6]. Representative examples of staining of the nevus tissues are shown in (d). Representative examples of staining of the primary (A, C and E) and metastatic melanoma lesions (B, D and F) with anti-PKCα (A and B), -PKCβ (C and D), and -PKCδ (E and F) monoclonal antibodies are shown in (e). A, B, E, and F are representative of clearly positive (+) staining, and C and D are representative of weakly positive (+/−) staining. G and H show negative controls (without primary antibodies) for primary and metastatic lesions, respectively. (f) High magnification of melanoma cells stained with anti-PKCα. Observation of the PKCα staining in metastatic melanoma tissue under higher magnification revealed that PKCα immunoreactivity was localized mainly in the cytoplasm. Similar results were obtained for PKCα, PKCβ, and PKCδ in both primary and metastatic melanoma tissues when positively stained (data not shown).