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## Cultured Human Dermal Fibroblasts do Produce Cortisol

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### TO THE EDITOR

Known steroidogenic functions of the skin that are represented by the synthesis and metabolism of androgens and estrogens (Chen *et al.*, 2002; Zouboulis and Degitz, 2004) probably include corticosteroids (Slominski *et al.*, 2000; Slominski, 2005). Thus, the skin expresses both the genes and proteins involved in the synthesis of adrenal corticosteroids (Slominski *et al.*, 1996, 1999, 2004; Rogoff *et al.*, 2001). Metabolism of progesterone to deoxycorticosterone and corticosterone has been documented in melanoma cells and in rat skin (Slominski *et al.*, 1999, 2000); corticotropin-releasing hormone- and ACTH-regulated cortisol production was demonstrated in histocultured human hair follicles (Ito *et al.*, 2005); and corticotropin-releasing hormone and ACTH enhancement of cortisol production was demonstrated in epidermal melanocytes (Slominski *et al.*, 2005b). In contrast, in human dermal fibroblasts cortisol production was not enhanced by corticotropin-releasing hormone and ACTH (there was only selective stimulation of corticosterone production; Slominski *et al.*, 2005a). However, since the cortisol-like immunoreactivity was detected by ELISA, we investigated the chemical nature of the antigen.

Normal human adult dermal fibroblasts (from lightly pigmented female: lot 1C0317 (Cascade Biologics Inc., Portland, OR)) were cultured in EpiLife medium with EpiLife Defined Growth Supplement free of serum (Cascade Biologics). The cells were washed twice with phosphate-buffered saline before incubation in supplement-free EpiLife medium with progesterone ( $10^{-6}$  M), ACTH ( $10^{-8}$  M, but added again after 12 h), and/or IBMX (phosphodiesterase inhibitor) ( $10^{-5}$  M). After 24 hours, media were extracted for cortisol identification and quantification as described previously (Slominski *et al.*, 2005b). Cortisol in supernatants was measured with ELISA (Bio-Quant Inc., San Diego, CA), whereas chemical nature of the compounds was assessed by liquid chromatography mass spectrometry analysis with auto-mass spectrometry/mass spectrometry as described previously (Slominski *et al.*, 2005b). Data were analyzed using Prism 4.00 (GraphPad Software), and is presented as mean + SEM ( $n = 4$ ).

Testing dermal fibroblasts for the release of cortisol into the media showed that addition of progesterone ( $10^{-6}$  M) stimulated significantly (4.3-fold) cortisol production; further enhancement (2.5 times) was observed after the addition of  $10^{-5}$  M IBMX (phosphodiesterase inhibitor) but not ACTH (11-fold increase over the basal values obtained from unstimulated cells; Figure 1). The addition of corticotropin-releasing hormone did not affect the production of cortisol (not shown). Liquid chromatography mass spectrometry analyses of extracted steroids showed the presence of an  $[M + H]^+$  ion at  $m/z$  363 (real mass 362) with retention time of 11 minutes identical to the cortisol standard (Figure 2). Final confirmation of the product identity as cortisol was obtained with mass spectrometry/mass spectrometry analysis that

yielded the same fragment ions as those of the cortisol standard:  $m/z = 345$  ( $[M + H]^+ - H_2O$ ),  $m/z = 327$  ( $[M + H]^+ - 2H_2O$ ), and  $m/z = 309$  ( $[M + H]^+ - 3H_2O$ ) (Figure 2).

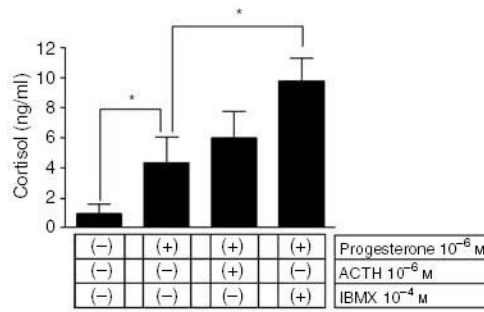
These data document the capability of human dermal fibroblasts to produce cortisol molecules identified by rigorous analysis with liquid chromatography mass spectrometry<sup>2</sup>. Moreover, and similar to normal (Slominski *et al.*, 2005b) and malignant melanocytes (Slominski *et al.*, 1999), fibroblasts also displayed a precursor substrate relationship in the accumulation of cortisol, for example, the addition of progesterone-stimulated production of the final corticosteroid cortisol, which was further enhanced by a phosphodiesterase inhibitor (IBMX). Nevertheless, in contrast to melanocytes ACTH and corticotropin-releasing hormone did not stimulate cortisol production in fibroblasts, related, perhaps, to defective coupling of MC-2R (if expressed) to intracellular signaling of the glucosteroidogenic pathway in cultured fibroblasts. Thus, these studies extend the *ex vivo* finding of cortisol production by hair follicles (Ito *et al.*, 2005), adding an important component of heterogeneity to the pleiotropic expression of hypothalamic-pituitary-adrenal mediators in the skin (Slominski and Wortsman, 2000; Slominski, 2005). The role of these factors in the cutaneous response to stress remains to be fully characterized.

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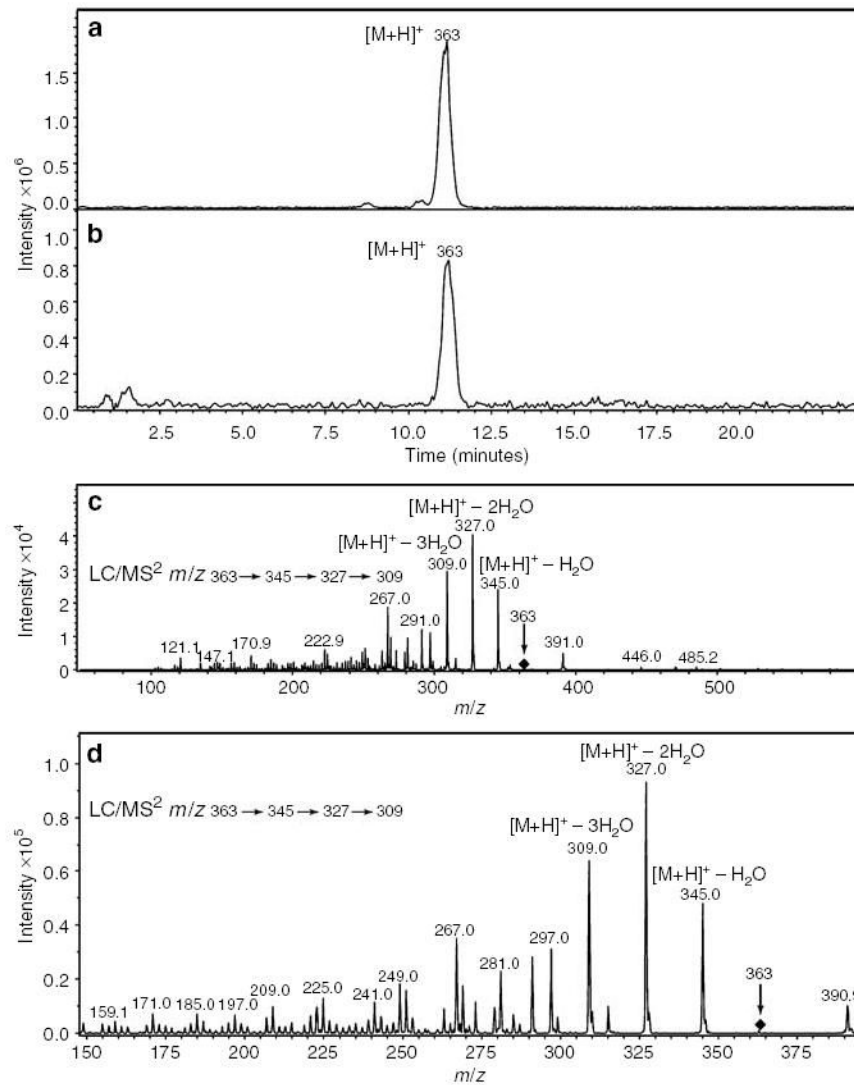
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**Figure 1. Effect of progesterone on cortisol synthesis**

Fibroblasts were cultured in 24-well plates seeded at density of 10<sup>5</sup> cells/well. Differences between control and experimental treatments: \**P*<0.05.



**Figure 2. Liquid chromatography mass spectrometry (LC/MS<sup>2</sup>) identifies cortisol in cultures of fibroblasts**

Fibroblasts were cultured in Petri dishes seeded at density of  $2 \times 10^6$  cells/dish. (a, c) Cortisol standard and (b, d) extracts from conditioned media.