Arch Dermatol Res (2002) 294 : 152–154
DOI 10.1007/s00403-002-0307-1

SHORT COMMUNICATION

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Expression of human *CYP1B1/lacZ* fusion gene in ultraviolet-irradiated human keratinocytes

Received: 3 December 2001 / Revised: 28 January 2002 / Accepted: 11 March 2002 / Published online: 24 April 2002

Keywords CYP1B1 · UVB · Ultraviolet responsive element

The cytochrome P450 enzymes (P450s or CYPs) are a superfamily of hemeproteins that catalyze the monooxygenation of a wide range of endobiotic and xenobiotic substrates. Among members of the superfamily 1, human CYP1B1 (hCY1B1) has been identified in rodent species and in humans [1, 2, 3]. The amino acid sequences in humans, rats, and mice are 80% similar between subfamilies [4], but there are considerable species differences in regulation, metabolic specificity, and tissue-specific expression of CYP1B1 [5]. The cDNA for hCYP1B1 has been isolated from human keratinocytes treated with TCDD [4]. In addition, it has been established that solar ultraviolet (UV) radiation is the major etiological agent in skin cancer development [6, 7]. Wavelengths in the UVB range of the solar spectrum are particularly associated with the induction of skin cancer by producing erythema and burns [8]. Recently, it has been demonstrated that UVB induces the expression of mRNA and protein of endogenous CYP1B1 in the epidermis [9], raising the possibility that the *hCYP1B1* gene is transcriptionally regulated by UVB.

In this study, we sought to determine whether UVB can direct the transcriptional activity of the *hCYP1B1/lacZ* reporter gene by UVB irradiation of human keratinocytes. In order to test whether the h*CYP1B1* gene promoter sequence is capable of influencing UVB-mediated transcription of the *lacZ* reporter gene, we constructed a fusion gene with this promoter linked to an *E. coli LacZ* gene with an SV40 polyadenylation signal (Fig. 1). The *lacZ* mRNA product (459 bp) is predicted from the *hCYP1B1/lacZ* fusion gene, transcription starting at the *hCYP1B1* promoter and terminating at the polyadenylation signal.

Human keratinocytes were transfected with the *hCYP1B1/lacZ* fusion gene, and the transfected cells were then exposed to UVB radiation (315 nm) for 1, 2 and 4 h after removal of the petri dish covers. It has been shown that UVB wavelengths in the range 280–320 nm are absorbed by the skin, producing skin cancer [8]. The RNA was isolated for determination of exogenous *lacZ* transcripts, and endogenous β-actin transcripts by RT-PCR (Fig. 2). UVB induced a threefold activation of transcription with a maximum at 1 h, followed by a rapid decline in the levels thereafter.

In parallel with the analysis of mRNA expression, we determined β-galactosidase protein levels by Western blotting of the same cells used for mRNA expression after exposure to UVB radiation for 1, 2 and 4 h. Protein levels of β-galactosidase were almost similar to those of *lacZ* transcripts as shown in Fig. 3A, although the levels did not rapidly decline. The activity of β-galactosidase was also determined using *o*-nitrophenyl-β-D-galactopyranoside in human keratinocytes by the measurement of the formation of galactose and *o*-nitrophenyl which is yellow. As shown in Fig. 3B, β-galactosidase activities in the keratinocytes were slightly induced by 1 h of UVB irradiation compared with the induction in cells not exposed to UVB.

In this study, we clearly showed that UVB exposure of human skin induces *hCYP1B1/LacZ* fusion gene in the transcripts at the protein level as well as the activity level (Figs. 2 and 3). These results suggest that a UV-responsive element-like (URE-like) element (ULE) within the *hCYP1B1* promoter may be the target binding site for an as-yet-unidentified UV-inducible cellular factor. In fact, the ULE (TGACTGGA) within the *hCYP1B1* functional promoter (–886 bp to –878 bp) was found with a 3-bp

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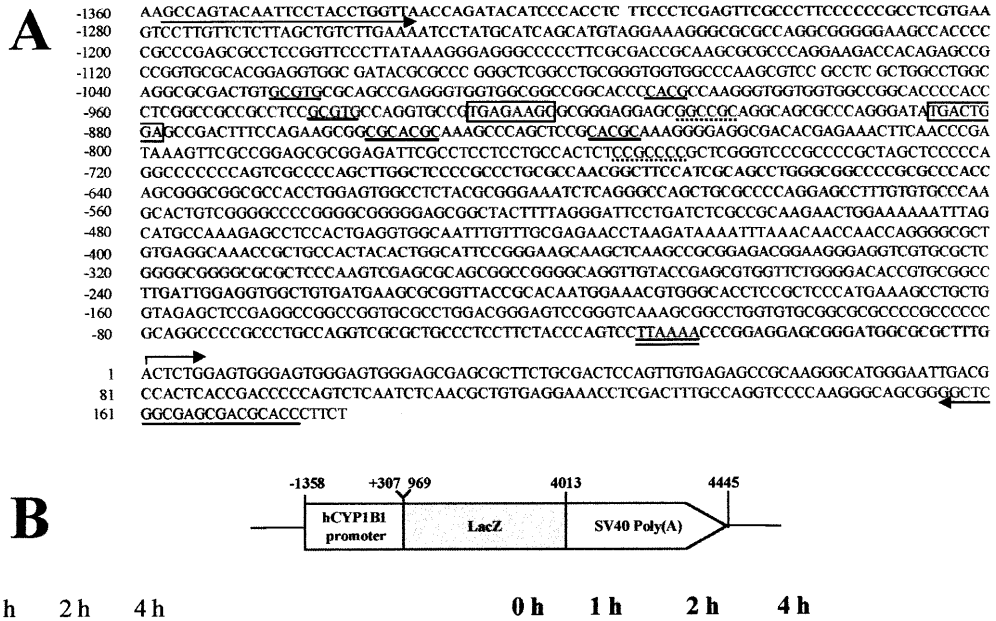

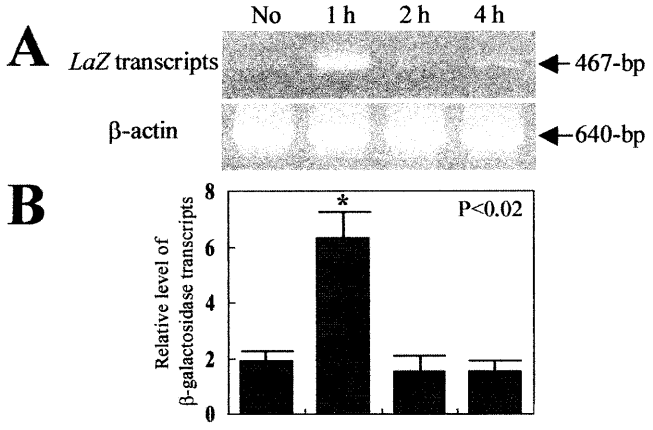

Fig. 2A, B Expression of *hCYP1B1*/*lacZ* fusion gene after exposure to UVB irradiation. Induction of the *lacZ* gene in the human keratinocytes was determined after exposure to UVB radiation for 1, 2 and 4 h. The β-actin signal served as control. The β-actin transcripts (640 bp) indicate RNA loading. Transcript levels at each time-point were quantified using a Kodak Electrophoresis Documentation and Analysis System 120. The significance of differences in the quantity of RNA (Fig. 3) were determined by one-way analysis of variance (using SPSS version 10.10 software). The values indicated are means±SD (n=3), **P*<0.02

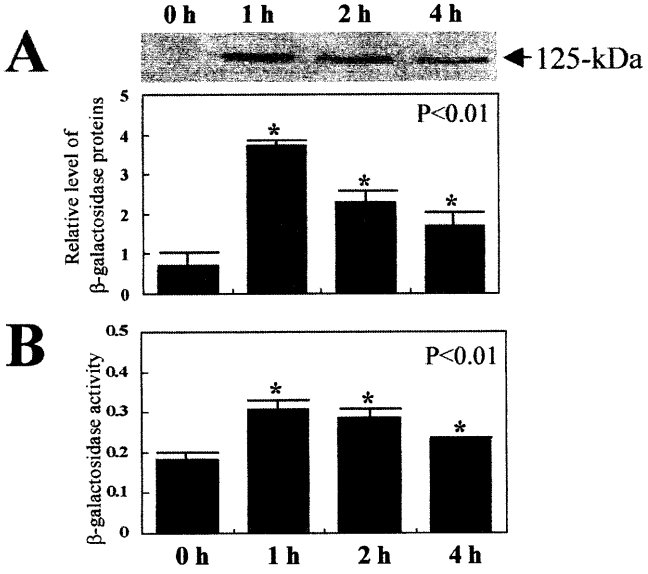

Fig. 3A, B Expression of β-galactosidase protein and β-galactosidase activity after exposure to UVB irradiation. **A** Protein (50 μg) from each sample was loaded for Western blot analysis. Nitrocellulose membrane was incubated with β-galactosidase primary antibody (Chemicon) at a dilution of 1:1000 in 2% powdered nonfat milk. Additional incubation was performed with horseradish peroxidase-conjugated goat α-rabbit IgG (GenTest) at a dilution of 1:1000 in 2% milk as a secondary antibody. The values indicated are means±SD (n=3), **P*<0.01. **B** Protein was assayed for β-galactosidase activity by fluorescence detection. The values indicated are means±SD (n=3), **P*<0.01

mismatch on the URE (Fig. 1A). UREs (TGACAACA) are present in human keratinocytes [10] and in polyoma DNA [11]. It will necessary to examine whether this ULE is a target for such factors or a novel factor increasing the transcriptional activity of the *hCYP1B1* gene promoter upon UVB irradiation. The gel mobility shift assay could be used to identify UVB-inducible factors binding to ULE within the human *CYP1B1* gene promoter. Microsomal CYP1B1 activity was slightly increased (more than 0.8-fold) in UVB-irradiated human keratinocytes compared to identical non-UVB exposed cells.

The results are affected by endogenous β-galactosidase activity in the keratinocytes used, but the introduction of a bacterial foreign β-galactosidase encoded by the *lacZ* gene may have little effect on the enhanced activity of β-galactosidase because of the background activity in human keratinocytes. The *hCYP1B1* promoter-activating system fused to a *lacZ* coding sequence should be of great utility for several reasons. The *hCYP1B1* promoter contains a ULE binding to UV-regulated factor (URF) in UVB-irradiated cells. It would necessary to clone this novel gene for URF which would be present in UV-irradi-

ated human keratinocytes. When it is cloned, an in vitro bioassay system expressing URF interacting with ULE within the hCYP1B1 promoter and *hCYP1B1/LacZ* fusion genes could be directly developed allowing the testing of UV sensitivity and possible carcinogens.

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