Ultraviolet Light (UVB and UVA) Induces the Damage-Responsive Transcription Factor CHOP/gadd153 in Murine and Human Epidermis: Evidence for a Mechanism Specific to Intact Skin

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C/EBP-homologous protein (CHOP)/gadd153 (or CHOP) is a transcription factor induced by endoplasmic reticulum (ER) stress. Forcible overexpression of CHOP causes apoptosis in keratinocytes in culture. Here, we asked whether CHOP might be increased in the skin after UVB (280–320 nm) exposure, thus implicating CHOP in sunburn cell (SBC) formation. SKH-1 hairless mice were exposed to a ultraviolet (UV) source (80 mJ per cm^2; $\sim$ 74% UVB, $\sim$ 16% UVA), and skin biopsies examined by immunohistology and immunoprecipitation. Compared with non-irradiated epidermis, CHOP expression was significantly increased at 30 min, and reached maximal levels by 24 h. Similar increases in CHOP following UVB exposure were observed in human buttock skin. The time course of CHOP expression preceded SBC formation and another marker of apoptosis, caspase-3 cleavage. Intracellular CHOP accumulated mainly in cytoplasmic and perinuclear locations, with little remaining in the nucleus. To examine mechanisms, cultured keratinocytes were irradiated *in vitro* and examined by western blotting. Under conditions that eliminated ER stress because of cell handling, CHOP did not accumulate (and was in fact decreased) in the cells. Thus, induction of CHOP in keratinocytes requires factors present only in the native skin. Overall, the data suggest that CHOP participates in adaptive responses of the epidermis following UVB/UVA exposure *in vivo*.

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In sunburn, a cutaneous reaction following excessive exposure to solar ultraviolet (UV) radiation (Rosario *et al*, 1979; Gange and Mendelson, 1981; Gilchrest *et al*, 1981), the death of epidermal keratinocytes occurs primarily by a mechanism of apoptosis. C/EBP-homologous protein (CHOP)/gadd153 (or simply CHOP) is a transcription factor with a strong connection to UV light, apoptosis, and the development of neoplasia. CHOP was originally identified among clones in a cDNA library derived from Chinese hamster ovary cells exposed to UVC (less than 280 nm) light (Fornace *et al*, 1988, 1989; Luethy *et al*, 1990), and was later identified as a new member of the C/EBP family of transcription factors (Ron and Habener, 1992). Recently, the concept has emerged that CHOP gene expression is associated with endoplasmic reticulum (ER) stress, i.e. many drugs and toxins that damage cellular membranes and cause protein unfolding in the ER can also trigger a signaling pathway which induces CHOP expression (Wang *et al*, 1996; Welihinda *et al*, 1999). Because of its strong inducible expression after toxic exposure and its suspected roles in growth-arrest, cell death, and carcinogenesis, CHOP has attracted considerable interest in the fields of toxicology (Johnson *et al*, 1997; Bernstein *et al*, 1999) and oncology. A specific role for CHOP has been described in the etiology of a particular human cancer, *myxoid liposarcoma*, in which CHOP is fused by chromosomal translocation to an RNA-binding protein (Crozat *et al*, 1993) which leads to the unscheduled expression of an adipocyte-specific gene (Kuroda *et al*, 1999) that disturbs the normal functioning of nuclear components of the cell (Thelin-Jarnum *et al*, 2002). Evidence for a connection between CHOP and neoplasia in other settings has been found in breast cancer (Arnal *et al*, 1999; Talukder *et al*, 2002) and in melanoma (Korabiowska *et al*, 2002).

The notion that CHOP may be involved in UVB (280–320 nm)-damage responses in keratinocytes of the skin seems logical, based upon the dual observations that UVB causes sunburn (apoptosis), and that many environmental stressors and toxins which induce CHOP, also cause apoptosis. An optimal apoptotic response in the skin is important to eliminate keratinocytes with persistent DNA mutations which might generate mutant clones and lead to the development of actinic keratoses and skin cancer (Ziegler *et al*, 1994; Rich *et al*, 2000). We and others have shown that forcible overexpression of CHOP in cultured keratinocytes and several other cell types, can induce apoptosis through

Abbreviations: CHOP, C/EBP-homologous protein; ER, endoplasmic reticulum; IP, immunoprecipitation; PBS, phosphate buffered saline; PMK, primary mouse keratinocytes; SBC, sunburn cell; TUN, tunicamycin; UV, ultraviolet light; UVA, 320–400 nm; UVB, 280–320 nm; UVC, less than 280 nm

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mechanisms that seem to involve downregulation of bcl-2 and impaired handling of oxidative stress (Matsumoto *et al*, 1996; Maytin *et al*, 2001; McCullough *et al*, 2001). Although it seems reasonable to ask whether CHOP participates in apoptosis in the skin, the basic question of whether UVB induces CHOP expression in the skin *in vivo* has not been adequately addressed up to now. Most previous studies on CHOP used non-physiologic UVC light, or when using solar-simulated UVB (Garmyn *et al*, 1991, 1992), examined CHOP mRNA but not levels of the protein.

In this study, we used two *in vivo* models of UVB-induced apoptosis: hairless mouse skin, and human buttock skin. The SKH-1 hairless mouse is a model we had previously characterized in terms of its UVB dose–response and time course of sunburn cell (SBC) formation in the skin (Kane and Maytin, 1995). We describe experiments to explore CHOP accumulation and translocation in the skin, the relationship of CHOP expression to the development of apoptosis *in vivo*, and the question of whether CHOP is inducible by UVB exposure in monolayer keratinocyte cultures.

Results

CHOP expression is induced in the epidermis *in vivo* after exposure to UVB light In these experiments, we employed the same strain of mice (SKH-1) and doses of UV used in our previous study of UVB exposure and apoptosis (Kane and Maytin, 1995). Note that our light source hits predominatly UVB ($\sim$ 74%) but also as small amount of UVA ($\sim$ 16%); hereafter we refer to this as "UVB light". SKH-1 mice were anesthesized and one flank was irradiated using 80 mJ per cm^2 of UVB (Fig 1*A*–*D*). The other flank was left unexposed to provide a matched control for each time point (Fig 1*A′*–*D′*, sham irradiation). At 30 min or 24 h, the skin was biopsied and analyzed. By hematoxylin–eosin staining, numerous SBC were detectable in the UVB-exposed skin (Fig 1*A*), whereas none were detectable in the sham control (Fig 1*A′*). Frozen sections, decorated with antisera to CHOP and visualized using an immunofluorescent probe (orange–yellow color), demonstrated a specific UVB-induced increase in CHOP expression that was weakly detectable at 30 min (Fig 1C *vs* C′), more pronounced at 4 h (not shown), and strongly detectable at 24 h (Fig 1D *vs* D′).

To ask whether induction of CHOP by UVB in mice might be generalizable to humans, we examined frozen sections from samples of human skin available from an unrelated study of UVB effects in human volunteers (C. R. Taylor, unpublished data). Immunostaining of control buttock skin revealed a very low signal (Fig 1*E*), whereas skin biopsied at 8 h and at 24 h after UVB revealed a sharp rise in the CHOP-specific signal (Fig 1*F*, *G*). Interestingly, brightly prominent CHOP-positive cells were often seen at 24 h in a distribution consistent with the appearance of SBC (Fig 1*G*, *arrows*).

Because frozen sections generally display poor morphologic detail, we strove to detect CHOP immunohistologically in paraffin-fixed tissue. An epitope-unmasking technique was developed (see Materials and Methods) that gives a strong, specific CHOP signal in the epidermis of paraffin-fixed skin (Fig 2*A*–*C*). Specificity of CHOP immunostaining was demonstrated by pre-incubation of the polyclonal antisera with an epitope-specific blocking peptide, either reducing (2 μg, Fig 2*B*) or completely eliminating (20 μg, Fig 2*C*) the CHOP signal. Time-course studies revealed a marked induction in CHOP in the epidermis at 30 min, increasing further at 4 and 24 h (Fig 2*D*–*G*). The CHOP signal remained elevated throughout the epidermis even after 3 d, a time when the original keratinocyte cell layers were being eliminated from the surface of the epithelium (Fig 2*H*, *arrow*).

Based upon our previous demonstration that SBC formation begins 4 h or more after UVB in this system (Kane and Maytin, 1995), it appeared that CHOP may coincide with the onset of apoptosis. To address this question we used a second marker of apoptosis, namely the appearance of activated caspase-3. By staining with an antiserum that primarily recognizes the cleaved form of caspase-3, a moderate increase in signal was noted in the expected cytoplasmic location at 30 min (Fig 2*J*) and 4 h (Fig 2*K*). By 24 h after UVB, the caspase-3 signal was very pronounced and highly concentrated in scattered, shrunken cells whose morphology was consistent with SBC (Fig 2*L*). To ask whether CHOP induction after UVB might correlate with events in the ER, staining for Grp78 revealed a time-dependent increase in this marker of ER stress (Fig 2*M*–*P*). The staining of keratin 14 (K14), a protein not expected to change significantly after UVB, served as an invariant control (Fig 2*Q*–*T*). To confirm the overall visual impressions, a semiquantitative analysis of all the experiments mentioned above was performed using image processing of digital fluorescence micrographs (Fig 3). The graphs illustrate the relative time courses of induction for CHOP and for the markers of apoptosis and ER stress, and confirm that UVB causes highly significant increases in the overall amounts of CHOP in the epidermis following irradiation. CHOP remains elevated for at least 5 d after UVB, long after keratinocytes apoptosis has occurred, suggesting that CHOP may have a role in epidermal recovery.

UVB exposure leads to a redistribution of CHOP from nuclear to cytoplasmic locations Prior to UVB irradiation, the intracellular distribution of CHOP in unirradiated skin is primarily nuclear (Fig 2*D*). But by 24 h after UVB exposure, CHOP appears to shift to a predominantly cytoplasmic location (Fig 2*G*, *H*). To confirm this impression, high-resolution confocal microscopy was used to examine specimens that had been double labelled for CHOP and Grp78 (Fig 4). At 30 min after UVB, the distribution of CHOP was nuclear (Fig 4*A*) and completely distinguishable from the cytoplasmic localization of Grp78 (Fig 4*B*). At longer times after UVB (i.e. after 24 h), CHOP appeared to redistribute into the cytoplasm (Fig 4*D*) with only a few CHOP-expressing nuclei remaining. The abundant regions of cytoplasm that showed costaining of CHOP and Grp78 (yellow–orange color) confirms that CHOP accumulates in keratinocytes in a perinuclear location, consistent perhaps with the ER and/or the Golgi.

Biochemical confirmation of CHOP accumulation in the living epidermis *in vivo* To strengthen the observation of changes in CHOP seen on histological sections, we examined levels of CHOP utilizing a second approach, immunoprecipitation (IP) followed by western blotting (Fig 5).