UVB induced EGF-R activation is mediated by the AhR: role of trypthophan

Abel J, Fritsche E, Schäfer C, Bernsmann T, Calles C, Wurm M, Hübenthal U, Cline JE, Hajimiragha H, Schroeder P, Rannug A, Klotz LO, Fürst P, Hanenberg H, and Krutmann J
[1]Institut für Umweltmedizinische Forschung (IUF), Auf'm Hennekamp 50, 40225 Düsseldorf, Germany. [2] Chemical and Veterinary Control Laboratory, Josef-König-Straße 40, 48147 Münster, Germany. [3]Department of Pediatric Oncology, Hematology & Immunology, Children's Hospital, Heinrich-Heine-University Medical Center, Moorenstr. 5, 40225 Düsseldorf, Germany.[4] Institute of Environmental Medicine, Karolinska Institutet, Box 210, S-171 77 Stockholm, Sweden.

Abstract

UVB radiation-induced signaling in mammalian cells involves two major pathways: one that is initiated in the nucleus through generation of DNA photoproducts and the second one is characterized by cell surface receptor activation. The chromophore for the latter one is unknown. Here, we show that this UVB response involves trypthophan as a chromophore for UVB resulting in intracellular generation of the AhR ligand 6-formylindolo(3,2-*b*)carbazole (FICZ) which triggers signaling to the nucleus and to the cell membrane. The UVB induced AhR activation induces CYP1A1 expression, internalization of the epidermal growth factor receptor (EGF-R), phosphorylation of the EGF-R-dependent mitogen-activated protein kinase (MAPK) ERK1/2 and the subsequent induction of cyclooxygenase-2 (COX-2). The role of the AhR in the UVB stress response was confirmed by *in vivo* studies using AhR KO mice.

Introduction

Exposure of mammalian cells to ultraviolet (UV) B radiation results in a signaling response called the UV response (1-3). This was previously shown to involve two major pathways. One that is initiated in the nucleus where UVB is absorbed by DNA and the subsequent formation of DNA photoproducts such as cyclobutane pyrimidine dimers are thought to represent the initiating signaling step (4-8). Cell enucleation experiments, however, have clearly demonstrated that a second part of the UV stress response occurs independently of nuclear DNA damage and is characterized by cell surface receptor clustering and subsequent activation of members of the mitogen-activated protein kinase family (MAPK) (1). The nature of the chromophore responsible for these non-nuclear UVB-induced signaling events has so far been enigmatic. In the present study we demonstrated for the first time the intracellular formation of the AhR ligand 6-formylindolo[3,2-*b*]carbazole (FICZ) from the chromophore tryptophan and provide the evidence that (i) UVB irradiation translocates the AhR into the

nucleus and induces CYP1A1 gene expression, (ii) the UVB-activated AhR additionally transfers the UVB signal to the cell membrane where it initiates EGF-R internalization and EGF-R dependent ERK1/2 phosphorylation and (iii) this signaling pathway is of *in vivo* relevance because AhR knockout mice show a compromised UVB responsiveness. Thus, AhR signaling is an integral part of the UVB stress response.

Material and Methods

The AhR antagonist 3´methoxy -4´nitroflavon was a kind gift of G. Vielhaber (Symrise, Holminden, Germany). FICZ was kindly provided by J. Bergman (Karolinska Institutet, Stockholm, Sweden). Cell culture media were purchased from Sigma-Aldrich (Munich, Germany). Antibodies used included: AhR (Affinity Bioreagents, Golden, Co, USA), Cox-2 (Oxford Biochemical Research, Oxford, MI, USA), EGF-R (Upstate Biotechnology, Lake Placid, NY, USA) and ERK1/2 (BioSource, Camarillo, Ca, USA). The immortalized keratinocyte cell line HaCaT was a gift from P. Boukomp (DKFZ, Heidelberg, Germany). UVB irradiation of cells was done with a TL20W/12RS lamp in PBS. Determination of intracellular trypthophan was achieved by GC-/MS/MS analyses after methylation and derivatization with trifluoracetic anhydride (TFA). FICZ was analysed by HPLC-MS/MS (13). The generation of AhR KO HaCaT cells were done as described (13). RT-PCR analyses were performed according to previous publications (10-11). Description of immunocytochemistry and Western blot analyses are given in (13).

Results and discussion

Irradiation of HaCaT cells with 10 mJ/cm^2 caused nuclear accumulation of a AhR-GFP fusion protein with was accompanied by nuclear accumulation of AhR protein, whereas almost no AhR protein was found in the nuclear compartment of sham-irradiated controls. In contrast to UVB, UVA radiation did not cause AhR translocation, indicating wavelength dependency.

UVB-induced AhR translocation was associated with transcriptional activation (3-fold increase in mRNA expression) of CYP1A1. Treatment of cells with the specific competitive AhR inhibitor 3‘Methoxy-4’Nitroflavone (10 µM) for 1 h prior to irradiation resulted in a 86% inhibition of UVB-induced CYP1A1 mRNA expression. In addition, AhR knockdown abolished the capacity of these cells to increase CYP1A1 mRNA expression upon UVB exposure. These data indicate that UVB radiation-induced translocation of the AhR from the cytoplasm into the nucleus leads to increased expression of an AhR-dependent gene. UVB radiation caused internalization of the EGF-R in a time dependent manner. Similar results

were seen after treatment of the cells with the AhR agonist Benzo(a)pyrene. Inhibition of AhR signaling by MNF pretreatment of AhR proficient cells or irradiation of AhR KO cells prevented UVB radiation-induced EGF-R internalization and partially antagonized the activation of well known EGF-R downstream targets like phosphorylation of ERK1/2 and induction of cyclooxygenase-2 (COX-2) mRNA and protein expression. These results indicate that activation of AhR signaling triggers UVB-induced EGF-R activation and subsequent downstream signal transduction events.

It was previously shown that tryptophan is a chromophore for UVB radiation and that under *ex vitro* conditions UVB irradiation of trypthophan leads to the formation of 6-formylindolo[3,2-*b*]carbazole (FICZ), which is a high affinity AhR ligand (12). To test the functional relevance of trypthophan for UVB-induced AhR signaling, the cells were incubated in trypthophan free medium. These tryptophan-deficient cells were compromised in their capacity to elicit a UVB response, as is shown for UVB-induced CYP1A1 and COX-2 expression as well as EGF-R internalization. This failure to mount a UVB response could be overcome if tryptophan was added back to the culture medium of tryptophan-starved cells 1 h prior to irradiation. In order to test whether the intracellular generation of FICZ, the photoproduct with the highest AhR affinity, is responsible for the UVB induced AhR activation, we analyzed the intracellular FICZ content in UVB treated HaCaT by HLPC-MS MS after loading of cells with $^{13}C_{11}{}^{15}N_2$-tryptophan 1 h prior to UVB irradiation. UVB radiation led to the generation of about 80 pM $^{13}C^{15}N$-labelled FICZ at the expected mass of 305.3. These data suggest that FICZ may be one of the photoproducts responsible for the AhR-dependent cellular signaling responses towards the nucleus and towards the cell membrane. Furthermore, in FICZ treated cells the cellular responses were similar to those observed after UVB irradiation implying that the AhR serves as a cytoplasmatic target which transfers the UVB signal. In order to assess the *in vivo* relevance of the AhR signaling pathway in the UVB response we have conducted comparative studies employing C57BL/6 mice and AhR knockout (KO) mice. UVB irradiation induces Cyp1a1 and Cox-2 mRNAs in wildtype, but not in AhR KO mice. Thus, AhR signaling appears to be involved in the UVB stress response *in vivo* as well.

Conclusion

UVB radiation is well known to be responsible for solar radiation-induced skin damage, most importantly skin cancer and premature skin aging (photoaging). Further studies are therefore needed to define the actual contribution of UVB radiation-induced AhR activation to these

detrimental effects. The UVB doses employed in the present study are about one third of the dose that is required to induce a visible erythema in a fair skinned individual and thus of physiological relevance. This hypothesis is supported by studies with AhR KO mice which fail to launch the UVB response.

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