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Melanocytes and keratinocytes have distinct and shared responses to ultraviolet radiation and arsenic

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HIGHLIGHTS

- Melanocytes are more resistant to UV toxicity than keratinocytes.
- Greater arsenic and UV are required for comparable ROS induction in melanocytes.
- Both cell types exhibit similar mechanism of PARP1 inhibition with As exposure.
- Melanocytes and keratinocytes retain DNA lesions after co-treatment with As and UV.

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ABSTRACT

The rise of melanoma incidence in the United States is a growing public health concern. A limited number of epidemiology studies suggest an association between arsenic levels and melanoma risk. Arsenic acts as a co-carcinogen with ultraviolet radiation (UVR) for the development of squamous cell carcinoma and proposed mechanisms include generation of oxidative stress by arsenic and UVR and inhibition of UVR-induced DNA repair by arsenic. In this study, we investigate similarities and differences in response to arsenic and UVR in keratinocytes and melanocytes. Normal melanocytes are markedly more resistant to UVR-induced cytotoxicity than normal keratinocytes, but both cell types are equally sensitive to arsenite. Melanocytes were more resistant to arsenite and UVR stimulation of superoxide production than keratinocytes, but the concentration of arsenite necessary to inhibit the activity of the DNA repair protein poly(ADP-ribose)polymerase and enhance retention of UVR-induced DNA damage was essentially equivalent in both cell types. These findings suggest that although melanocytes are less sensitive than keratinocytes to initial UVR-mediated DNA damage, both of these important target cells in the skin share a mechanism related to arsenic inhibition of DNA repair. These findings suggest that concurrent chronic arsenic exposure could promote retention of unrepaired DNA damage in melanocytes and act as a co-carcinogen in melanoma.

1. Introduction

The skin is a major target organ for arsenic toxicity and chronic exposure has been linked to increased incidence of keratinocytic tumors, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (Chervona et al., 2012; Morton and Dunnette, 1994; Platanias, 2009; Shannon and Strayer, 1989; Tokar et al., 2010). There is less evidence for potential contributions of arsenic exposure to the development of melanoma. However, many populations associated with areas of high endemic arsenic, such as Taiwan and Bangladesh, are highly resistant to ultraviolet radiation (UVR)-induced melanoma. Thus, the many epidemiological studies focused on these global sites would be unlikely to detect increases in the rate of malignant melanoma, leaving the question of arsenic involvement in melanoma risk unresolved.

There is emerging evidence that environmental or occupational exposures to arsenic may contribute to malignant melanoma risk. A small increase in melanoma risk was detected in socioeconomically disadvantaged areas of Australia and associated with soil arsenic exposure (Pearce et al., 2012). A suggested, but not statistically significant, association between melanoma and exposure to arsenic-containing pesticides in the U.S. was also noted (Dennis et al., 2010). These studies corroborate a previous report of a significant positive association between toenail arsenic levels and melanoma risk in a predominantly Caucasian Iowa population (Beane Freeman et al., 2004).

Exposure to UVR is well recognized as the major etiologic factor for development of nonmelanoma skin cancer and melanoma (IARC, 1992; Pfeifer and Besaratinia, 2012). Epidemiologic data

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indicate an association between sun exposure and nonmelanoma skin cancer in arsenic exposed populations (Chen et al., 2006, 2003; Melkonian et al., 2011; Watanabe et al., 2001) and there is strong in vivo evidence that arsenic acts as a co-carcinogen with UVR for development of SCC in mice (Rossman and Klein, 2011; Rossman et al., 2004). Nude mice chronically exposed to arsenite in drinking water develop significantly more skin tumors following UVR exposure than mice exposed to arsenite or UVR alone (Rossman et al., 2004). Potential mechanisms to account for these observations include generation of oxidative stress by arsenic and UVR (Cooper et al., 2009; Rossman and Klein, 2011; Wiencke et al., 1997; Yager and Wiencke, 1997) and inhibition of UVR-induced DNA repair (Beyersmann and Hartwig, 2008; Cooper et al., 2013; Ding et al., 2008; Ebert et al., 2011; Piatek et al., 2008; Zhou et al., 2011). Poly(ADP-ribose)polymerase (PARP)-1 is a recognized direct molecular target for arsenic and a key protein in the base excision repair arm of DNA repair which is responsible for resolution of oxidative lesions and strand breaks (Beyersmann and Hartwig, 2008; Cooper et al., 2013; Zhou et al., 2011). Oxidative DNA damage was greatly increased in the skin and tumors of the mice exposed to both arsenite and UVR, suggesting that both proposed mechanisms may be involved in the enhanced carcinogenesis (Rossman and Klein, 2011).

Melanin is an important regulator of the balance of reactive oxygen species in melanocytes that could alter response of melanocytes to arsenic and UVR when compared to keratinocytes (Cunha et al., 2012; Jenkins and Grossman, 2013; Suzukawa et al., 2012). In this study, we investigate similarities and differences between purported mechanisms underlying arsenic and UVR-induced DNA damage in these two important target cells within the skin. We find that normal melanocytes are markedly more resistant to UVR-induced cytotoxicity than normal keratinocytes, whereas cell viability following arsenite exposure is similar in the two cell types. Melanocytes are also more resistant to arsenite and UVR stimulation of superoxide with greater exposure levels required to generate responses comparable to keratinocytes. In contrast, the arsenite concentration dependence for zinc loss from PARP-1 and inhibition of PARP-1 enzyme activity was essentially equivalent in both cell types. These findings suggest that although melanocytes are less sensitive to initial UVR-mediated genotoxic insult, if UVR exposure is sufficient to generate DNA damage, melanocytes and keratinocytes are equally sensitive to arsenite inhibition of DNA repair mediated by PARP-1. The interaction between UVR-induced DNA damage and inhibition of DNA repair by arsenic could account, in part, for the epidemiologic findings suggesting increased risk of melanoma upon exposure to arsenic in non-Hispanic whites.

2. Materials and methods

2.1. Cell culture and treatment

Normal human neonatal epidermal keratinocytes (HEKn), normal human neonatal epidermal melanocytes (HEMn) and DermaLife culture medium with supplements were purchased from Lifeline Cell Technologies (Oceanside, CA). This medium contains no serum or phenol red indicator and is clear with little UV absorptive properties. Cells were cultured at 37 °C in 95% air/5% CO_2-humidified incubators. All experiments were performed on cells at passage 9 or less. 10 mM stock solution of sodium arsenite (99%; Fluka Chemie, Buchs, Germany) was prepared in milliQ water and sterilized using a 0.22-μm syringe filter. Working solutions were prepared by diluting the stock with complete cell growth medium. Cells were rinsed and placed in complete medium containing arsenite, then exposed to solar simulated (ss)UVR at doses and times indicated in the figure legends. Cell viability for both cell lines and all treatment conditions and exposure times was performed using the CellTiter 96 Non-radioactive cell proliferation assay kit following the manufacturer's instructions (Promega, Madison, WI).

2.2. UV source

UVR exposures were performed using an Oriel 1000 W Watt Solar Ultraviolet Simulator (Oriel Corp., Stratford, CT). This solar simulator produces a high intensity UVR beam in both the UVA (320–400 nm) and UVB (280–320 nm) spectrum with an emission ratio of 14:1 (UVA:UVB). The proportion and intensity of UVA/UVB was measured using a radiospectrometer (Optronics laboratories, Inc., Orlando, FL) and exposure times were calculated to give the desired doses. Measurements were made with Erythema UV and UVA intensity meter (Solar Light Co., Inc., Philadelphia, PA) in order to verify daily lamp output consistency and to estimate minimum erythema dose (MED). The dose of 3 kJ/m^2 used for the keratinocytes is approximately 1 MED and the highest dose used for melanocytes (10 kJ/m^2) is about 1.5 MED. These values are supported by measurements reported by Ciren and Li showing that the average daily human exposures are 3–5 kJ/m^2 for 1 MED exposure at mid-latitudes (Ciren and Li, 2003).

2.3. Reactive oxygen species (ROS) detection

Cells were cultured in 96 well plates in complete medium. When cells reached approximately 40% of confluent density, cultures were placed in fresh media and treated with arsenite (1 or 5 μM), UVR (3 or 5 kJ/m^2) or both for the times indicated in the figure legends. Thirty minutes prior to collection, dihydroethidium (DHE, 5 μM) was added as a fluorescent indicator of ROS generated in response to the described treatment. Cells were rinsed with PBS and relative fluorescence intensity was quantified by measuring the intensity of fluorescence emission using a Wallac Victor 2 fluorescence spectrophotometer equipped with 390 nm excitation and 410 nm emission filters. A minimum of 3 independent samples were analyzed per treatment and time point. Values were normalized to total DNA fluorescence as previously described (Rago et al., 1990). Briefly, plates previously analyzed for ROS were rinsed with Krebs Ringer buffer (20 mM HEPES, 10 mM dextrose, 127 mM NaCl, 5.5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgSO_4$, pH 7.4) then frozen at −80 °C overnight. Plates were thawed for at least 2 h at room temperature, stained with Hoechst dye (10 μg/ml bis Benzimide) overnight and fluorescence determined using a Wallac Victor 2 fluorescence spectrophotometer equipped with 350 nm excitation and 460 nm emission filters. This method of fluorescence quantification was validated by comparison with data obtained using Metamorph software (version 6.3r6) as previously described (Cooper et al., 2007).

2.4. NADPH oxidase activity

Cells were cultured in 12-well plates and treated as described for ROS experiments. NADPH oxidase activity was detected using the Lucigenin Illumination method. Briefly, following treatment and incubation time cells were rinsed thoroughly with PBS, removed by scraping, resuspended in 500 μl PBS and placed in microfuge tubes. Cells were frozen at −80 °C overnight to lyse cells. Samples were aliquoted (100 μl) in quadruplicate, placed in luminometer tubes and incubated with diethyldithiocarbamate (DTC, 1 M) for 20 min at 37 °C to inhibit superoxide dismutase activity. Immediately before measuring, 1 μl lucigenin (0.5 mM) was added to the sample followed by 1 μl NADPH (10 mM) and mixed. Luminescence was measured in a TD20/20 Luminometer (Turner Designs, Sunnyvale, CA) with ten 30 s counts. Sample values were integrated and average units calculated. Parallel samples were analyzed with the addition of Tiron (1 M) to scavenge superoxide and confirm measurement of NADPH oxidase activity.

2.5. Assessment of PARP-1 activity

Cells were treated with arsenite for 24 h, then exposed to ssUVR (3 kJ/m^2) and incubated for an additional 1 h. Whole cell extracts were assayed for PARP activity using the HT Colorimetric PARP/Apoptosis Assay kit (Trevigen, Inc., Gaithersburg, MD) according to manufacturer's instructions. Briefly, PARP standards and total protein (100 ng) were placed in duplicate in rehydrated, histone coated wells of a 96 well plate and incubated for 30 min. Samples were incubated with the substrate cocktail containing activated DNA and NAD for an additional 30 min, washed and PAR antibody added. HRP conjugated secondary antibody followed by TACS-Sapphire reagent was subsequently added and the reaction measured using a SpectraMax M2 plate reader (Molecular Devices, Sunnydale, CA) at 450 nm. Sample absorbance was compared to the standard curve generated and activity reported as mUnits per mg protein.

2.6. Isolation of zinc finger proteins and zinc content measurement

PARP-1 was isolated from treated cells by immunoprecipitation. Cells were cultured as described above and treated as described in the figure legend (Fig. 5). Cells were no more than 75% confluent at the time of collection. Immunoprecipitaiton and determination of zinc content was performed as previously described (Cooper et al., 2013; Zhou et al., 2011). Briefly, total protein was collected and PARP-1 isolated via immunoprecipitation (500 μg in 500 μl) with 5 μl of rabbit polyclonal antibody (PARP-1; Cell Signaling #9542). Protein samples and zinc standards (100 μl) were transferred to 96 well plates and zinc content measured by adding 10 μl of 1 mM 4,(2-pyridylazo)-resorcinol and absorbance at 493 nm was determined by a SpectraMax M2 plate reader (Molecular Devices; Sunnyvale, CA) with