Mutation Research 775 (2015) 10–18

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

The effect of low dose ionizing radiation on homeostasis and functional integrity in an organotypic human skin model

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ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 10 February 2015
Accepted 3 March 2015
Available online 16 March 2015

Keywords:
3D skin equivalent
Differentiation profile
Ionizing radiation
Heavy ion
Radiation quality

ABSTRACT

Outside the protection of Earth's atmosphere, astronauts are exposed to low doses of high linear energy transfer (LET) radiation. Future NASA plans for deep space missions or a permanent settlement on the moon are limited by the health risks associated with space radiation exposures. There is a paucity of direct epidemiological data for low dose exposures to space radiation-relevant high LET ions. Health risk models are used to estimate the risk for such exposures, though these models are based on high dose experiments. There is increasing evidence, however, that low and high dose exposures result in different signaling events at the molecular level, and may involve different response mechanisms. Further, despite their low abundance, high LET particles have been identified as the major contributor to health risk during manned space flight. The human skin is exposed in every external radiation scenario, making it an ideal epithelial tissue model in which to study radiation induced effects. Here, we exposed an *in vitro* three dimensional (3-D) human organotypic skin tissue model to low doses of high LET oxygen (O), silicon (Si) and iron (Fe) ions. We measured proliferation and differentiation profiles in the skin tissue and examined the integrity of the skin's barrier function. We discuss the role of secondary particles in changing the proportion of cells receiving a radiation dose, emphasizing the possible impact on radiation-induced health issues in astronauts.

1. Introduction

Outside Earth's atmosphere, all astronauts will be exposed to space radiation, giving rise to NASA's concern with associated health risks. Current risk estimates for manned space flight are based predominantly on human epidemiological data following low linear energy transfer (LET) and high dose radiation exposures. However, space radiation is composed of high LET, low dose particle irradiation resulting in large uncertainties in health risk estimates. Although the average daily dose accumulated in space from particle irradiation is not high enough to cause acute radiation exposure health effects, several studies have demonstrated an increased cancer risk in animal models (summarized in [1]). Despite the relatively low abundance of high atomic number and energy (HZE) particles in space, they have an enhanced relative biological effectiveness (RBE) due in part to their high ionization potential [2,16] and pose and increased health risk relative to more abundant protons.

A three dimensional (3-D) human organotypic skin model was employed in this study. The choice of skin as an epithelial model is three-fold, (1) skin is a relatively radiation sensitive organ, (2) it will receive a radiation dose in all external exposure scenarios, and (3) it is easily biopsied for validation of results in the astronaut population. The ICRP has estimated a nominal risk coefficient (cancer cases/10,000 persons/Sv) for skin in a sex specific population of exposed 18–64 years olds [3]. The nominal risk coefficient for skin is 670 versus 116 for the second most sensitive tissue, the female breast (see Ref. [3] table A.4.19, page 210). This makes skin the most highly sensitive tissue to ionizing radiation exposures. However, ionizing radiation induced skin cancers are almost exclusively non-lethal basal cell carcinomas [4], thus accounting for its relatively low tissue weighting factor (~3) and the common belief skin is a "radio-resistant" tissue. The high sensitivity of radiation induced responses makes skin an excellent model for examining low dose

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http://dx.doi.org/10.1016/j.mrfmmm.2015.03.003
0027-5107/

effects at a molecular level where the endpoint is not metastatic disease.

The human organotypic skin model is composed of fibroblasts expressing extracellular matrix proteins in the dermis, and keratinocytes in the fully developed epidermis. New epidermal cells are exclusively formed through mitosis in the stratum basale (basal layer) and move up though the strata spinosum, granulosum, and corneum as they differentiate. Keratinocytes reach terminal differentiation at the air interface and are converted into corneocytes. Disturbances to the homeostatic regulation of keratinocyte proliferation, differentiation and death leads can lead to skin diseases such as atopic dermatitis, psoriasis and ichthyosis [5,6]. The complex homeostasis of proliferation and differentiation is characterized by changes in protein expression that define cell phenotype. Keratin 10 (K10) expression is a marker for the spinous and the granular layers [7]. Filaggrin is a major component of both the cornified layer and the granular layer where it is expressed. The skin protects the body against pathogens as well as oxidative, chemical and mechanical stresses. Mutations in the filaggrin gene are associated with a variety of skin diseases with disrupted skin barrier function [8]. External factors that induce alterations in the homeostatic regulation such as space relevant ions [9], environmental toxins as well as chronic trauma and inflammation [10] can contribute to benign and malignant epidermal cell growth leading to the most frequent human cancer type, epidermal tumors [11].

Our central hypothesis is that low doses of HZE radiation are capable of disturbing the homeostatic regulation of epithelial development, resulting in an LET dependent alteration in the proliferation and differentiation profiles. To address this hypothesis, the total number of cells in the basal layer, their proliferative activity, and the overall area of viable and differentiating keratinocytes in the epidermis of a 3-D organotypic skin model post exposure to space relevant low doses of high LET radiation were measured. In addition, the spatial distribution of the differentiation markers K10 and filaggrin in tissue sections as well as the epidermal barrier function, a functional outcome, were evaluated. Our data will contribute to the improvement of health risk estimates for astronauts on deep space missions, and to the development of a mechanistic understanding of the effects of low dose, high LET radiation exposures in epithelial tissues.

2. Material and methods

2.1. Tissue culture and irradiation

In vitro 3-D organotypic skin model consisting of epidermal keratinocytes and dermal fibroblasts were obtained from MatTek (EpiDermFT 400, MatTek Corp., Ashland, MA, USA). Tissue samples were randomized upon receipt to exclude production specific alterations. Samples were grown at 37 °C, 95% humidity and 5% CO_2 in a 6-transwell insert system and maintained in 3 ml of maintenance medium (MatTek Corp.). Three hours prior to irradiation, the culture media was replaced with 2 ml fresh media. Due to constraints in beam time availability, sample age and equilibration times (minimum 24 h) could not be held constant. Ion exposures were performed at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL, Uptown, NY, USA, campaigns IIA, IIB and IIC). Table 1 summarizes the ion exposure conditions, including ion species, LET, energy, and dose. Exposures were designed to deliver either a mean value of one primary ion traversal/basal cell (F2 = 1.1×10^{-3} ions/μm^2) or one traversal for every third cell in the basal layer (F1 = 3.6×10^{-4} ions/μm^2) [12]. Neon information from previous experimental exposures is included for the purpose of discussion only [13]. Samples were oriented at 45° from the incident particle beam to provide a more

Table 1
Summary of ion exposure conditions employed at NSRL. Beam radii and the δ-ray mean hits per cell were determined using GERMCode [19]. Neon data included for discussion purposes only [12].

Ion	LET (keV/μm)	Energy (MeV/u)	Range (g/cm^2)	Fluence 1	Fluence 2	Beam radius (μm)		Fluence 1 (F1) 3.6×10^{-4} ions/μm^2		Fluence 2 (F2) 1.1×10^{-3} ions/μm^2	
								Mean hits/cell		Mean hits/cell	
				3.6×10^{-4} ions/μm^2	1.1×10^{-3} ions/μm^2						
				Dose (cGy)	Dose (cGy)	Core	Penumbra	Core	δ-rays > 0.1 cGy	Core	δ-rays > 0.1 cGy
O^{+8}	18	500	29.05	0.11	0.31	0.239	45	0.33	0.51	1	1.56
Ne^{+10}	35	300	10.23	0.2	0.6	0.205	63	0.33	0.62	1	1.88
Si^{+14}	60	400	11.69	0.35	1.06	0.248	73	0.33	0.71	1	2.16
Fe^{+26}	174	600	16.25	1	3	0.268	137	0.33	1.04	1	3.16