MICRONUCLEUS FORMATION IN HUMAN SKIN KERATIONCYTES EXPOSED TO DIFFERENT RADIATION QUALITIES IN 2D AND IN 3D

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BACKGROUND

One of the main concerns associated with manned missions to space is the increased risk for developing cancer from space radiation exposure. In particular, the physical characteristics and the biological effects of the radiation qualities in space make it difficult to predict their biological effects on the basis of data from γ- and X-ray exposures. In the same manner, it is also very difficult to extrapolate the risk for radiation (IR) carcinogenesis due to charged particle exposure from existing epidemiological data for radiation-induced cancers.

Our study is aimed at further elucidating the Relative Biological Effect (RBE) of charged particle radiation, encompassing the Linear Energy Transfer (LET) range from 44-250 keV/μm. To assess genotoxicity from low doses of different radiation qualities the cytokinesis-block micronucleus assay is used. This assay is high-throughput and highly sensitive, and allows the accumulation of all cells at the bi-nucleate stage, regardless of their division kinetics. Importantly, in humans, micronuclei (MN) are one of the four main endpoints, together with chromosomal aberrations, aneuploidy, and sister chromatid exchanges (SCE), in the identification of cancer initiation processes [1, 2].

MODEL SYSTEMS

Normal primary human keratinocytes and immortalized HaCaT keratinocytes, grown in 2D cell monolayers, were exposed to graded doses of 300 or 1000 MeV/n Si or Fe ions and MN frequency was assessed. The advantage of using primary keratinocytes is that their response to IR most closely resembles that of normal human skin cells. Immortalized HaCaT cells were used as a model for intraepithelial neoplasia. HaCaT cells carry a UVB fingerprint mutation in their *p53* gene [3], and similar mutations, induced from the sunlight, reside in many skin cells of healthy middle-aged individuals, such as astronauts.

Although 2D *in vitro* assays are high-throughput and can be very informative, they do not appropriately recapitulate the complex interactions between different cell types and the extracellular matrix within tissues. For this reason, to more realistically simulate the complex architecture of the skin, we have also investigated 3D reconstructed multi-cellular organotypic skin epithelia, in which the keratinocytes form a stratified squamous epithelium and a proliferative basal cell layer. These tissues were exposed to the same radiation qualities as described above, and MN frequency was assessed in the proliferative keratinocyte compartment.

RESULTS

We have established dose response curves for MN induction of 300 and 1000 keV/μm Si and Fe ions. ^{137}Cs γ-rays were used as low LET reference radiation. Although the shapes and magnitudes of the dose response curves differ, exposure to 300 and 1000 MeV/n Fe ions led to the highest fraction of MN induced in both primary and initiated keratinocytes grown in 2D. RBE_{max} values obtained range from 2.8 to 5.6 and from 2.9 to 7.2 for primary and initiated keratinocytes, respectively. At radiation doses ≤ 0.4 Gy, 300 MeV/n Fe ions were most effective in inducing MN in normal keratinocytes. However, at doses larger than 0.5 Gy, for 300 MeV/n Fe ions an "over-kill effect" was observed, and 1000 MeV/n Fe ions were more efficient in inducing MN. In initiated keratinocytes, 300 MeV/n Fe ions produced MN with greater frequency than 1000 MeV/n Fe ions up to 0.8 Gy. Compared to primary keratinocytes, MN formation was higher in immortalized keratinocytes for all doses and radiation qualities investigated, reflecting the greater tolerance of persistent DNA damage in initiated cells.

Compared to keratinocytes grown in 2D, RBE_{max} values for MN induction were generally lower in keratinocytes recovered from irradiated 3D skin epithelia and, for Fe ion exposure, ranged from 1.5 to 1.6 and from 1.6 to 4.0 for primary and initiated epidermal keratinocytes, respectively. Our results suggest that tissue architecture plays an important role in preventing the persistence of genotoxic lesions from space radiation exposure, possibly by enhancing DNA repair fidelity.

REFERENCES

[1] Hagmar L., et al. 2001, Int. J. Hyg. Environ. Health 204: 43-7. [2] Tucker J.D. and Preston R.J., Mutat. Res. 365: 147-59. [3] Lehman T. A., *et al*. 1993, Carcinogenesis 14:833-9.