

UVA and UVB genetic damage in the p53 gene occur at different depths within solar keratosis

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Introduction

Australia has the highest incidence of skin cancer in the world. In this country skin cancer incidence significantly exceeds that of all other malignancies combined. It is accepted that ultraviolet radiation is the main causative factor in skin cancer formation. Till now most research has attributed these effects to the UVB fraction of sunlight. We hypothesised that UVA in addition to UVB may be playing an important role in skin cancer formation. In order to examine this we have mapped the pattern of mutational damage within the premalignant lesion solar keratosis in our target gene of interest -the tumour suppressor gene p53.

Methods

Frozen sections were prepared from 8 Solar Keratosis excised from human volunteers attending the Dermatology Outpatients Clinic at RPAH. Utilising the Pixcell II Laser Capture Microdissection Apparatus, several areas were dissected from within each skin. Histologically 2 epithelial rete pegs representative of each lesion were chosen. Superficial and deep cells from each vertical peg were sampled with the removal of 20 cells per microdissection. DNA was extracted from microdissected cells using protocols previously established (1). 2ul of crude cell lysate was then directly used as a DNA template. Exons 5-9 were amplified in all samples using primers designed by Schifter (2) under the following conditions: Exons 5-9: Denatured at 95^o C for 15min, then 94^oC for 3min, followed by Annealing over 50 cycles at 94^oC for 20 sec and 64^oC for 20 sec. A final extension step of 72^oC for 10 minutes was then performed for Exon 5 only, after which the specimens were kept on hold at 4^oC. PCR product was purified using a Qiaquick Gel extraction kit prior to automated sequencing (Applied Biosystems Model 373A). Each amplicon was successfully sequenced completely in forward and reverse directions and the results repeated to confirm mutations. Negative controls without DNA and positive controls (K562 cell line DNA-known to contain the wild type p53 genomic sequence) were also included in each PCR run.

RESULTS

Utilising the methods described above we were able to reliably sequence 100% of microdissected keratinocytes. Both UVA and UVB fingerprint mutations were detectable in Solar Keratosis and showed a striking spatial distribution (See Fig 1). The majority of UVB fingerprint mutations (87.5%) were found in the spinous layer or above, in contrast to the basal distribution of UVA fingerprint mutations (88%) in SK. When the distribution of UV induced mutations was calculated as depth per mm from the skin surface and a Chi square analysis performed it was found that 100% of UVA mutations had a depth \leq 0.4mm. Whereas only 45.3% of UVB mutations were \leq 0.4mm deep. This difference was statistically significant ($p < 0.001$).

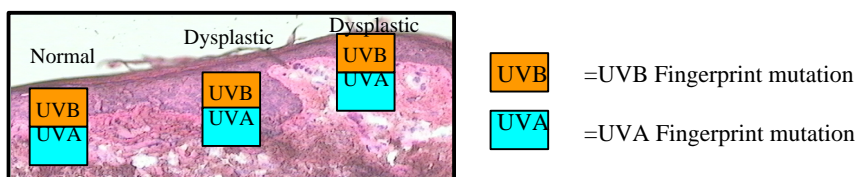


Fig 1 Schematic representation of microdissection results in SK

DISCUSSION

Utilising laser capture microdissection we report for the first time a marked spatial distribution of UVB and UVA induced genetic damage in the premalignant skin lesion the solar keratosis. The deeper localisation of UVA fingerprint mutations in the basal germinative layer of the epidermis suggests that UVA rather than UVB may be the dominant carcinogen in this formative region. A lack of sensitivity in previous whole tissue sampling and failure to sample the basal layers where UVA is possibly exerting its maximal effects may explain why UVA induced DNA lesions have been difficult to identify in the past.

REFERENCES

1. www.ARCTUR.com

2. Schifter M et al, Epithelial P53 gene expression and mutational analysis combined with growth fraction assessment, in oral lichen planus. J Oral Pathol Med, 27:318 (1998).

This study implicates a much earlier and more significant genotoxic role for UVA in skin cancer formation than previously described. Our results may help explain why the use of principally UVB blocking sunscreen has not altered the growing skin cancer incidence both here in Australia and worldwide.