Development of Biomarkers to Predict Response to Ultraviolet-B Treatment in Psoriasis.

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Aims

- Confirm apoptosis occurs after UVB exposure at the clinically effective wavelength of 311nm
- Validate the expression of response genes at the protein level after UVB exposure to give insight into the mechanism of plaque clearance.

Introduction

One treatment of the inflammatory skin disease psoriasis is UVB therapy using the clinically effective wavelength of 311nm however, the mechanism behind this treatment is unknown.

Apoptosis is controlled cell death and thought to be a major part of plaque clearance induced by UVB therapy. Several genes have been identified which are involved in apoptosis and regulated by this UVB exposure.

The aim was to look at how these genes respond at the protein level, to see how and where the changes were occurring.

This information will help to understand the mechanism by which this treatment works and could potentially lead to the development of a biomarker. This would allow patients who would respond well to this therapy to be easily identified.

One of the proteins stained for was Bub1 as this had been found to be up-regulated in the gene array data. Bub1 is a mitotic checkpoint kinase so has a function in the cell cycle, and so it is plausible that this protein could be involved in the plaque clearance and regulated by the apoptosis pathway.

Methods

Skin biopsies from normal, non-psoriatic skin were used in all procedures.

To view any changes in the levels of protein before and after UVB exposure immunofluorescent staining was used.

The antibodies used were against the proteins of interest. These were Caspase-3, a marker for apoptosis and Bub1 and Aurora A, two genes previously identified in gene array studies to be regulated by the UVB therapy.

The immunostaining methods for Bub1 and Aurora A had to be optimised, which involved testing several fixatives (methanol, acetone, 4% paraformaldehyde and 1:1 acetone:methanol mix).

A section from tumour cells was used as a control in the optimisation studies.

Results

Caspase-3 Immunostaining.

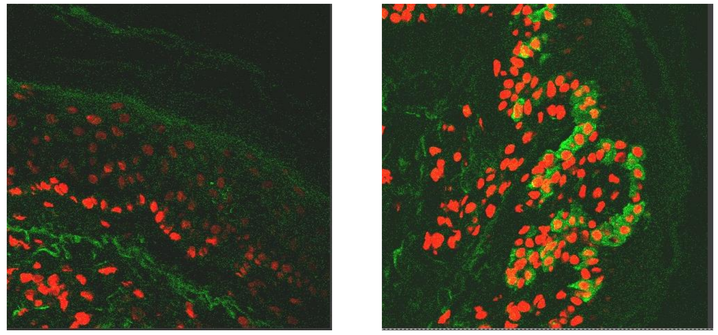

Figure 1. The left image shows the control (not exposed to UVB) stained and imaged for caspase-3. The image on the right shows the staining for caspase-3 but in skin which had been exposed to UVB.

This shows that there is more apoptosis in the basal layers of the epidermis in the sample exposed to UVB, confirming that UVB exposure at 311nm induces apoptosis. This was also carried out in another donor to validate this, although it is not shown here.

Bub1 Immunostaining

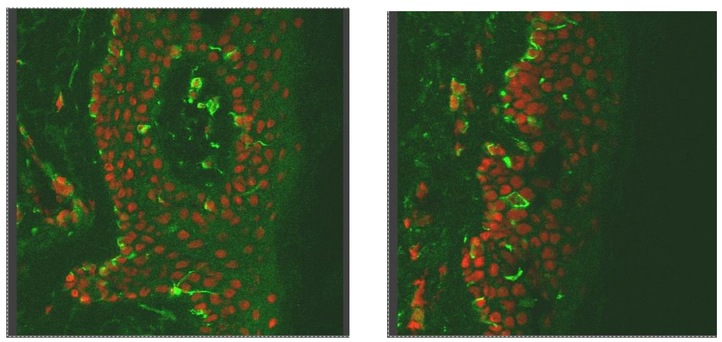

Figure 2. Both are stained for Bub1 and fixed in acetone as this was the best fixative. The left image shows the control (no UVB exposure), the right shows the UVB treated skin sample.

The images show that there is positive staining for Bub1 in the basal layers of the epidermis in the nucleus as expected from our knowledge of Bub1 in proliferative cells. There is also staining of dendritic (Langerhans) cells, as this was unexpected a co-localisation study should be carried out to confirm this.

Aurora A Immunostaining

The optimisation of the antibody could not be achieved in the time constraints of this project. There are no results to draw any reliable conclusions from.

Conclusions

- Caspase-3 is up-regulated by exposure to the clinically effective UVB as shown in multiple donors.
- This shows that it is likely that apoptosis has a major role in the clearance of psoriatic plaques.
- The antibody for Bub1 was optimised, however further study is required to confirm the presence of Bub1 in Langerhans cells.
- Although there was positive staining for Bub1, it could not be determined from this technique whether there was a significant difference in the levels of Bub1 between the non-treated and the UVB treated skin.
- The Aurora A optimisation was unsuccessful due to the time constraints so it was not possible to draw any conclusions from this.

Further Study

Co-localisation of Bub1 and Langerhans cells and a western blot to show if there was a significant difference in Bub1 levels.

Other genes from the gene array will also be studied in a similar way to expose the mechanism and genes involved in plaque clearance by UVB therapy.

The same procedures should be carried out in psoriatic skin.

The long term aim is to provide a biomarker to allow prediction of a patients response to this therapy.

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References

Sophie C. Weatherhead et al. (2011) *Keratinocyte apoptosis in epidermal remodelling and clearance of psoriasis induced by UV radiation* Journal of investigative dermatology **131** 1916-26