BIOMARKERS OF THE MOLECULAR RESPONSE TO ULTRAVIOLET RADIATION B IN PSORIASIS

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Master by Research 2016-2017

[Word Count – 926]
**BACKGROUND**

Psoriasis is an autoimmune skin disorder affecting approximately 2% of people worldwide.\(^1\) It is classed as a chronic inflammatory dermatosis. A number of subtypes exist – with chronic plaque psoriasis making up around 90% of cases.

Narrowband UVB radiation is a safe and effective treatment for psoriasis and is therapeutic at a wavelength of 311nm. When used at 290nm however, UVB is not effective.

Epidermal keratinocyte apoptosis appears central to resolution of psoriatic plaques in response to UVB\(^2\), However the underlying molecular mechanisms of clearance remain to be defined. Gene array studies have identified differential regulation of apoptotic genes following effective UBV therapy compared to ineffective UVB.\(^3\) By comparing transcriptional responses in psoriatic skin 24 hours after irradiation with effective (311nm) and non-effective (290nm) radiation, putative regulatory pathways and potential biomarkers have been identified.

This project aims to study whether the proteins associated with these gene changes are also modified in effective UVB treated skin. This may provide insight into the mechanism of action by which UVB clears psoriatic plaques, leading to more targeted systemic therapies with fewer side effects.

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![ultraviolet spectrum](image)

**Figure 1** – The ultraviolet spectrum. 290nm is ineffective at producing clearance of psoriasis (even when used at very high doses), while 311nm is effective at relatively low doses. Both cause erythema.\(^2\)
HYPOTHESIS AND AIMS
The overall hypothesis is that genes differentially expressed by irradiation of psoriatic epidermis in vivo by 311 nm UVB (effective) versus 290nm UVB (ineffective) may provide insight to mechanisms involved in clearing psoriatic plaques. Moreover, by using matched erythemogenic doses of 290nm and 311nm UVB, bystander effects are minimised. To validate key genes I studied their protein expression, hypothesising that they would also be differentially expressed by 311nm versus 290 nm. Such proteins may then act as biomarkers of therapeutic response.

- To compare protein expression of BUB1, Aurora-A, FRA1 and MMP3 in lesional psoriasis irradiated with effective (311nm) and ineffective (290nm) wavelengths of UVB and in control (un-irradiated) psoriatic skin.
- To evaluate these proteins as biomarkers of psoriatic plaque clearance.
- Optimise immunohistochemistry protocols for BUB1, Aurora-A, FRA1 and MMP3.

MATERIALS AND METHODS
Immunostaining was performed on frozen sections of human psoriatic skin that had been irradiated 24 hours prior to biopsy with effective UVB (311nm), ineffective UVB (290nm) or was unirradiated. Confocal microscopy Images were analysed to determine protein expression in control, 311nm and 290nm treated skin.

Skin biopsies were obtained from patients presenting with psoriasis at tertiary referral clinics at the Royal Victoria Infirmary, Newcastle upon Tyne. This was carried out as part of previous research.

Positive cells were identified and quantified using Volocity. Images were cropped using the region of interest tool in order to exclude dermis and stratum corneum. A protocol was set for each antibody to quantify the levels of protein per square μm of epidermis, and in the case of Aurora-A, also the number of positive cells per square μm of epidermis. Nuclear and cytoplasmic colocalisation measurements were performed by setting a background threshold and creating a separate protocol.

Using Graphpad Prism 5 software, One-way analysis of variance (ANOVA) was used to compare the levels of immunofluorescence between control, 311nm and 290nm treated skin. Additionally, paired t-tests were carried out to detect differences between the two wavelengths in matched samples. Statistical significance was set at p-value ≤ 0.05.
RESULTS AND DISCUSSION

AURORA-A

Overall, epidermal fluorescence per μm² and number of positive cells were highest in the untreated skin, followed by 311nm and then 290nm. Compared to untreated skin, Aurora-A expression in 311nm treated skin was lower, both when measured by overall epidermal signal and number of positive cells. Aurora-A is an anti-apoptotic protein when expressed in keratinocytes and is highest during mitosis, acting to promote centrosome maturation and spindle formation. My results conflict with the evidence that AURKA is down-regulated to a certain extent. Gene array data showed a decrease in AURKA expression at four hours post irradiation, but a increase 18 hours after. This could indicate a time lag between mRNA expression and protein expression.

Figure 2. Quantification of Aurora-A confocal images using epidermal signal per μm². Data shows mean ± SEM of control un-irradiated skin vs 290nm UVB treated skin vs 311nm UVB treated skin. One-way ANOVA test gave p=0.09. Bonferroni’s Multiple Comparison Test did not produce a significant p-value. Control n=4, 290nm n=6 311nm n=11

Figure 3. Quantification of Aurora-A confocal images using positive epidermal cells per μm². Data shows mean ± SEM of control un-irradiated skin vs 290nm UVB treated skin vs 311nm UVB treated skin. One-way ANOVA test gave p=0.13. Bonferroni’s Multiple Comparison Test did not produce a significant p-value. Control n=4, 290nm n=6 311nm n=11

Figure 4 Quantification of Aurora-A confocal images using epidermal signal per μm². Data shows all paired data points with mean 290nm UVB treated skin vs 311nm UVB treated skin. Paired t-test gave p=0.02.

Figure 5 Quantification of Aurora-A confocal images using positive epidermal cells per μm². Data shows all paired data points with mean 290nm UVB treated skin vs 311nm UVB treated skin. Paired t-test gave p=0.007
Figure 6 - Confocal images of lesional psoriatic skin irradiated with effective UVB (311nm), ineffective UVB (290nm) and control (un-irradiated lesional psoriatic skin). Note reduction in strongly expressing cells by 311nm, and disappearance of strongly expressing cells in 290nm irradiated skin. White dotted lines indicate epidermal limits. Scale bars = 100μm in large images and 50 μm in zoomed images. These images are all taken from one donor, however are representative of at least five donors.
FRA1

FRA1 stained strongly in the nuclei of control skin, with little or no cytoplasmic staining. In order to discern whether protein had translocated to the cytoplasmic space from the nuclei, the nuclei to cytoplasmic ratio was calculated. It plays a complex role in cell proliferation, differentiation and apoptosis and mainly acts through its role in AP-1 activation.

Figure 7: Quantification of FRA1 confocal images using epidermal signal per μm². Data shows mean ± SEM of control un-irradiated skin vs 290nm UVB treated skin vs 311nm UVB treated skin. One-way ANOVA test gave p=0.36. Bonferroni’s Multiple Comparison Test did not produce a significant p-value. Control n=5, 290nm n=7, 311nm n=11.

Figure 8: Quantification of FRA1 confocal images using epidermal signal per μm². Data shows all paired data points with mean 290nm UVB treated skin vs 311nm UVB treated skin. Paired t-test gave p=0.02.

Figure 9: Quantification of FRA1 confocal images using nuclear to cytoplasmic ratio. Data shows mean ± SEM of control un-irradiated skin vs 290nm UVB treated skin vs 311nm UVB treated skin. One-way ANOVA test gave p=0.40. Bonferroni’s Multiple Comparison Test did not produce a significant p-value. Control n=5, 290nm n=7, 311nm n=11.
Figure 10 - Confocal images of lesional psoriatic skin irradiated with effective UVB (311nm), ineffective UVB (290nm) and control. Note reduction in FRA1 nuclear staining in 311nm > 290nm. White dotted lines indicate epidermal limits. Scale bars = 100μm in large images and 50 μm in zoomed images. These images are all taken from one donor, however are representative of at least five donors.
MMP3

MMP3 was shown to localise in the cytoplasm and extracellular space, where it acts to break down extracellular matrix proteins. MMP3 was found in particularly high concentrations around the dermal-epidermal borders. MMP3 is predominantly involved in the breakdown of ECM within the dermis, and is found in higher quantities in psoriatic skin.

![Figure 11 Quantification of MMP3 confocal images using epidermal signal per μm². Data shows mean ± SEM of control un-irradiated skin vs 290nm UVB treated skin vs 311nm UVB treated skin. One-way ANOVA test gave p=0.74. Bonferroni’s Multiple Comparison Test did not produce a significant p-value. Control n=4, 290nm n=3 311nm n=6](image)

![Figure 12 Quantification of MMP3 confocal images using epidermal signal per μm². Data shows all paired data points with mean 290nm UVB treated skin vs 311nm UVB treated skin. Paired t-test gave p=0.06](image)
Figure 13 - Confocal images of lesional psoriatic skin irradiated with effective UVB (311nm), ineffective UVB (290nm) and control. Note reduction in MMP3 cytoplasmic staining at the dermal-epidermal border in 311nm > 290nm. White dotted lines indicate epidermal limits. Scale bars = 100μm in large images and 50 μm in zoomed images. These images are all taken from one donor, however are representative of at least five donors.
BUB1

On viewing at low power during optimisation, BUB-1 stained a number of cells strongly. During high power viewing however it appeared these strongly expressing cells were all dendritic cells present in the epidermis.

Figure 14 Confocal images of lesional psoriatic skin irradiated with effective UVB stained with anti-BUB1. Note the pattern of staining in images A.i) and A.iii). The only positive cells within the epidermis look to be dendritic in appearance. Background staining was present in all images so was not taken to be true staining. Scale bars = 100μm in large images and 50 μm in zoomed images. These images are representative of all images taken of anti-BUB1.
CONCLUSIONS

This study confirmed FRA1 and Aurora-A as potential biomarkers of UVB induced psoriatic plaque clearance.

- FRA1 protein expression in lesional psoriatic skin significantly decreased when exposed to effective (311nm) UVB compared with ineffective (290nm) UVB. This result suggests that FRA1 may be acting anti-apoptotic manner when expressed in keratinocytes in response to UVB.
- Aurora-A protein expression in lesional psoriatic skin significantly decreased in response to effective UVB. This project highlights the predictive value of gene expression when determining protein expression.
- MMP3 failed to show potential as a biomarker as there was no significant difference in protein expression between the effective and non-effective UVB treated skin. Nevertheless it confirmed the presence of MMP3 in psoriatic epidermis.
- BUB1 was found to be absent in keratinocytes, but present in Langerhans cells within the epidermis.

Overall these findings provide further evidence for the role of keratinocyte apoptosis in mediating the therapeutic effects of narrowband UVB therapy in psoriasis.
**BIBLIOGRAPHY**

