UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2024/25

NB: This whole report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.

Name of student:

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Twitter Handle*:
*(*optional)*

Name of supervisor(s):

Dr Helen Dodson

Project Title: (no more than 220 characters)

Analysis of the DNA damage response, proliferation and collagen remodelling in Rac1 deficient mouse skin following irritant induced inflammation

Project aims: (no more than 700 words)

This project aimed to investigate how loss of the GTPase, Rac1, would influence responses to inflammation. We specifically looked at collagen remodelling, keratinocyte proliferation and the DNA damage response in the epidermis.

Collagen remodelling was explored using Masson's trichome staining, allowing us to visualise collagen deposition within the epidermis. Using QuPath software we defined the collagen and calculated this relative to total tissue area. Tissue was stained using immunohistochemistry with the proliferation marker, Ki67 and the DNA damage response marker ɣH2AX. The proportion of Ki67 and ɣH2AX positive nuclei in the epidermis was calculated using a positive detection filter in QuPath.

Background

Rac1 is a GTPase with many cellular functions including control of cell motility and regulation of oxidative stress. It is upregulated in many pathologies including cancer and is therefore a potential therapeutic target [1]. Reactive oxygen species (ROS) are generated during normal cellular metabolism, but despite anti-oxidant mechanisms they can accumulate during ageing and during cancer development leading to oxidative stress [2]. We are interested in the interplay between reactive oxygen species and the DNA damage response in cancer [reviewed in 3]. The DNA damage response (DDR) is a conserved mechanism which limits mutations and genome instability following cellular exposure to endogenous and exogenous DNA damaging agents. DDR components recognise DNA damage and activate DNA repair pathways [4]. There is evidence that H2AX, a key DDR protein, plays a role in ROS generation [5]. H2AX is a histone protein which is a component of chromatin. It is phosphorylated on Ser 139 in response to DNA damage (ɣH2AX) and plays a key early role in the recruitment of key DDR mediators to damage sites [6]. The maintenance of genome stability is very important to prevent disease, including the development of cancer. Understanding these mechanisms is the basis for disease diagnosis and future improvements in treatment. ɣH2AX and DDR activation will be explored in mouse keratinocytes which are lacking Rac1. Ear skin treated with croton oil, as a model of contact dermatitis, will be compared to vehicle treated control in both wild-type and Rac1 null tissue. Previous work with this model has shown a requirement for Rac1 in hair follicle integrity [7]. Also, keratinocytes lacking Rac1 show increased sensitivity to inflammatory stimuli [8] and a resulting changes in the dermal matrix [9].

References

[1] Christian Bailly C et al., (2022), Rac1 as a therapeutic anticancer target: Promises and limitations. Biochemical Pharmacology. Volume 203.

[2] de Almeida AJPO, et al,. (2022) ROS: Basic Concepts, Sources, Cellular Signaling, and its Implications in Aging Pathways. Oxid Med Cell Longev. 2022 Oct 19;2022:1225578. doi: 10.1155/2022/1225578. PMID: 36312897; PMCID: PMC9605829.

[3] Srinivas US, et al., (2019). ROS and the DNA damage response in cancer. Redox Biol. 2019 Jul;25:101084.

[4] Ciccia A and Elledge SJ. (2010). The DNA damage response: making it safe to play with knives. Mol Cell. 2010 Oct 22;40(2):179-204.

[5] Kang MA et al.,(2012) DNA damage induces reactive oxygen species generation through the H2AX-Nox1/Rac1 pathway. Cell Death Dis. Jan 12;3(1):e249.

[6] Turinetto V and Giachino C (2015). Multiple facets of histone variant H2AX: a DNA double-strand-break marker with several biological functions. Nucleic Acids Res. Mar 11;43(5):2489-98.

[7] Chrostek A et al., (2006). Rac1 is crucial for hair follicle integrity but is not essential for maintenance of the epidermis. Mol Cell Biol. 26 (18): 6957-70.

[8] Pedersen E et al., (2012). RAC1 in keratinocytes regulates crosstalk to immune cells by Arp2/3-dependent control of STAT1. J Cell Sci. 125 (22):5379-90.

[9] Stanley A et al., (2016). Changes in dermal matrix in the absence of Rac1 in keratinocytes. J Anat. 228 (5):826-37.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Project outcomes

Proliferation in the epidermis was measured using Ki67 immunostaining in each mouse group. At least two animals were analysed per group and the percentage of positive nuclei in the epidermis is presented as percentage of the total (Figure 1). Keratinocyte proliferation was highest in the vehicle control treated groups, with 30.5% positive nuclei in the epidermis of WT (con) and 29.5% in Rac 1 KO. Treatment with ICD reduced the Ki67 index in WT to 14.4%, however, the Rac1 KO seemed protective with 28.3% positive nuclei following ICD treatment. The data was analysed by ANOVA and no statistically significant differences were found between the animal groups.

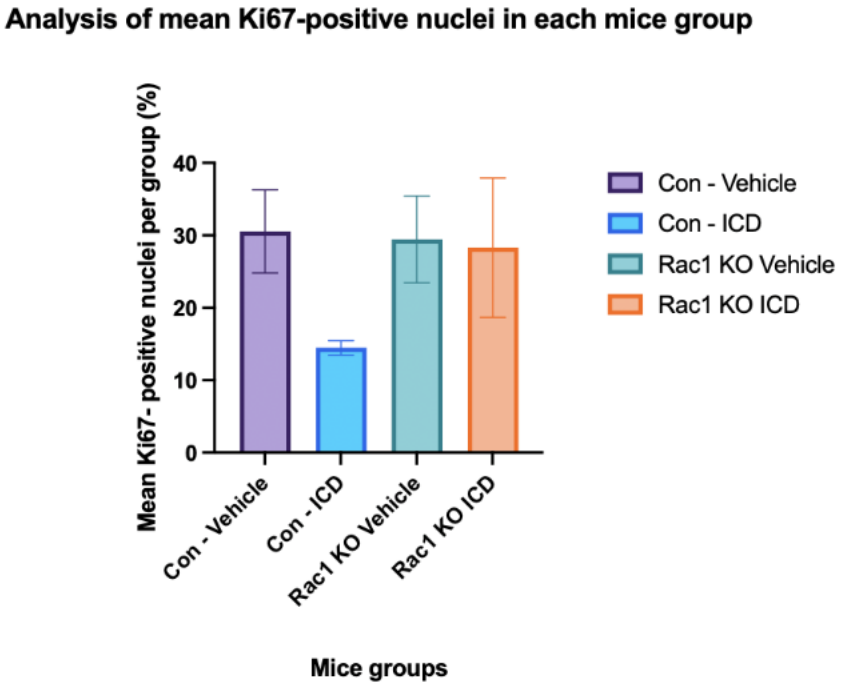

***Figure 1**: Quantification of Ki67 positive nuclei in the mouse epidermis. Wild type (Con) animals and animals lacking Rac1 expression in keratinocytes (Rac1 KO) were either treated with a vehicle control or 2% croton oil (ICD) for 8 hrs before fixation. Data is presented as mean +/- SDV.*

Collagen in the mouse skin sections was quantified following Masson's trichrome staining. QuPath software was used to segregate the tissue components and the percentage of collagen within the tissue was determined for at least two animals in each group (Figure 2). The highest average collagen deposition was seen in the Rac 1 knockout animals following croton oil treatment, however when tested via ANOVA no statistically significant differences were detected between the groups.

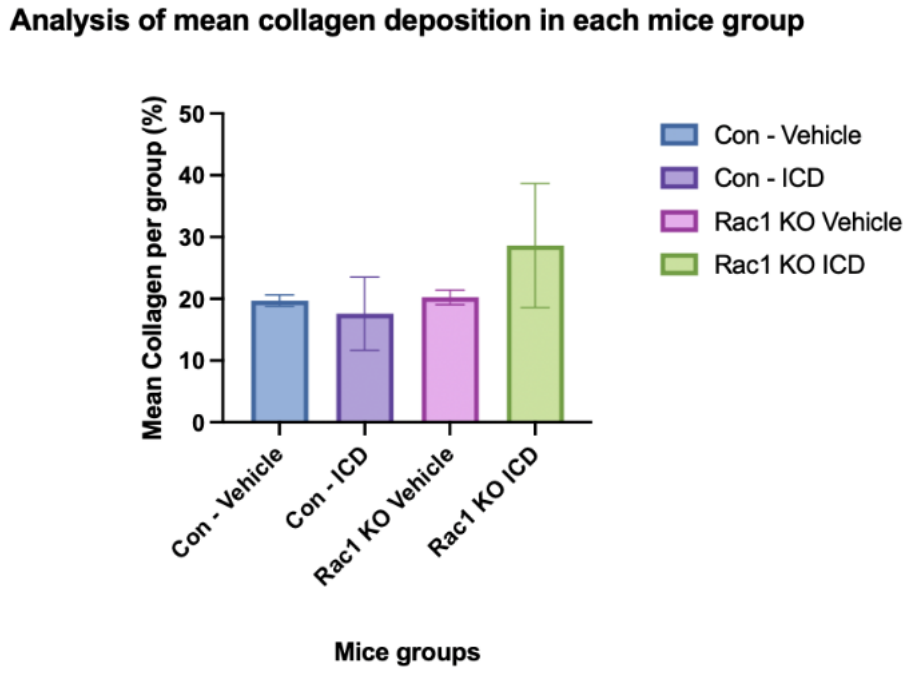

Figure 2*: Quantification of collagen in the mouse skin. Wild type (Con) animals and animals lacking Rac1 expression in keratinocytes (Rac1 KO) were either treated with a vehicle control or 2% croton oil (ICD) for 8 hrs before fixation. Data is presented as mean +/- SDV.*

The percentage of ɣH2AX positive nuclei in the epidermis was quantified (data not shown). The highest levels were detected in tissues lacking Rac1 following treatment with croton oil.

These findings suggest the deletion of Rac1 plays a protective role in sustaining keratinocyte proliferation and activation of the DNA damage response and further increasing collagen deposition in the epidermis following inflammation. The lack of significance observed may be due to the short timeframe of 8hrs croton oil treatment or due to the fact that Rac1 depletion is restricted to keratinocytes only.

Experience gained

This project has allowed me to obtain a valuable training experience in both wet-lab and digital pathology analysis techniques. I was able to develop proficiency in histology techniques through staining methods such as Masson's trichrome for collagen detection and Ki67 immunostaining. Learning how to optimise staining methods and follow the protocol with precision to obtain results allowed me to gain insight into how, from a technical standpoint, small changes seem to affect the quality of results greatly. A key part of the project was the use of QuPath, an analysis software which I upload digital microscopy images. This allowed me to gain confidence in scanning slides, applying regions of interest on samples and obtaining quantitative data. Gaining expertise in the area of digital pathology has allowed me to have a transferable skill which will be invaluable in the future.

This summer project has strengthened my ability to critically evaluate data and to successfully present my findings. While differences between the mice groups was measured which suggested effects of Rac1 loss, when statistical testing was carried out we found no statistically significant difference between the groups. Learning how to interpret results whilst carefully balancing biological relevance with statistical outcomes has helped me gain a multi-faceted approach to data interpretation. In addition to this, collaboration between myself, my research peer and my supervisor provided insights into my project, including how to present data, discuss further actions and

incorporating feedback. This was critical in helping me communicate my ideas clearly and learning how to present my research in a nuanced way along with helping me craft next steps for my project. An essential part of this research experience was being able to manage my work independently and having full rein over planning experiments, setting deadlines for staining and consistently documenting my work. Lastly, this project has allowed me to gain interpersonal skills, which will allow me to carry out further health research in the future.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Summer

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

The GTPase Rac1 is involved in regulating cellular processes, including proliferation, reactive oxygen species production and responses to inflammation. To further explore the role of this protein in skin, we used a mouse model lacking Rac1 expression in keratinocytes. We investigated keratinocyte proliferation and collagen modeling as a response to irritant contact dermatitis. Skin samples which had been previously fixed, processed and sectioned were available for this research. Rac1 keratinocyte specific knock out (KO) and wild-type mice (WT) were treated on the right ear with 2% croton oil for 8 hours as a model of irritant contact dermatitis (ICD) and treated on the left ear with a vehicle control (VEH). Samples from two animals were available for each of the four groups; WT-VEH, WT-ICD, Rac1 KO-VEH, Rac1 KO-ICD. Masson's trichrome staining was used to assess collagen remodeling and we explored the impact on proliferation by Ki67 immunohistochemistry. Slides were scanned, evaluated and quantified using Qu Path software. The mean area of collagen relative to the total tissue was calculated as a percentage. The WT-VEH group had slightly less collagen (19.7%) compared to Rac 1 KO-VEH (20.3%). Collagen deposition decreased following ICD treatment in the control (WT-ICD, 17.6%), but was the highest of all groups in the Rac1 KO-ICD (23.7%). No statistically significant differences were detected between the groups based on an ANOVA. Ki-67 analysis revealed that keratinocyte proliferation was highest in the control groups, with 30.5% positive nuclei in the epidermis of WT-VEH and 29.5% in Rac 1 KO-VEH. Treatment with ICD reduced the Ki67 index in WT to 14.4%, however, the Rac1 KO seemed protective with 28.3% positive nuclei following ICD treatment. These findings suggest the deletion of Rac1 plays a protective role in sustaining keratinocyte proliferation and further increasing collagen deposition in the epidermis following inflammation.

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

This project aimed to investigate the role of a critical cellular component called Rac1 in skin tissue. Rac1 acts like a molecular switch controlling many important functions such as cell structure and movement, cell growth and division as well as communication and immune function. We investigated the impact of the absence of this protein in a mouse model where the Rac1 protein is not expressed in skin cells called keratinocytes. The animals were treated with croton oil for 8 hrs, which causes local inflammation and is a model for irritant contact dermatitis. We investigated the impact of Rac1 loss on cell proliferation, the DNA damage response and on collagen deposition. Skin cells normally have a great capacity to divide and replace damaged tissue. Collagen is a key component of the dermis,

providing structural support and required for wound healing. We measured expression of the proliferation marker Ki67 and the DNA damage response marker 53BP1 in the epidermis. In addition, we quantified collagen deposition in the tissue.
We found no difference in the number of Ki67 positive nuclei when comparing Rac1 knockout animals with wild-type controls. We observed a decrease in Ki67 expression following croton oil treatment in control animals, which appeared to be rescued in the absence of Rac1. Surprisingly, we did not see any differences in collagen deposition following treatment with croton oil or in animals lacking Rac1.
We hope to understand more about the interplay between Rac1 and the DNA damage response with further experimentation.

Other comments: (no more than 300 words)

The original plan for the project was to focus on analysis of the DNA damage response. Unfortunately, we found it very difficult to optimse one of the markers, 53BP1 on the skin tissue. Another student working in the lab at the time was working on analysis of gamma-H2AX and this data will be included in the final poster presentation as part of a more complete story.

The original experimental design included 4 animals in each experimental group. Unfotuntely, for one of the groups two tissue blocks were missing. This resulted in a lower n number than initially planned.

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