Medicine | UNIVERSITY OF Southampton

UV-induced CD39 expression promotes DNA damage and development of cutaneous squamous cell carcinoma

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Introduction

- Cutaneous squamous cell carcinoma (cSCC) results from UV-induced DNA damage and immunosuppression.
- Regulatory T cells (Tregs), which are present in cSCC, can suppress immune responses via CD39 (ENTPD1), which catalyses the generation of adenosine from extracellular ATP.
- The purpose of the current study was to investigate the role of CD39 in UV-induced epidermal DNA damage and cSCC.

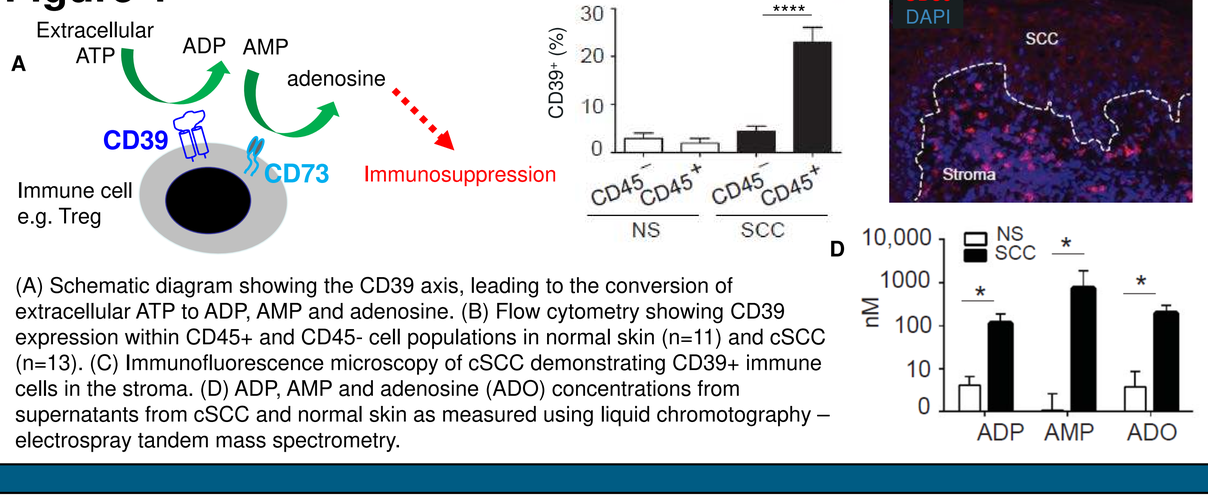
Methods

- Flow cytometry was conducted on immune cells isolated from cSCC, peripheral blood and normal skin.
- Immunofluorescence microscopy was performed on frozen cSCC sections.
- Immunohistochemistry was performed on formalin-fixed paraffin embedded primary cSCCs which had not metastasised after 5 years and primary cSCCs which did metastasise.
- Wild type and IL27RA knockout mice were irradiated with 100mJ/cm^2 UVB and *Entpd1* measured by qPCR.
- Normal human keratinocytes were stimulated with supernatants from anti-CD3 activated skin resident T cells primed with/without 100 ng/ml rhIL-27 and irradiated with 10mJ/cm^2 UVB prior to immunofluorescence staining for γH2AX as a marker of DNA damage.
- Murine ears were excised, irradiated with 100mJ/cm^2 UVB in culture and epidermal sheets were obtained for immunofluorescence staining for γH2AX.
- Keratinocytes were cultured with 10 μM adenosine and microarray was performed for gene expression analysis.

Results

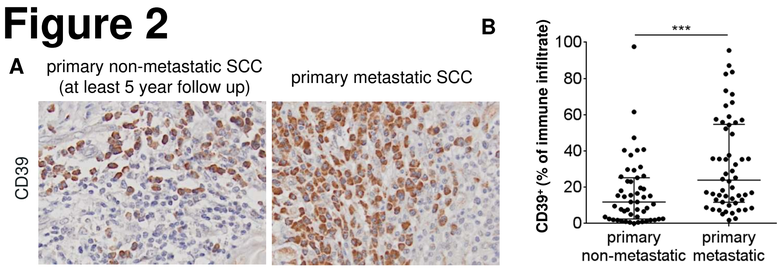
- CD39 expression is increased in human cSCC compared to normal skin (figure 1).
- Increased CD39 expression in primary cSCCs is associated with metastasis (figure 2).
- CD39 is upregulated on T cells in cSCC and is highly expressed by Tregs (figure 3).
- UV-induced CD39 expression in murine skin is IL-27 dependent and IL-27 is present on CD14+, CD207+ (Langerin) and CD209+ (DCSIGN) antigen presenting cells in human cSCC (figure 4).
- IL-27 signalling suppresses UV-induced DNA damage repair (shown by γH2AX staining) in human keratinocytes and murine skin (figure 5).
- γH2AX is expressed in human cSCCs and perilesional skin infiltrated with CD39+ immune cells (figure 6).
- Adenosine downregulates *NAP1L2*, a nucleosome assembly protein required for DNA repair (figure 7).

Figure 1



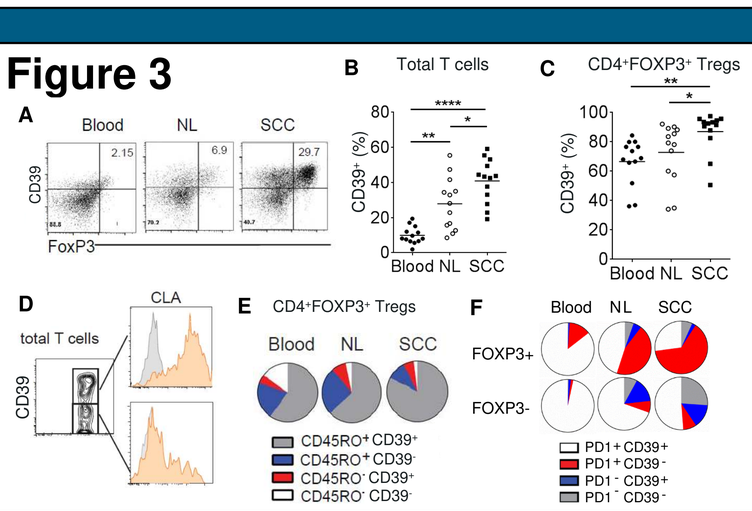
(A) Schematic diagram showing the CD39 axis, leading to the conversion of extracellular ATP to ADP, AMP and adenosine. (B) Flow cytometry showing CD39 expression within CD45+ and CD45- cell populations in normal skin (n=11) and cSCC (n=13). (C) Immunofluorescence microscopy of cSCC demonstrating CD39+ immune cells in the stroma. (D) ADP, AMP and adenosine (ADO) concentrations from supernatants from cSCC and normal skin as measured using liquid chromotography – electrospray tandem mass spectrometry.

Figure 2



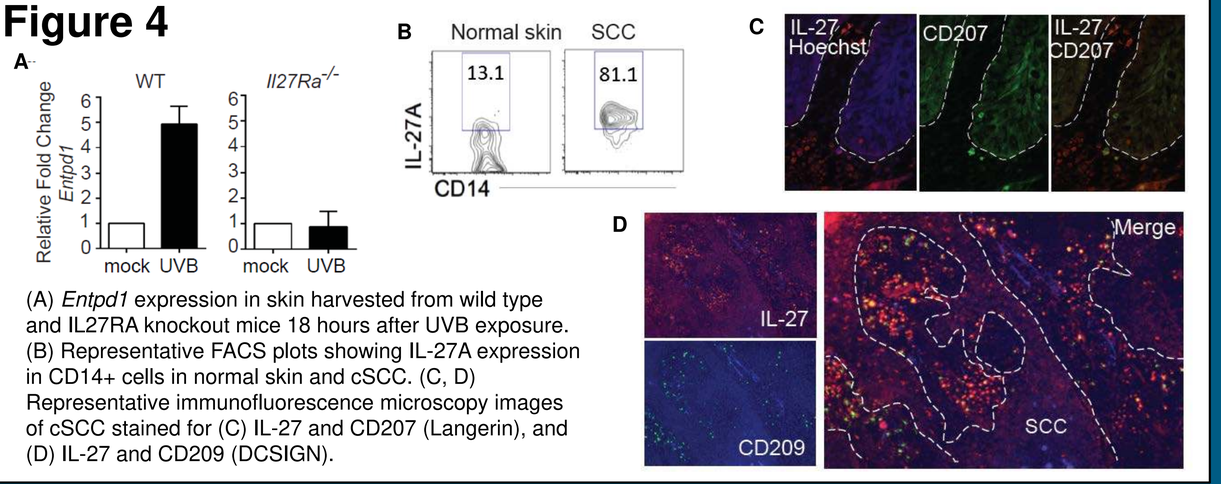
(A) Representative immunohistochemistry images of cSCC stained for CD39. (B) Percentages of tumoral immune cells that are CD39$^+$ in primary cSCCs that did not metastasise (P-NM, n=51) and primary cSCCs that metastasised (P-M, n=54).

Figure 3



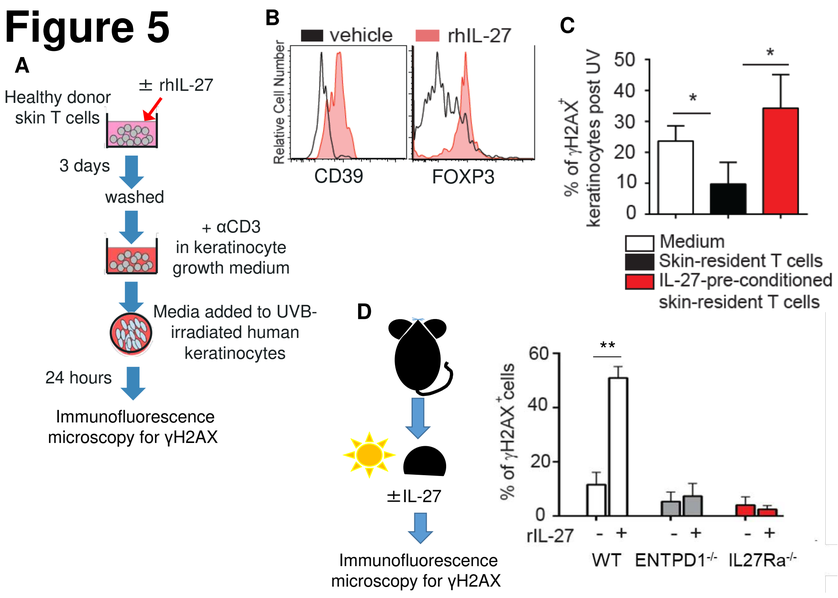
(A) Representative FACS plots showing expression of FOXP3 and CD39 on CD3+ T cells from blood, non-lesional skin and cSCC. (B, C) CD39+ frequencies in the (B) total CD3+ T cell and (C) CD4+FOXP3+ Treg populations in blood, non-lesional skin and cSCC (n=13). (D) Representative FACS plot showing expression of cutaneous lymphocyte antigen (CLA, a skin homing marker) within the CD39+ and CD39- T cell populations in cSCC. (E) CD45RO and CD39 coexpression in CD4+FOXP3+ Tregs in blood, non-lesional skin and cSCC (n=7). (F) PD-1 and CD39 co-expression in FOXP3+ Tregs and FOXP3- T cells in blood, non-lesional skin and cSCC (n=8).

Figure 4



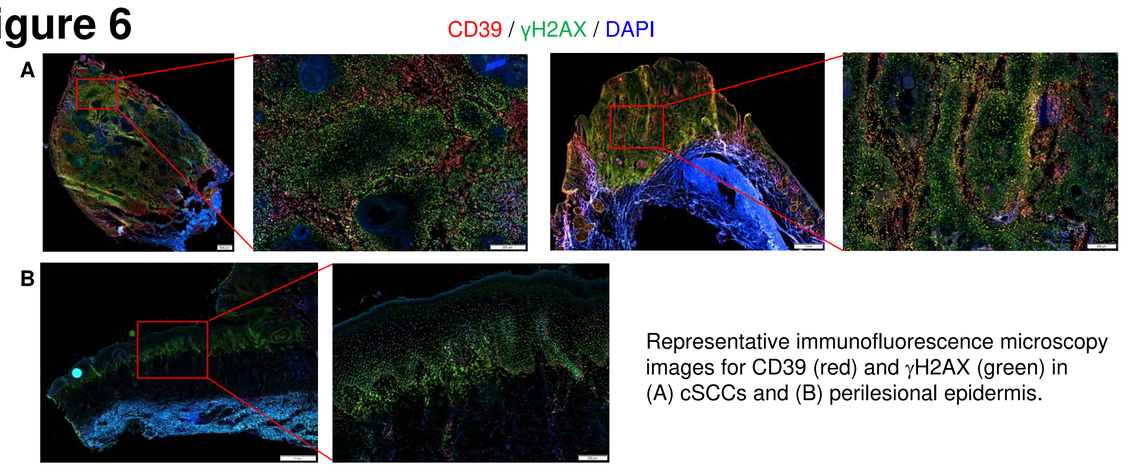
(A) *Entpd1* expression in skin harvested from wild type and IL27RA knockout mice 18 hours after UVB exposure. (B) Representative FACS plots showing IL-27A expression in CD14+ cells in normal skin and cSCC. (C, D) Representative immunofluorescence microscopy images of cSCC stained for (C) IL-27 and CD207 (Langerin), and (D) IL-27 and CD209 (DCSIGN).

Figure 5



(A) Schematic diagram for experiments performed in (B) and (C). (B) CD39 and FOXP3 expression in healthy donor skin T cells after stimulation with rhIL-27 or vehicle. (C) Human keratinocytes were cultured with medium (n=3), medium from anti-CD3 stimulated skin-resident T cells (n=4), or medium from IL-27 primed and anti-CD3 stimulated skin-resident T cells (n=3) followed by UVB irradiation and measurement of DNA damage by immunofluorescence quantification of γH2AX after 24 hours. (D) Ears from WT, ENTPD1$^{-/-}$, and IL27Ra$^{-/-}$ mice were irradiated with UVB and treated with or without IL-27. Immunofluorescence quantification of γH2AX$^+$ in epidermal sheets after 24 hours was performed (n = 3 per group).

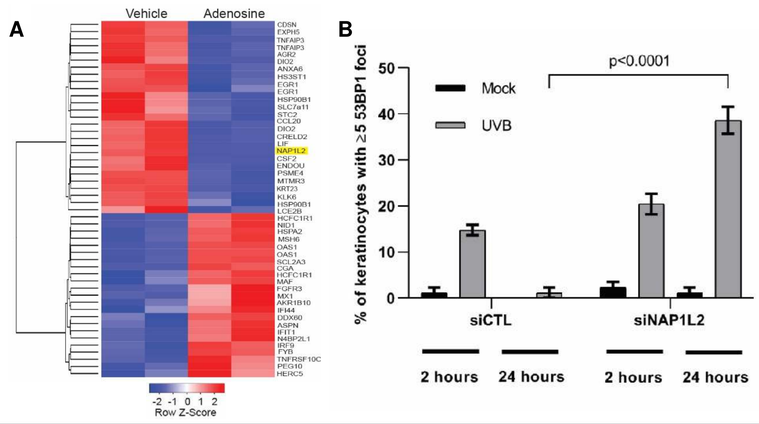
Figure 6



Representative immunofluorescence microscopy images for CD39 (red) and γH2AX (green) in (A) cSCCs and (B) perilesional epidermis.

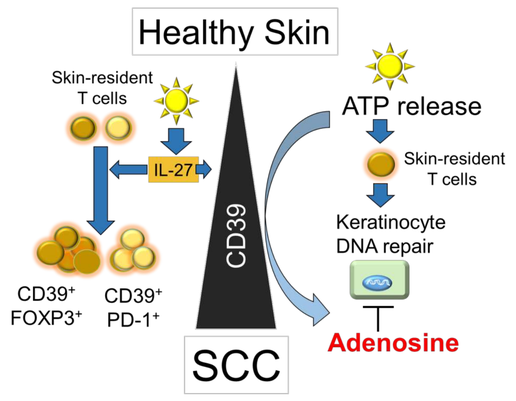
Figure 7

(A) Heat map of the top 49 differentially expressed genes in normal human keratinocytes stimulated with vehicle or 10 μM adenosine, showing downregulation of *NAP1L2*.
(B) Measurement of DNA damage (based on cells with ≥5 53BP1 foci) 2 and 24 hours after UVB irradiation of keratinocytes with or without siRNA knockdown of *NAP1L2* (n=4 for all conditions).



Conclusions

- CD39 is highly expressed by Tregs in cSCC and CD39 and adenosine are upregulated in cSCC compared to normal skin.
- Higher CD39 expression in primary cSCCs is associated with metastasis.
- UV radiation induces CD39 in an IL-27 dependent manner.
- Adenosine downregulates the expression of NAP1L2, which is important for repair of UV-induced DNA damage.
- These results indicate a key role for CD39 in UV-induced DNA damage and promoting cSCC development and metastasis.



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