Poster 403

Increased Expression of Oncostatin M Receptor β in Chronic Pruritic Diseases

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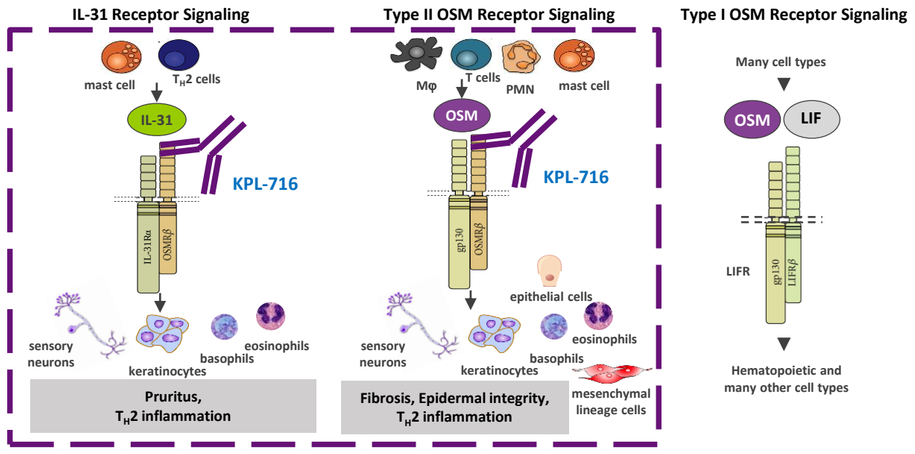
BACKGROUND

- Chronic Pruritus (CP), a common symptom in dermatological conditions, is often intractable and negatively impacts patient quality of life[1]
- A significant unmet medical need remains in CP, as current treatment options are limited
  - The Global Burden of Disease project listed pruritus as one of the 50 most common interdisciplinary symptoms leading to high burden levels[2]
  - CP, defined as pruritus lasting longer than 6 weeks, affects almost one fifth of the general population, leading to great impairment of quality of life[3]
- Recent evidence suggests that the cytokine interleukin (IL)-31 is a major driver of pruritic responses[4,5,6]
- Notably, IL-31 mRNA has been reported to be significantly overexpressed in pruritic atopic skin compared with nonpruritic psoriatic skin[7]
  - Highest IL-31 levels were detected in Prurigo Nodularis, one of the most pruritic forms of chronic skin inflammation[7]
- IL-31 and Oncostatin M (OSM) signal through the common receptor subunit, OSM Receptor β (OSMRβ) (**Figure 1**)
- OSMRβ gain-of-function mutations have been shown to be associated with chronic pruritus and deposition of epidermal keratin filaments in the dermis in cutaneous forms of amyloidosis[8]
- In this study, we examined the expression of IL-31 and OSM cytokines and IL-31-Rα and OSMRβ receptor subunits in CP skin compared to healthy skin at the mRNA and protein level

Figure 1: KPL-716 Impact on IL-31 and OSM signaling[9]



IL = interleukin, LIF, leukemia inhibitory factor, OSM = oncostatin M, T_H2 = type 2 T helper cell.
Image adapted from Richards C. ISRN Inflammation. 2013;2013:1-23.

METHODS

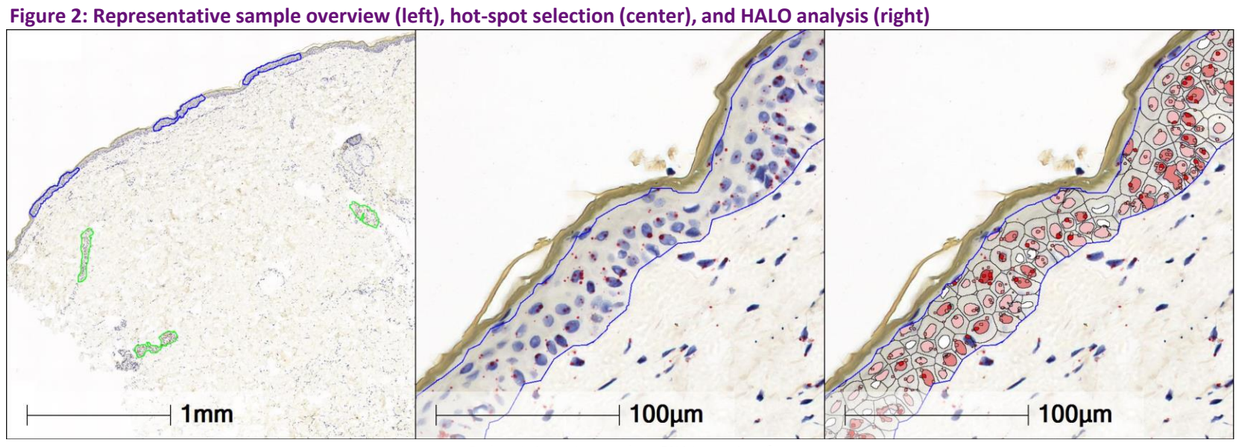
Samples Used for Analysis

- Archived FFPE skin punch biopsies from patients with Chronic Idiopathic Urticaria (CIU), Chronic Idiopathic Pruritus (CIP), Lichen Simplex Chronicus (LSC) and Lichen Planus (LP) (n=10-16 per cohort) and from healthy controls (n=8) were selected for analysis; samples had heterogeneous disease severities and clinical histories
- Samples were obtained from University of Florida, University of California San Francisco, and Washington University in accordance with local IRB-approved protocols for archived sample analysis; identical CIU, LP and LSC samples were used for RNAscope® and NanoString analysis; identical CIP samples were for RNAscope®, NanoString, and IHC analysis; an independent set of CIU and LP samples was used for IHC analysis

mRNA Expression by RNAScope®

- RNAscope® *in situ* hybridization was performed on 5 µm sections of FFPE skin biopsies from patients with CP to detect mRNA transcripts associated with IL-31 and OSMRβ (Advanced Cell Diagnostics, CA, USA; ACD); each mRNA transcript is represented as a single red dot
- Transcript analysis was performed using the hot-spot method. For each sample analyzed, a pathologist identified 3 regions of interest (each ~18,500 ±500µm^2) in each of the dermis and epidermis, based on zones (hot-spots) best representing histological features of the disease; in case of biopsies showing minimal disease, hot-spots were selected based on highest percentage of cells positive for the target of interest. The same hot-spots were used for both IL-31 and OSMRβ analyses
- To evaluate heterogeneity in marker expression, H-Score calculation was performed in HALO® (Indica Labs, NM, USA) ISH image analysis module. Using all 3 hot-spots in each region, a single score was assigned to either the dermis or epidermis region. For this calculation, cells were classified into 1 of 5 bins based on the number of dots per cell by the software. The H-score for each region was then calculated using a weighted formula algorithm (ACD) based on the percentage of cells positive in each bin and provided on a scale of 0–400 (**Figure 2**)

Figure 2: Representative sample overview (left), hot-spot selection (center), and HALO analysis (right)



Blue Lines = Epidermal hot spot regions analyzed
Green Lines = Dermal hot spot regions analyzed

Red spots in center panel = mRNA transcript of target receptor

Pink/red nuclei in right panel = Cells expressing target receptor

mRNA Expression by NanoString

- RNA isolated from the samples (8 slices of 5 µm thickness) was analyzed for IL-31, OSM, IL-31-Rα and OSMRβ mRNA expression by NanoString (Core Diagnostics, CA, USA)
- Absolute transcript counts were normalized to housekeeping genes using nSolver software (NanoString Technologies, Seattle, WA, USA)

Protein Expression by Immunohistochemistry

- FFPE blocks were prepared from fresh skin biopsies according to standard practices
- Tissue sections were cut (5 µm), dewaxed and rehydrated using standard protocols and then incubated at 4 °C overnight with primary antibodies against IL-31, OSM, IL-31-Rα and OSMRβ. The slides were washed and incubated with HRP conjugated species specific secondary antibodies
- Slides were developed with 3,3'-Diaminobenzidine (DAB) chromagen solution and counter stained with hematoxylin. Staining was evaluated by grading the intensity of staining in 3 representative "hot spots" on a 0-4 scale and then the average score and standard error for the epidermis and dermis was calculated for each biopsy

RESULTS

- OSMRβ mRNA transcript levels and protein levels were significantly increased in the epidermis, but not the dermis, of all diseases tested compared to healthy controls (**Figure 3** and **Figure 4**)
  - OSMRβ mRNA transcript levels (HALO H-scores) were significantly higher in epidermis of CIU, CIP, LSC, and LP skin compared to healthy skin
  - Normalized OSMRβ count (NanoString) was significantly higher by 1.5-fold in CIP and 2.5-fold in CIU patient skin compared to healthy skin
  - OSMRβ protein levels were significantly higher (2-3-fold) in epidermis of CIU, LP, and CIP samples, and the dermis of CIU (1.7-fold) and LP (5.4-fold), compared to healthy skin
- IL-31 mRNA transcript expression and protein levels were increased in the epidermis of LP samples compared to healthy skin (**Figure 5**)
  - Mean IL-31 mRNA transcript levels (HALO H-scores) trended higher (2-3-fold) in epidermis of CIU, CIP and LP samples, and in the dermis of CIP (1.5-fold); similar trends were not observed in the epidermis of LSC and the dermis of LP and LSC
  - IL-31 counts were below limit of detection by NanoString
  - IL-31 protein levels were significantly higher (3-fold) in epidermis of LP samples compared to healthy skin, but were similar in CIU and CIP
- OSM counts were below limit of detection by NanoString; OSM protein levels were significantly elevated in the epidermis of LP samples but not in CIU or CIP samples compared to healthy skin controls (**Figure 6A**)
- IL- 31Rα counts were below limit of detection by NanoString; IL-31Rα protein levels did not show significant changes in LP, CIU or CIP compared to healthy skin controls (**Figure 6B**)
- Protein levels in LSC samples were not tested by IHC for any markers evaluated

RESULTS, continued

Figure 3: Representative OSMRβ RNAscope® Staining (Healthy vs. CIP epidermis)

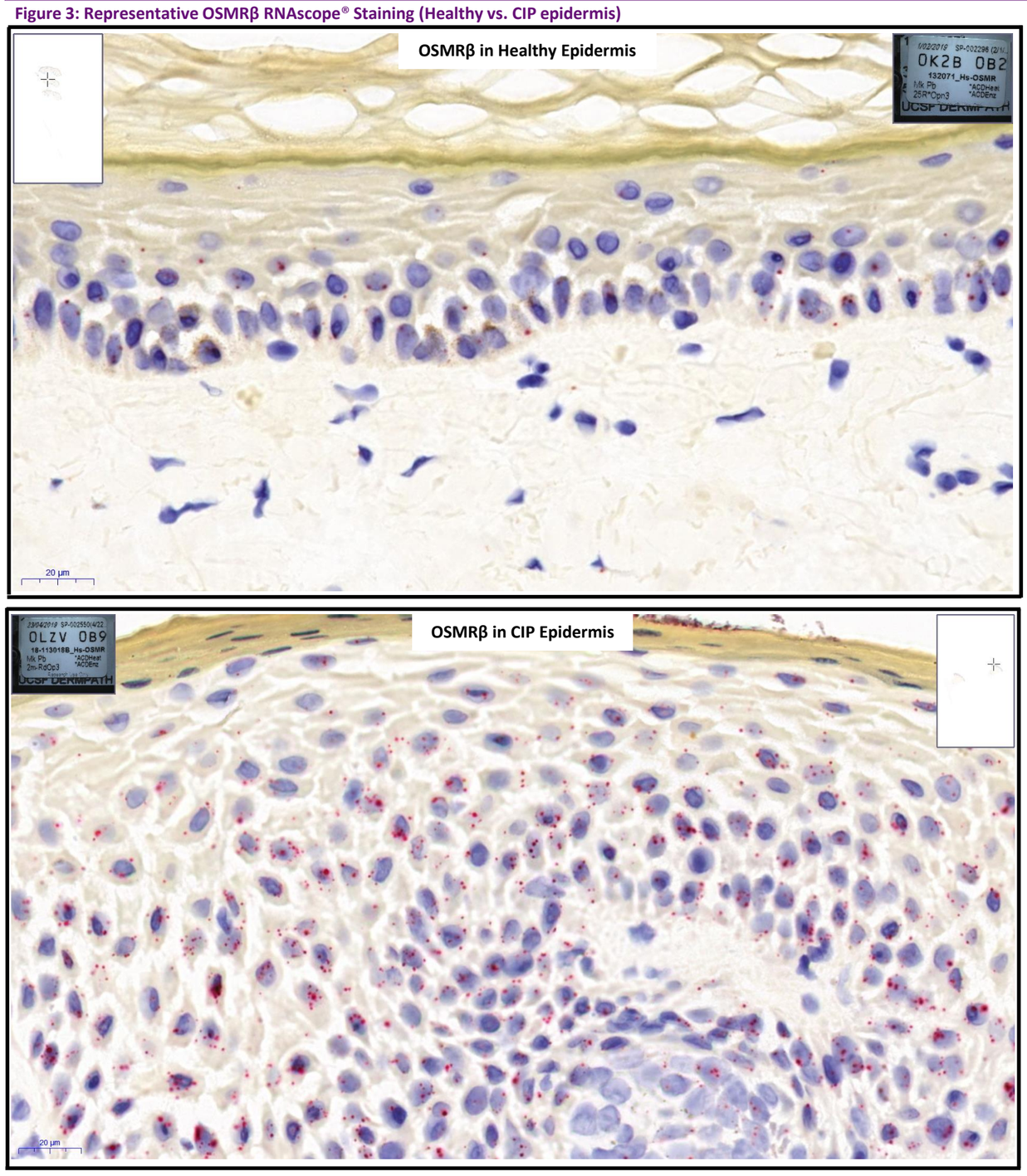
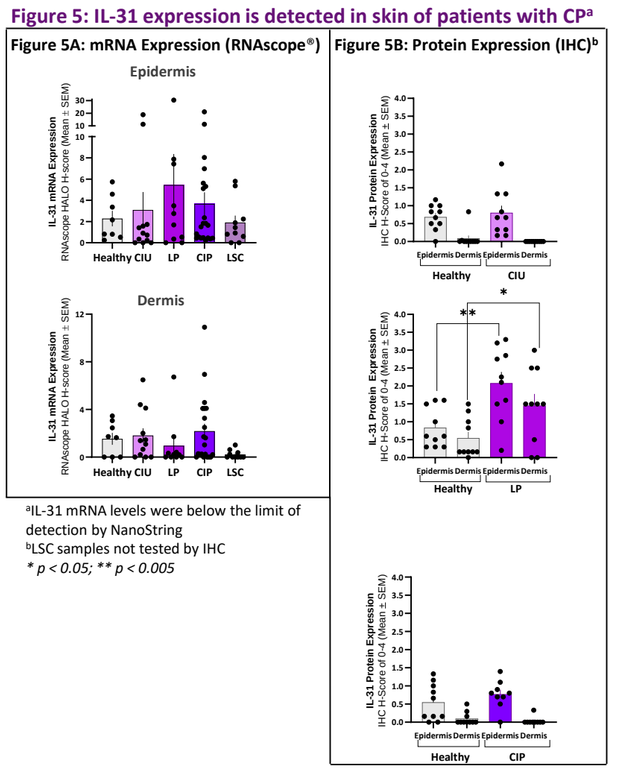


Figure 4: OSMRβ expression is significantly higher in skin of patients with CP compared to healthy skin

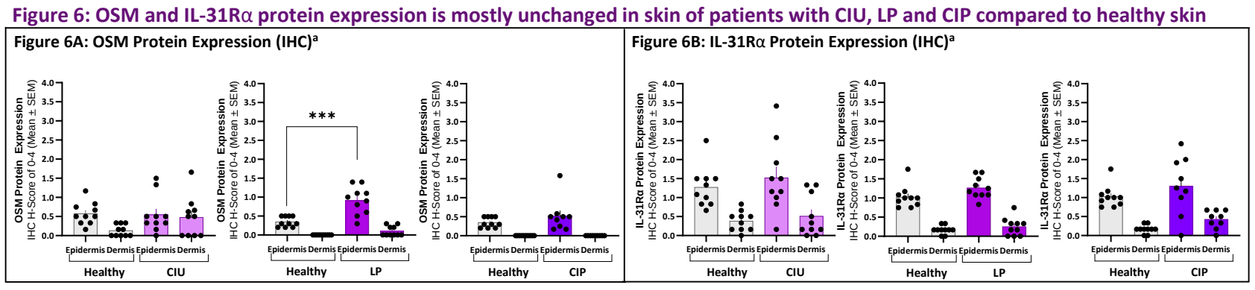
[a]LSC samples not tested by IHC
** p < 0.05; ** p < 0.005*

Figure 5: IL-31 expression is detected in skin of patients with CP[a]



[a]IL-31 mRNA levels were below the limit of detection by NanoString
[b]LSC samples not tested by IHC
** p < 0.05; ** p < 0.005*

Figure 6: OSM and IL-31Rα protein expression is mostly unchanged in skin of patients with CIU, LP and CIP compared to healthy skin



[a]OSM and IL-31Rα were not analyzed by RNAscope®; OSM and IL-31Rα were below the limit of detection by NanoString
**** p < 0.0005*

CONCLUSIONS

- Increased mRNA transcript levels and protein levels of OSMRβ, the signaling subunit of the IL-31 and OSM Type 2 receptors, in FFPE biopsies from patients with CIU, CIP, LP and LSC, relative to healthy control skin samples, suggest the IL-31/OSM signaling axis is associated with these pruritic diseases
- Elevated levels of OSMRβ mRNA and protein observed in regions of inflammatory infiltrate of all chronic pruritic diseases tested relative to healthy controls suggests that the OSMRβ axis may be active in, and contributing to, these skin disorders, particularly in view that OSM and IL-31 mRNA and protein are present in each disease evaluated for these cytokines
- Even though IL-31 was detected in all healthy and disease samples, the significance of this expression relative to the contributions of IL-31, IL-31Rα vs. OSM in OSMRβ-driven pathways in chronic pruritic diseases needs to be further explored in research and clinical studies
- These data provide a rationale for correlating the presence, or absence, of a biomarker signature and targeting the IL-31/OSM axis with KPL-716, a mAb targeting OSMRβ, for potential therapeutic benefit in chronic pruritic diseases
- KPL-716 is currently being evaluated for attenuation of itch across multiple pruritus diseases in Phase 2 clinical studies (NCT03858634, NCT03816891)

References

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Disclosures

This project was funded by an unrestricted grant from Kiniksa Pharmaceuticals, Ltd. Medical writing assistance was provided by Emily Plummer, PhD, who is employed by Kiniksa Pharmaceuticals, Corp.

We would like to thank the team at Advanced Cell Diagnostics for their assistance in data analysis and image acquisition.