Good correlation of a murine model of oxazolone-induced chronic dermatitis with major gene hallmarks of atopic dermatitis in human patients

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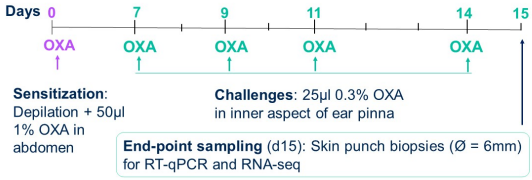
Introduction

- Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease characterized by pruritus and eczematous skin lesions.
- We had previously characterized and pharmacologically validated a model of dermatitis, induced by repeated topical applications of oxazolone (OXA) in the ears of sensitized mice. Ear skin inflammation, redness and histological changes typical of AD were reduced by commercial formulations of standards of care.
- Our aim was to analyze the similarity of the gene expression signature of the OXA model in mice and the human data from clinical studies.
- We performed RNA-seq analysis, as well as qPCR of a set of relevant genes in human AD. To assess the transferability of our model to human, we calculated a similarity score (Pearson correlation) between the gene expression from our RNA-seq results with previously published human transcriptomic data in AD and psoriasis (PS). Also, we identified upregulated pathways by GSEA enrichment analysis.
- Overall, this model displays great resemblance to human AD human, making it suitable for screening and molecule profiling purposes.

Material and Methods

- 6-week-old Balb/c mice were topically sensitized in the abdomen with 1% OXA in acetone:olive oil (4:1).
- 0.3% OXA was applied topically on the inner aspect of the right ear pinna on days 7, 9, 11 and 14 and 6-mm ear skin punches were collected on day 15.
- Non-sensitized mice (Healthy) were treated with vehicle only, following the same protocol as OXA mice.



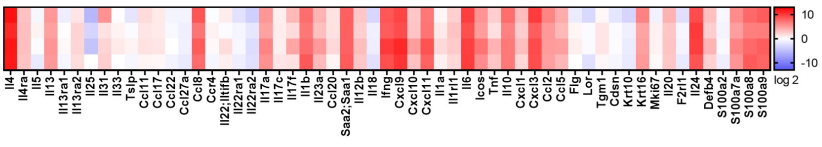
All *in vivo* experiments were approved and monitored by the Animal Ethical committee of Almirall following ARRIVE guidelines and in accordance with EU Directive 2010/63/EU.

- TaqMan assays were used for qRT-PCR. Expression of each transcript calculated relative to the housekeeping gene, β-actin (Actb). Fold change relative to Healthy by the $2^{-\Delta\Delta Ct}$ method.
- RNA sequencing was performed by Biogazelle (Belgium). QuantSeq 3' mRNA-Seq Library Prep Kit was used followed by Illumina single end sequencing with read length of 75 bp on the NextSeq 500 Sequencing System (Illumina). 20 million reads were obtained on average per sample
- RNA-seq fold-inductions were calculated between OXA and contralateral (non-challenged) ear or OXA and Healthy ear.

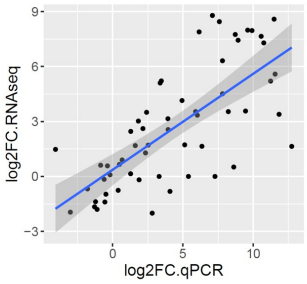
Skin markers gene expression and similarity analysis

Skin markers gene expression in ear (log_2 of fold *vs* Healthy)

qPCR results



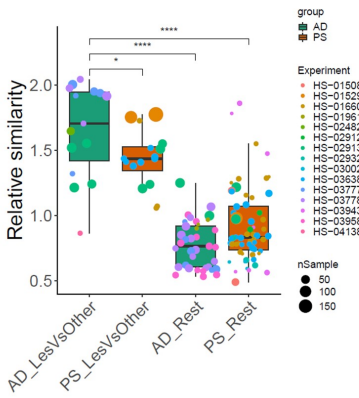
qPCR-RNA-seq correlation



- Key driver type 2 cytokines involved in the pathophysiology of the disease, IL-4, IL-13 and IL-31, were upregulated in the skin, along with cytokines related to type 1, 17 and 22 responses, which have been reported to be relevant in the chronic stages of the disease.
- Loricrin (Lor) was downregulated in the OXA-treated skin while there were no robust changes in filaggrin (Flg).
- Good correlation between qPCR gene expression and RNA-seq data is observed in the model.

Pearson correlation: similarity of OXA model in mice and human AD/PS

RNA-seq data

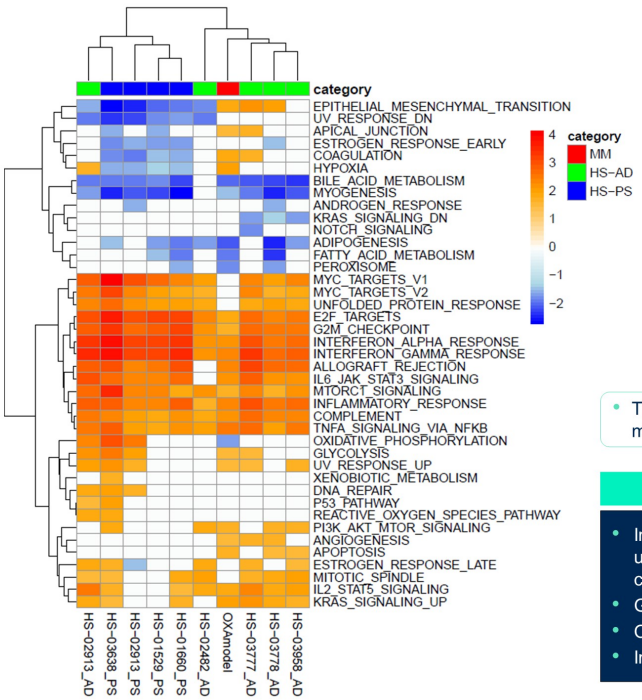


AD: atopic dermatitis
PS: psoriasis

- All fold changes derived from comparing Lesional vs Non-lesional/Healthy are labeled as LesVsOther. All the other comparisons (Non-lesional vs Healthy, Healthy vs Lesional…) are labeled as Rest.
- As expected, lesional vs non-lesional/healthy fold changes are more similar to OXA model fold changes, regardless of skin affection.
- For the fold changes between lesional vs non-lesional/healthy, OXA model shows greater significant similarity to the transcriptomic impact of human AD studies than for PS.

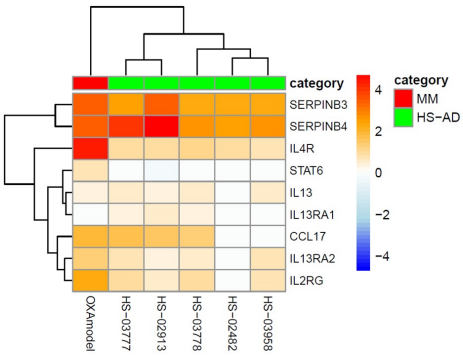
GSEA enrichment pathway analysis

Pathways involved in the OXA model and human disease



- Clustering with GSEA enrichment using MsigDB hallmarks shows OXA model tends to cluster more with AD human studies.
- Pathways like interferon, TNFα, complement or mTORC1 are consistently upregulated across all studies whereas upregulation of EMT or hypoxia is specific of OXA model and AD studies.
- IL-13 pathway genes shows consistent overexpression (except for STAT6) across AD human studies and OXA mouse model.

IL-13 pathway genes



- This analysis shows a great resemblance of our OXA model to AD human studies and pinpoints the specific mechanisms involved.

Conclusions

- In the OXA mouse model, cytokines related to AD signature in humans, such as IL-4, IL-13, IL-31, are upregulated in skin, as well as genes associated with the chronicity of the disease like type 1 and type 17 cytokines. In addition, epidermal barrier proteins like loricrin are reduced.
- Good correlation between qPCR gene expression and RNA-seq data is observed in the model.
- OXA model shows greater similarity to the transcriptomic signature of human AD studies than for PS.
- In the IL-13 pathway OXA overexpression of different genes correlates with AD human studies.

References:

1. Gavaldà A., et al., JID 2022. Vol 142 (12): S186
2. Liu B et al. FASEB J. 2013. Sep 27 (9):3549-63
3. Jegal J et al. Nutrients. 2017. 9(12). E1324
4. Lundberg R et al. Comp Med. 2012. 62 (5):371-80

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