Investigation of a set of dysregulated tear proteins from Sjögren's syndrome patients using in vitro models uncovers new insights and potential candidate biomarkers

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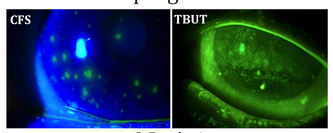
Background

Sjögren's syndrome (SS) is a systemic, chronic and progressive **autoimmune disease** characterized by a lymphocyte infiltration into exocrine glands. Its prevalence is 10 times higher in women. It causes a severe form of **aqueous deficient dry eye (DE)**. There is no effective treatment that can hinder progression.

The hallmarks of SS are:

- B cell activation, autoantibody production such as anti-SSA, anti-SSB
- xerostomia (dry mouth) and xerophthalmia (dry eyes)



C. Baudouin

Why do we need tear biomarkers for SS?

- ✓ Early diagnosis and management are challenging
- ✓ Serological markers are not specific to the disease
- ✓ Biomarker investigations from tear fluid are non invasive
- ✓ Proteomic investigations may reveal new insights in the pathophysiology and progression of the disease
- ✓ For more accurate and rapid diagnosis
- ✓ For patient stratification and follow-up

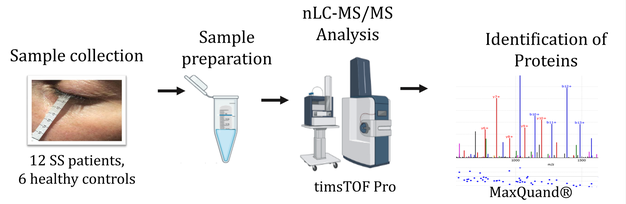
Aim of the study:

➔ To explore dysregulated tear proteins of SS patients and describe new targets of interest (TOI) as potential biomarker candidates using a bottom-up proteomic approach.

➔ Subsequent investigations of selected TOI using *in vitro* models to reveal their early response at cellular level.

Methodology

(1) Shotgun proteomic analysis of SS patients



➢ Proteomics analysis enabled **detection of dysregulated proteins** in SS patients (Limma statistical test)

(2) Selection of targets of interest (TOI)

Functional annotation and enrichment analysis
Bioinformatic tools
reactome
Metascape
Cytoscape

Selection criteria of 25 targets of interest (TOI)

- ✓ Significantly modulated in SS
- ✓ Cellular function
- ✓ Signaling pathways
- ✓ Protein interaction network
- ✓ Description in the literature
- ✓ Available validation tools

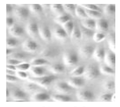
(3) Investigations of TOI in *in vitro* models

➢ Five in vitro models were used.

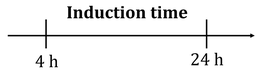
(1) Hyperosmolar (HO) model (500 mOsM) and Inflammation models (10 ng/ml)

(2) TNF-α, (3) IL-1β,

(4) IFN-γ, (5) IL-17A



Immortalized human corneal epithelial cells (HCEs)



Characterization of the in vitro models
- Release of proinflammatory cytokines (ELISA, n=6)
- ERK and JNK MAPK activation (Western blot, n=3)

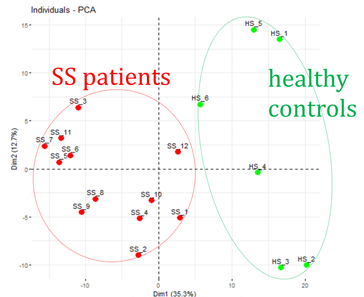
Gene expression analysis of TOI
- A customized plate was used to analyze gene expression of TOI (RT-qPCR, n=1)
- Gene expression of modulated TOI in the customized plate was repeated (RT-qPCR, n=3)

Protein expression analysis of TOI
- Western Blot
- Immunocytochemistry

Results

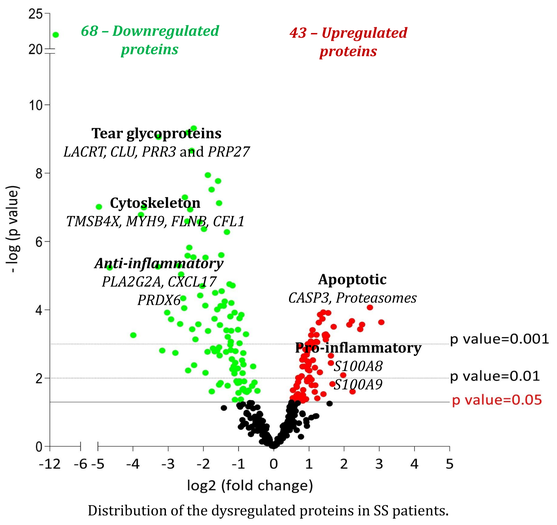
Part I: Dysregulated tear proteins in SS patients

➢ **Significant proteome segregation between the two groups**



Principal component analysis

➢ **111 dysregulated proteins in SS patients**



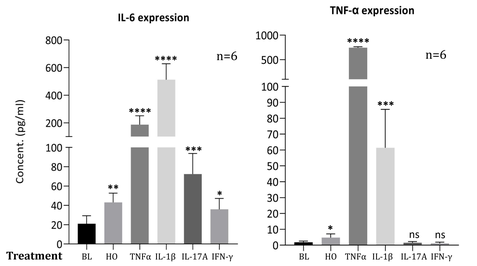
Distribution of the dysregulated proteins in SS patients.

➢ **25 targets of interest (TOI) were selected among 111 dysregulated proteins in SS patients**

Part II: Investigations of TOI in *in vitro DE* models

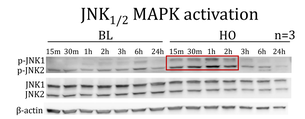
(1) Characterization of models

Release of pro-inflammatory cytokines after 24 h



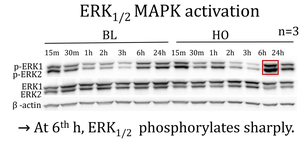
Release of IL-6 and TNF-α from *in vitro* models.

$JNK_{1/2}$ MAPK activation



→ $JNK_{1/2}$ start to phosphorylates at 15 min. or earlier, reaching its highest level at 1 h.

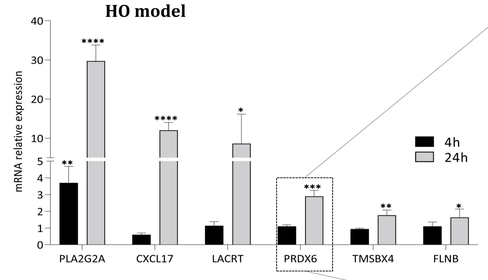
$ERK_{1/2}$ MAPK activation



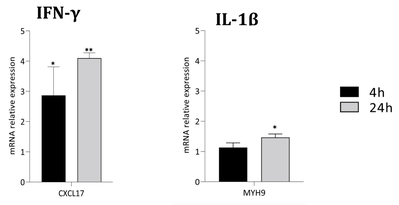
→ At 6th h, $ERK_{1/2}$ phosphorylates sharply.

(2) Gene expression of the TOI

HO model



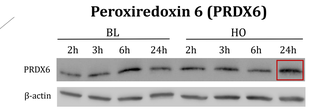
Inflammation models



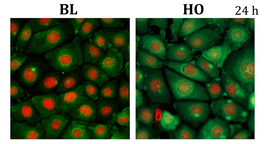
➢ Cellular survival mechanisms are activated as an acute response in vitro models.
➢ Cytoskeletal reorganization

(3) Protein expression of the TOI

Peroxiredoxin 6 (PRDX6)

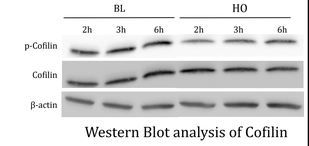


Western Blot analysis of PRDX6



PRDX6 staining in HCEs

Cofilin (CFL1)



Western Blot analysis of Cofilin

➢ **Under HO stimulation,** the expression of PRDX6 was increased and it localized more around the nucleus. Expression of CFL1 did not change, it was activated (dephosphorylated) under HO stress.

Discussion and conclusions

- ➢ The dysregulated proteins in SS patients were formed by the cytoskeleton, anti-inflammatory (PRDX6, PLA2G2A) and cell-protective proteins (CLU, LACRT). Inversely, overexpressed proteins were those involved in apoptosis (CASP3) and inflammation (S100A8, S100A9).
- ➢ HO treatment resulted in a significant increase in gene expressions of several TOI in HCEs.
- ➢ HO and IL-1β might play a role in cytoskeleton reorganization. MYH9 was modulated specifically by IL-1β exposure.
- ➢ Downregulated TOI in DE patients were overexpressed in some *in vitro* models as a response to acute inflammatory or hyperosmotic stress, a part of cell survival mechanisms.
- ➢ These TOI are probably exposed to the same stimulants in DE patients as *in vitro* models but in a chronic way and with additional factors.
- ➢ Protective cell survival mechanisms observed in these acute models might be impaired in chronic stages of DE.
- ➢ *In vitro* models reflect an early response formed by cell-survival mechanisms rather than the state of chronic DE pathology.
- ➢ Decreased protective mechanisms in DE patients might deteriorate the OS homeostasis and modulatory feedback.

References

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