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KRT14-EXPRESSION AS A MARKER OF LUNG REGENERATION/REPAIR

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Abstract — Currently, little is known about the cellular and molecular mechanisms that promote alveolar epithelial repair. A recent study suggests that Keratin14-expression could represent a marker of alveolar regeneration, since its expression was immunoistochemically detected in hyperplastic pneumocytes in human samples of ARDS-related DAD. Here we shown the analysis on lung samples of various human diseases in order to confirm the hyphotesis of the previous study. We used two molecular biology tecniques, Real Time Pcr and Western Blot, on autoptical and bioptical lung specimens. As a result, we demonstrate here that Krt14-expression is only found in pathological samples with alveolar regeneration or repair, while all normal control samples are Krt14 negative.

Index Terms — Lung, regeneration, repair, Keratin 14

1 BACKGROUND

To date, little is known about the mechanisms that promote lung regeneration and the identification of the adult stem cells in the alveolar region is still under debate. Acute Respiratory Distress Syndrome (ARDS) represents a model for the study of lung regeneration, since its natural history includes the resolution and repair of diffuse alveolar damage (DAD). A previous study of ours investigated in human samples of DAD the immunophenotypical profile of the regenerating pneumocytes, in order to identify possible markers of alveolar regeneration. The analysis revealed the expression of Keratin14 in a remarkably high percentage of proliferating tipe 2 pneumocytes in almost all the DAD samples, suggesting that Krt14 could represent a marker of alveolar regeneration.

2 OBJECTIVES

The main objective of this study is to confirm the Krt14-expression as a marker of lung regeneration/ repair, using molecular biology techniques on lung samples of various human diseases. The secondary objective is to lay the groundwork for studying biomechanisms that underlie the Krt14 expression in proliferating pneumocytes and its role in the process of regeneration.

3 APPROACH & METHODS

General approach: We collected 12 various lung diseases (ARDS, NSIP, interstitial lung disease) specimens and 7 normal lung control specimens. 17 of the 19 samples were autoptical lung samples and the additional two were in vivo pulmonary biopsies. All samples were frozen and stored at -80°C. We then performed Real Time Quantitative PCR, to asses mRNA Krt14 expression and Western Blot, to asses protein Krt14 expression.

Methods: Real Time PCR -
1. mRNA extraction from lung samples.
2. cDNA syntesis.
3. Amplification and quantification of Krt14 mRNA.

Western Blot -
1. Proteins extraction from lung samples.
2. Gel Electrophoresis.
3. Transfer of proteins onto a nitrocellulose membrane.
4. Incubation with primary antibody.
5. Incubation with secondary antibody.
6. Chemiluminescent detection.

4 RESULTS

Real Time PCR showed that only one of the samples expressed Krt14 and that all the control specimens were negative for Krt14.

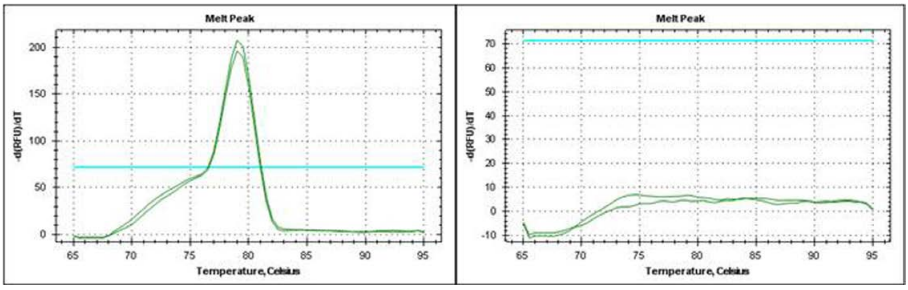

Figure 1: Real Time PCR result for single Krt14+ sample compared to a control Krt14-

Considering the greater susceptibility to degradation of RNA compaired to proteins, we performed Western Blot. This analysis showed that 5 of the 12 cases expressed Krt14 (41,6%), while in all controls were always absent specific signals.

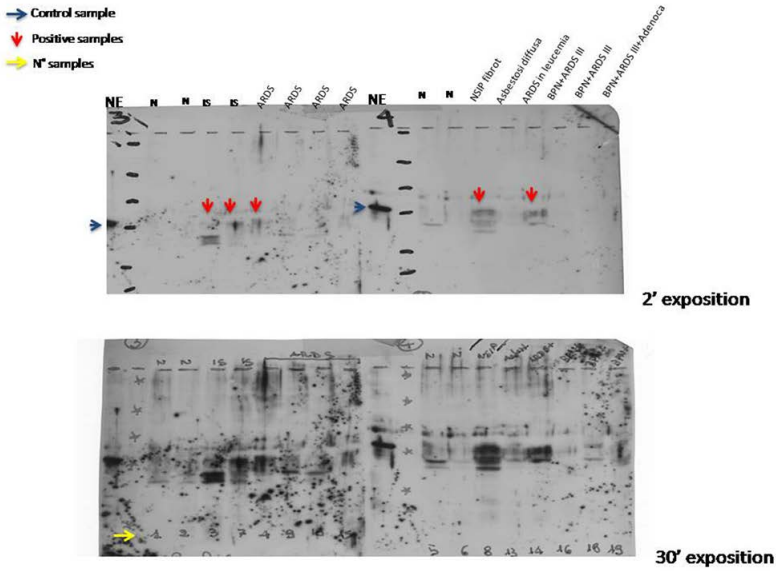

Figure 2: Western Blot Krt14

5 CURRENT COLLABORATIONS

5.1 With hospitals

University Hospital of Cattinara, Trieste, to obtain pathological/biological samples.

5.2 With other researchers

ICGEB, Area Science Park,Trieste, for the analysis on the samples.

6 UNS NEEDED

6.1 For basic research (investigation of biological mechanisms): 20.000 €

6.2 For applied research (solutions for real-world problems): 5.000 €

6.3 For pilot & demonstrator activities (to develop a prototype): 10.000 €

7 CONCLUSION

Many lung diseases, such as COPD, IPF and ARDS, remain incurable and continue to have substantial morbidity and mortality. Understanding cellular and molecular mechanisms that promote alveolar epithelial regeneration/repair may provide novel potential therapeutic approaches.