Project:
An alternative cell-based assay for the characterization of protein allergens, ref. nr 15-392

The goal of this project has been to further develop the Genomic Allergen Rapid Detection (GARD) [1-6] test system for the prediction of protein allergens able to sensitize the respiratory tract using the established cell model MUTZ-3. We furthermore aimed to test a primary cell model of the bronchial epithelium, and to challenge both cell models with representative enzymes used in the detergent industry provided by Novozymes A/B, Bagsvaerd, Denmark. Different methods, such as whole-genome analyses and several bioinformatical tools were used in order to develop a predictive biomarker signature and to investigate involved mechanisms in sensitization caused by this type of substances more in detail.

The results have been summarized in the "Delrapport", sent December 2016, and due to lack of further funding, no more experiments could be performed since then.

Based on the results, we have published the following article:

Zeller KS, Johansson H, Lund TØ, Kristensen NN, Roggen EL, Lindstedt M. An alternative biomarker-based approach for the prediction of proteins known to sensitize the respiratory tract. Toxicol In Vitro. 2018 Feb;46:155-162. doi: 10.1016/j.tiv.2017.09.029. Epub 2017 Oct 7. PubMed PMID: 29017774.

The article is attached separately.

Below, we summarize our results for workpackage 1 and 2 in a **short English abstract** as required for the final report.

To date, no validated test system exists that can predict the airway-sensitizing potential of natural and industrial protein allergens. The aim of this project was to develop a protocol based on the Genomic Allergen Rapid Detection (GARD) assay that can be used to assess and predict the capacity of protein allergens known to induce sensitization in the airways and to combine this with airway epithelial cells (a "co-culture") in order to elucidate molecular mechanisms involved in protein sensitization.

A predictive signature consisting of 391 potential biomarkers was successfully identified based transcriptional profiling, flow cytometry and multiplex cytokine using the dendritic cell-like system, MUTZ-3, challenged with eight selected proteins. A series of cross-validations supported the validity of the model. These results together with biological pathway analysis of the transcriptomic data indicated that MUTZ-3 is able to capture relevant events linked to respiratory sensitization. In a similar approach, MucilAir™, a primary cell system modelling bronchial epithelium, was not able to capture differences between the here used protein allergen preparations and controls, however, protein allergen controls influenced cytokine levels, indicating that it could be useful for co-culture with a dendritic cell-based model after further adaptations. As an alternative co-culture compound, we investigated the bronchial epithelial cell line 16HBE14o$^-$ and established protocols e.g. to check the integrity of the cell layer.

In summary, we have successfully adapted the GARD system to predict protein allergens known to sensitize the respiratory tract and our data indicate that co-culture with a bronchial cell model could further increase the sensitivity of the method.

References

1. Forreryd, A., et al., *Prediction of chemical respiratory sensitizers using GARD, a novel in vitro assay based on a genomic biomarker signature.* PLoS One, 2015. **10**(3): p. e0118808.
2. Forreryd, A., et al., *From genome-wide arrays to tailor-made biomarker readout - Progress towards routine analysis of skin sensitizing chemicals with GARD.* Toxicol In Vitro, 2016.
3. Johansson, H., et al., *A genomic biomarker signature can predict skin sensitizers using a cell-based in vitro alternative to animal tests.* BMC Genomics, 2011. **12**: p. 399.
4. Johansson, H., et al., *Genomic allergen rapid detection in-house validation--a proof of concept.* Toxicol Sci, 2014. **139**(2): p. 362-70.
5. Zeller, K.S., et al., *The GARD platform for potency assessment of skin sensitizing chemicals.* ALTEX, 2017. **34**(4): p. 539-559.
6. Johansson, H., et al., *Evaluation of the GARD assay in a blind Cosmetics Europe study.* ALTEX, 2017. **34**(4): p. 515-523.