Signature biomarker analysis for prediction of skin sensitizers using a cell-based in vitro alternative to animal experimentation

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Introduction

Allergic contact dermatitis is an inflammatory skin disease caused by immunological responses towards chemical haptens. Current test of sensitizing chemicals rely on animal experimentation. New legislations on the registration and use of chemicals within pharmaceutical and cosmetic industries have stimulated significant research efforts to develop alternative, human cell-based assays for the prediction of sensitization.

We have developed a novel cell-based assay for the prediction of sensitizing chemicals, based on differentially regulated transcripts in the myeloid cell-line MUTZ-3.

Conclusion

We have identified a biomarker signature with accurate predictive power, which represents a compelling readout for an in vitro assay useful for the identification of human sensitizing chemicals. The biomarker signature include transcripts involved in relevant biological pathways, such as oxidative stress and xenobiotic induced responses, which sheds light on the molecular interactions involved in the process of sensitization.

Results

By analysing the transcriptome of the human cell line MUTZ-3 after 24h stimulation, using 20 sensitizing chemicals, 20 non-sensitizing chemicals (table 1) and vehicle controls, a biomarker signature of 200 transcripts with potent discriminatory ability was identified (figure 1). Using a support Vector Machine for supervised machine learning, the prediction performance of the assay was estimated to an average area under the ROC curve of 0.98 (figure 2).

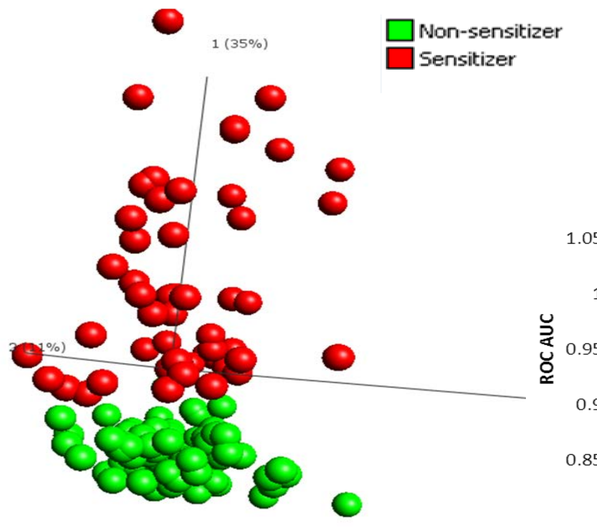

Figure 1. PCA of differentialy expressed transcripts: Principal component analysis of the 200 genes identified using one-way ANOVA and backward elimination. Sensitizing chemicals (red) vs. non-sensitizing (green) chemicals are displayed.

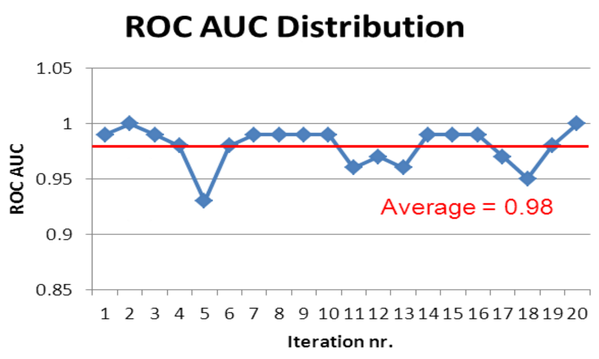

Figure 2. Test performance*:* An SVM was trained on 70% of the data set (training set), and validated with 30% (test set), yielding an average test performance of ROC AUC 0.98..

In addition, categorizing the chemicals according to the Local Lymph Node Assay (LLNA), this gene signature could also predict sensitizing potency (figure 3 & 4). The identified markers are involved in biological pathways with immunologically relevant functions, including innate immune responses and dendritic cell maturation. Such pathways include the NF-E2 related factor 2 (NRF2)-pathway, Aryl hydrocarbon receptor (AHR)-pathway and Toll-like receptor (TLR)-signalling. The developed assay has been termed GARD – *Genomic Allergen Rapid Detection test,* and the testing strategy is summarized in figure 5.

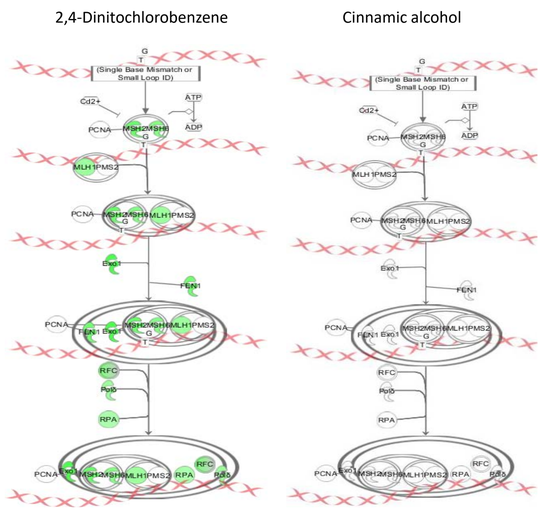

Figure 3. Mechanisms of action: Sensitizers with different potency regulate specific pathways. In this example, regulation of the Mismatch repair pathway is shown.

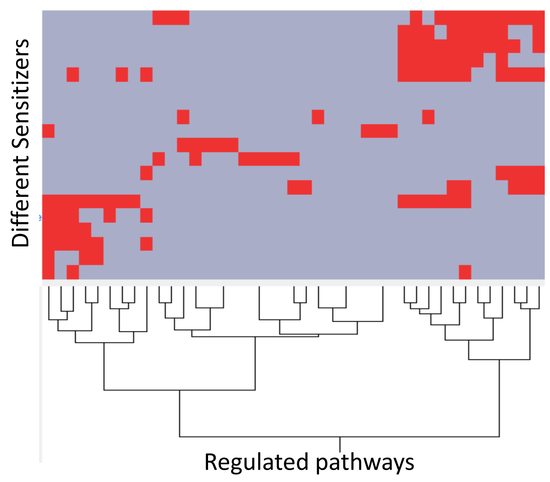

Figure 4. Relation between potency and mechanisms of action: Hierarchical clustering of sensitizer-activated pathways indicates that the potency of a sensitizer is related to specific pathways.

Chemical	Potency classification according to LLNA
2,4-Dinitrochlorobenzene	Extreme
Oxazolone	Extreme
Potassium dichromate	Extreme
Kathon CH (MC/MCI)	Extreme
Formaldehyde	Strong
2-Aminophenol	Strong
2-nitro-1,4-Phenylendiamine	Strong
p-Phenylendiamine	Strong
Hexylcinnamic aldehyde	Moderate
2-Hydroxyethyl acrylate	Moderate
2-Mercaptobenzothiazole	Moderate
Glyoxal	Moderate
Cinnamaldehyde	Moderate
Isoeugenol	Moderate
Ethylendiamine	Moderate
Resorcinol	Moderate
Cinnamic alcohol	Weak
Eugenol	Weak
Penicillin G	Weak
Geraniol	Weak
1-Butanol	Non-sensitizer
4-Aminobenzoic acid	Non-sensitizer
Benzaldehyde	Non-sensitizer
Chlorobenzene	Non-sensitizer
Diethyl phthalate	Non-sensitizer
Dimethyl formamide	Non-sensitizer
Ethyl vanillin	Non-sensitizer
Glycerol	Non-sensitizer
Isopropanol	Non-sensitizer
Lactic acid	Non-sensitizer
Methyl salicylate	Non-sensitizer
Octanoic acid	Non-sensitizer
Propylene glycol	Non-sensitizer
Phenol	Non-sensitizer
p-Hydroxybenzoic acid	Non-sensitizer
Potassium permanganate	Non-sensitizer
Salicylic acid	Non-sensitizer
Sodium dodecyl sulphate	Non-sensitizer
Tween 80	Non-sensitizer
Zinc sulphate	Non-sensitizer

Table 1. Reference chemicals used for assay development.

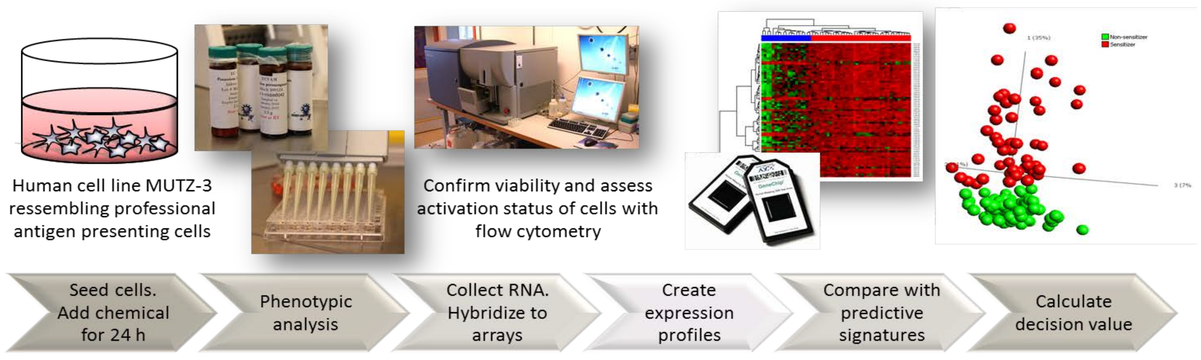

Figure 5. The GARD test principle: The MUTZ-3 cell line are seeded in wells, and incubated with test substances for 24h. RNA is isolated and hybridized to arrays. Following data acquisition, the transcription levels of the predictive signature (200 genes) are analyzed, and each sample is given a decision value as either sensitizer or non-sensitizer, based on SVM classifications.

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