GARD

a genomic in vitro assay for assessment of chemical sensitizers

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SIXTH FRAMEWORK PROGRAMME

INTRODUCTION

Allergic contact dermatitis is an inflammatory skin disease caused by immunological responses towards chemical haptens affecting almost 20% of the western population. 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 a human myeloid dendritic cell-line.

CONCLUSIONS

We have identified a genomic biomarker signature with accurate predictive power (AUC ROC 0.98), representing a compelling readout for an *in vitro* assay useful for the identification of human sensitizing chemicals.

The biomarker signature include 200 transcripts involved in cellular pathways that are active during immunological reactions involved in the process of sensitization.

The assay is denoted GARD – Genomic Allergen Rapid Detection.

RESULTS

By analysing the transcriptome of the cell line 24 h after stimulation, using 20 sensitizing chemicals, 20 non-sensitizing chemicals (**Tab. 1**) and vehicle controls, a biomarker signature of 200 transcripts with discriminatory ability was identified (**Fig. 1**). Using a Support Vector Machine (SVM) for supervised learning, the prediction performance of the assay was estimated to an average area under the ROC curve of 0.98.

Table 1. Reference chemicals used for assay development.

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

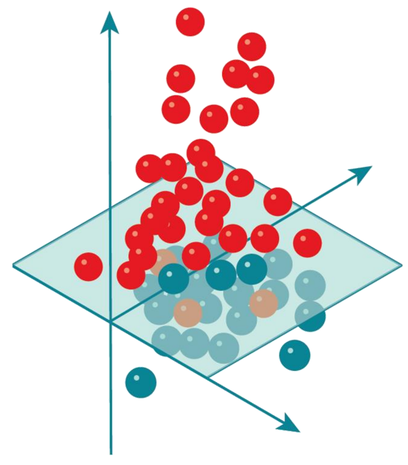

Figure 1. Illustration of the gene expression displayed after PCA. The principal component analysis of the transcript levels identified is utilized in the data analysis (one-way ANOVA, backward elimination). Sensitizing chemicals and non-sensitizing chemicals are represented by red and blue dots respectively.

Table 2. The function of identified markers activated by the sensitizers.

CELLULAR RESPONSES
Recognition of foreign substances
Activation of immunological self-defence mechanisms of the host
Cellular stress response
Communication with other cells of the immune system

The identified genomic biomarkers are involved in biological signalling pathways induced by chemical sensitizers, leading to dendritic cell maturation and a subsequent immunologic response (**Tab. 2**). The developed assay is denoted GARD – Genomic Allergen Rapid Detection test, and is summarized in **Fig. 2**

Figure 2. The GARD test principle. The cultivated cells are exposed to test substances for 24 h. mRNA is isolated and hybridized to reporter probes. After automated measurement and 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.

Acknowledgement
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Additional reading
Johansson H., Albrekt A.S., Lindstedt M., Borrebaeck C.A.K. *A genomic biomarker signature can predict skin sensitizers using a cell-based in vitro alternative to animal tests.* **BMC Genomics, 2011**.
Johansson H., Lindstedt M. *Prediction of skin sensitizers using alternative methods to animal experimentation.* **Basic Clin Pharmacol, 2014**.