SkinSensDB: a curated database for skin sensitization assays

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Abstract
Skin sensitization is an important toxicological endpoint for chemical hazard determination and safety assessment. Prediction of chemical skin sensitizer had traditionally relied on data from rodent models. The development of the adverse outcome pathway (AOP) and associated alternative in vitro assays have reshaped the assessment of skin sensitizers. The integration of multiple assays as key events in the AOP has been shown to have improved prediction performance. Current computational models to predict skin sensitization mainly based on in vivo assays without incorporating alternative in vitro assays. However, there are few freely available databases integrating both the in vivo and the in vitro skin sensitization assays for development of AOP-based skin sensitization prediction models. To facilitate the development of AOP-based prediction models, a skin sensitization database named SkinSensDB has been constructed by curating data from published AOP-related assays. In addition to providing datasets for developing computational models, SkinSensDB is equipped with browsing and search tools which enable the assessment of new compounds for their skin sensitization potentials based on data from structurally similar compounds. SkinSensDB is publicly available at http://cwtung.kmu.edu.tw/skinsensdb.

Background
Skin sensitization associated with allergic contact dermatitis (ACD) is the second most common occupational illness accounting for 10–15% of all occupational disease worldwide [1]. The disease not only impairs the quality of life for the patients but also results in high costs in healthcare systems and economy [2]. Skin sensitization is thereby an important toxicological endpoint in chemical safety assessment and a focus in regulatory decision making. Chemical sensitizers, which may be detergents, preservatives, or fragrances in household and personal care products or active ingredients, impurities from synthetic process and industrial materials in the pharmaceutical products, act as haptons binding to protein molecules. These chemically modified proteins may trigger T cell-mediated immune reactions and lead to ACD [3].

Traditionally, guinea pig maximization test (GPMT) and Buehler assay (BA) are utilized as predictive animal models for the identification of skin sensitizers and are widely accepted by regulatory authorities due to their reliable detection of potential human contact allergens. Nevertheless, these protocols are relatively long and complex with some limitations [4]. The murine local lymph node assay (LLNA) has been established as an alternative animal model to traditional guinea pig methods to provide important animal welfare benefits and has been successfully validated and incorporated into a regulatory guideline described as Organization for Economic Cooperation and Development (OECD) Test Guideline Number 429 [5]. The assay measures the proliferation of lymphocytes during the induction phase of skin sensitization and provides EC3 values (effective concentration for a stimulation index of threefold in lymphocyte proliferation compared to vehicle controls) to make comparisons of the relative potency of different chemical sensitizers. The animal tests have been recently banned for cosmetic ingredients in 2013 by the European Union. There is a strong need for the development of alternative testing methods for identifying skin sensitizers [6, 7].

Many quantitative structure–activity relationship (QSAR) methods have been proposed to predict skin
sensitizers based on descriptors of chemical structures [8–18]. All of the QSAR models were developed for a single type of sensitization endpoints mostly from either LLNA or GPMT. Although reasonably good prediction performance was obtained from the QSAR models, the prediction gave no insights into the detailed mechanism of actions. Recently, skin sensitization has been formulated as an adverse outcome pathway (AOP) to address the complex nature of sensitization [19]. Four key events including protein binding, keratinocyte activation, dendritic cell activation and T cell activation were clearly defined. Several alternative methods have been developed according to the key events such as Direct Peptide Reactivity Assay (DPRA) and Peroxidase Peptide Reactivity Assay (PPRA) [20, 21] for quantification of chemical peptide reactivity, KeratinoSens [22, 23] and LuSens [24] for keratinocyte activation by determining the activation of antioxidant response element (ARE) reporter genes, and h-CLAT [25, 26] for activation of dendritic cells by measuring the increased level of CD54 and CD86 maturation markers, respectively. The integration of the key events of the AOP for predicting skin sensitizers showed improved prediction performance over single models [27–30]. In addition, the data from individual steps of the sensitization progress can be interpreted and evaluated by experts for hazard determination and risk assessment.

To facilitate the development of AOP-based computational prediction methods, a novel curated database named SkinSensDB was constructed by manual curation of published literature. A total of 710 unique chemicals were curated into SkinSensDB with corresponding reactivities of 2078, 467, 1323 and 1060 assay values for peptide reactivity, keratinocyte activation, dendritic cell activation, and T-cell activation, respectively. Search tools have been implemented with exact, similarity, and substructure search functionality. The SkinSensDB is expected to be a useful database supporting the development of AOP-based computational models for predicting skin sensitizers.

Construction and content
The SkinSensDB database was implemented using MongoDB version 3.0.7. The web interface and search functions were implemented using PHP, Python, HTML and JavaScript programming languages and frameworks of AngularJS version 1.4.6 (https://angularjs.org/) and Angular Material version 1.1.1 (https://material.angularjs.org/latest/). The SkinSensDB website is publicly available at http://cwtung.kmu.edu.tw/skinsensdb.

Database content
SkinSensDB database consists of chemical information and four types of well-developed assays associated with the four events of the AOP for skin sensitization reported by OECD [19]. For each chemical, its basic structure and physicochemical property information was extracted from PubChem Compound database using PUG REST functions from PubChem [31]. Chemical-related information provided at SkinSensDB included the CAS number, IUPAC name, INCHI, INCHIKEY, formula, SMILES, hydrogen-bond acceptor, hydrogen-bond donor, molecular weight and topological polar surface area (TPSA). In addition, external links to PubChem database and SDF files for 2D and 3D structures were available for further exploration of chemical-specific information and development of QSAR models, respectively.

For protein binding ability of chemicals, results of peptide reactivity assays of DPRA/PPRA were collected. For each chemical, its corresponding curated information included source literature, assay types, markers, peptide concentrations, chemical concentrations, the addition of horseradish peroxidase (HRP) enzymes and the percentage of peptide depletion with standard deviations. Peptide depletion assays with and without enzymatic activation using HRP enzymes are so called PPRA and DPRA, respectively. The keratinocyte activation ability of chemicals was represented by data from KeratinoSens and LuSens assays. Curated information for keratinocyte activation included the maximum fold induction of luciferase activity (Imax), concentrations for the n-fold induction of luciferase (EC1.5, EC2, and EC3) and cytotoxicity (IC50). For the activation potential of dendritic cells, information related to h-CLAT was curated including CD54 and CD86 markers, the induction criteria for the markers (EC type), the effective concentrations for the markers (EC), relative fluorescence intensity (RFI), and the concentrations that produced 75% cell viability (CV75). The LLNA assays representing the T-cell activation ability of chemicals were collected from NICEATM LLNA database [32]. LLNA-associated data consisted of vehicles, effective concentration for threefold induction of draining lymph node cell proliferation, the result of LLNA, evaluated concentrations and corresponding stimulation index (SI).

Utility and discussion
SkinSensDB is a curated database for AOP-associated skin sensitization assays aiming to provide an easily accessible resource for the development of AOP-based computational models. Currently, there are 710 unique chemicals with 4928 assay values of AOP-related skin sensitization assays from the literature. A typical record of SkinSensDB is shown in Fig. 1 where basic information of chemicals and associated skin sensitization assays can be found. The full record can be exported as an Excel file via the download button. Both the 2D and 3D
structure files are downloadable for each chemical. With the integration of chemical structure and physicochemical property information, it is expected to be useful for developing AOP-based QSAR models. Users who wish to contribute their data to this database can submit their data using an Excel file provided at SkinSensDB.

For the exploration of chemicals and corresponding skin sensitization assays, a browse tool providing searchable summarized information of the four assays has been implemented. The criteria for defining the summarized information were shown in Table 1. The data table can be easily browsed by sorting and filtering on specific columns. Functions for batch queries and exporting the summarized information were also implemented.

Based on the assumption that structurally similar chemicals could have similar bioactivities, three types of tools for exact, similarity and substructure searches have been implemented to facilitate the search of structurally similar chemicals. Figure 2 shows the user interface of search functions. For the input of chemical structure for search, users can either draw a structure in the JSME [33] editor or enter a SMILES text that can be automatically converted into chemical structures. The search functions were implemented based on RDKit [34], an open-source cheminformatics library. The similarity search was based on Tanimoto similarity between topological fingerprints of two chemicals. In addition to the summarized information, the similarity score between 0 and 1 will be available in the search results as shown in Fig. 3.

In addition, a file summarizing essential information for developing QSAR models is available at the SkinSensDB website. The summarized file consists of fields of the chemical name, CAS number, PubChem CID, the highest peptide depletion of DPRA/PPRA, the lowest

Table 1: The classification criteria of positive and negative responses to a chemical

| Assay type       | Criteria                                      | Classification |
|------------------|-----------------------------------------------|----------------|
| DPRA/PPRA        | Peptide depletion ≤ 6.38%                     | Negative       |
|                  | Peptide depletion > 6.38%                     | Positive       |
| KeratinoSens/LuSens | EC1.5 ≥ 1000 µM                                 | Negative       |
|                  | EC1.5 < 1000 µM                                | Positive       |
| h-CLAT           | Neither CD86 EC150 nor CD54 EC200 was determined | Negative       |
|                  | CD86 EC150 ≤ CV75 or CD54 EC200 ≤ CV75         | Positive       |
| LLNA             | SI ≤ 3                                        | Negative       |
|                  | SI ≥ 3                                        | Positive       |

EC effective concentration, CV75 75% cell viability, SI stimulation index
**Fig. 2** The search functions. Users can search the SkinSensDB database using functions of exact, substructure and similarity searches with a user-supplied chemical structure by either drawing a chemical structure or converting from a SMILES string.

**Fig. 3** An illustrated example of the similarity search in SkinSensDB. A similarity score between query and target chemicals is available and sortable in the second column. All the other columns are the same as the browse tool in SkinSensDB consisting of name, CAS number, PubChem CID, summarized results for the four key events.
EC1.5 value of KeratinoSens/LuSens, the lowest EC and CV75 values of h-CLAT, and the EC3 value of LLNA for all chemicals in SkinSensDB. The structure files can be easily downloaded from PubChem database using the CIDs and transformed into descriptors using softwares such as PaDEL-Descriptor [35]. QSAR models can be subsequently constructed to study the relationship between structure descriptors and response values from assays of four key events.

Conclusions
SkinSensDB is a web-based resource providing useful information of chemical structures, physicochemical properties and experimental data from both alternative in vitro and in vivo skin sensitization assays. Browse and search tools were implemented to facilitate the exploration of skin sensitization data. The integration of chemical structures, physicochemical properties, and experimental results from these AOP-related assays could be helpful for the development of an AOP-based prediction system integrating all models corresponding to the four major events.

Authors’ contributions
CWT conceived the project. CCW, YCL and CWT wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability and requirements
SkinSensDB is freely available at http://cwtung.kmu.edu.tw/skinsensdb without restrictions for academic use. The website has been tested with browsers of latest versions including Opera, Internet Explorer Edge, Firefox and Google Chrome.

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References
1. Baskette DA, White IR, McFadden JP, Kimber J (2015) Skin sensitization: implications for integration of clinical data into hazard identification and risk assessment. Hum Exp Toxicol 34:1222–1230. doi:10.1177/0960327115601760
2. Peiser M, Tralau T, Heidler J et al (2012) Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects. Current knowledge assembled at an international workshop at BIR, Germany. Cell Mol Life Sci CMLS 69:763–781. doi:10.1007/s00018-011-0846-8
3. Karberg A-T, Bergström MA, Borje A et al (2008) Allergic contact dermatitis–formation, structural requirements, and reactivity of skin sensitizers. Chem Res Toxicol 21:53–69. doi:10.1021/tx7002239
4. Baskette DA, Scholes EW (1992) Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 30:65–69. doi:10.1016/0278-6915(92)90138-8
5. Kimber I, Hilton J, Dearman RJ et al (1995) An international evaluation of the murine local lymph node assay and comparison of modified procedures. Toxicology 103:63–73
6. Mehlng A, Ericksson T, Elze T et al (2012) Non-animal test methods for predicting skin sensitization potentials. Arch Toxicol 86:1273–1295. doi:10.1007/s00204-012-0867-6
7. Vandebriel RJ, van Loveren H (2010) Non-animal sensitization testing: state-of-the-art. Crit Rev Toxicol 40:389–404. doi:10.3109/10408440903524262
8. Li Y, Tseng YJ, Pan D et al (2007) 4D-fingerprint categorical QSAR models for skin sensitization based on the classification of local lymph node assay measures. Chem Res Toxicol 20:114–128. doi:10.1021/tx6002535
9. Ren Y, Liu H, Xue C et al (2006) Classification study of skin sensitizers based on support vector machine and linear discriminant analysis. Anal Chim Acta 572:272–282. doi:10.1016/j.aca.2006.05.027
10. Liu J, Kern PS, Gerberick GF et al (2008) Categorical QSAR models for skin sensitization based on local lymph node assay measures and both ground and excited state 4D-fingerprint descriptors. J Comput Mol Des 22:345–366. doi:10.1007/s10822-008-9190-y
11. Li Y, Pan D, Liu J et al (2007) Categorical QSAR models for skin sensitization based upon local lymph node assay classification measures part 2: 4D-fingerprint three-state and two-state logistic regression models. Toxicol Sci Off J Soc Toxicol 99:532–544. doi:10.1093/toxsci/kfm185
12. Golla S, Madhilly S, Robinson RL, Gasm KAM (2009) Quantitative structure-property relationship modeling of skin sensitization: a quantitative prediction. Toxicol Vitr Int J Publ Assoc with BIBRA 23:454–465. doi:10.1016/j.tiv.2008.12.025
13. Yuan H, Huang J, Cao C (2009) Prediction of skin sensitization with a particle swarm optimized support vector machine. Int J Mol Sci 10:3237–3254. doi:10.3390/ijms10073237
14. Nandy A, Kar S, Roy K (2013) Development and validation of regression-based QSAR models for quantification of contributions of molecular fragments to skin sensitization potency of diverse organic chemicals. SAR QSAR Environ Res 24:1009–1023. doi:10.1080/10408483.2013.812142
15. Alves-VM, Muroto V, Fourches D et al (2015) Predicting chemically-induced skin reactions: Part I: QSAR models of skin sensitization and their application to identify potentially hazardous compounds. Toxicol Appl Pharmacol 284:262–272. doi:10.1016/j.taap.2014.12.014
16. Dearden JC, Hewitt M, Roberts DW et al (2015) Mechanism-based QSAR modeling of skin sensitization. Chem Res Toxicol 28:1975–1986. doi:10.1021/acs.chemrestox.5b00197
17. Sarath Kumar KL, Thangapalliwar SR, Desai A et al (2016) Integrated computational solution for predicting skin sensitization potential of molecules. PLoS ONE. doi:10.1371/journal.pone.0155491
18. Li S, Fedorowicz A, Singh H, Soderholm SC (2005) Application of the random forest method in studies of local lymph node assay based skin sensitization data. J Chem Inf Model 45:952–964. doi:10.1021/ci0493199
19. OECD (2012) The adverse outcome pathway for skin sensitisation initiation and risk assessment. Hum Exp Toxicol 34:1222–1230. doi:10.1177/0960327115601760
21. Gerberick GF, Vassallo JD, Bailey RE et al (2004) Development of a peptide reactivity assay for screening contact allergens. Toxicol Sci Off J Soc Toxicol 81:332–343. doi:10.1093/toxsci/kfh213
22. Emter R, Ellis G, Natsch A (2010) Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers in vitro. Toxicol Appl Pharmacol 245:281–290. doi:10.1016/j.taap.2010.03.009
23. Natsch A, Emter R (2008) Skin sensitizers induce antioxidant response element dependent genes: application to the in vitro testing of the sensitization potential of chemicals. Toxicol Sci Off J Soc Toxicol 102:110–119. doi:10.1093/toxsci/kfm259
24. Bauch C, Kolle SN, Ramirez T et al (2012) Putting the parts together: combining in vitro methods to test for skin sensitizing potentials. Regul Toxicol Pharmacol RTP 63:489–504. doi:10.1016/j.yrtph.2012.05.013
25. Sakaguchi H, Ashikaga T, Miyazawa M et al (2006) Development of an in vitro skin sensitization test using human cell lines; human Cell Line Activation Test (h-CLAT). II. An inter-laboratory study of the h-CLAT. Toxicol Vitr Int J Publ Assoc with BIBRA 20:774–784. doi:10.1016/j.tiv.2005.10.014
26. Ashikaga T, Hoya M, Itagaki H et al (2002) Evaluation of CD86 expression and MHC class II molecule internalization in THP-1 human monocyte cells as predictive endpoints for contact sensitizers. Toxicol Vitr Int J Publ Assoc with BIBRA 16:711–716. doi:10.1016/S0887-2333(02)00060-7
27. Patlewicz G, Kuseva C, Kesova A et al (2014) Towards AOP application—implementation of an integrated approach to testing and assessment (IATA) into a pipeline tool for skin sensitization. Regul Toxicol Pharmacol 69:529–545. doi:10.1016/j.yrtph.2014.06.001
28. Pirone JR, Smith M, Kleinstreuer NC et al (2014) Open source software implementation of an integrated testing strategy for skin sensitization potency based on a Bayesian network. ALTEX 31:336–346. doi:10.14573/altex.1310151
29. van der Veen JW, Ronije E, Emter R et al (2014) Evaluating the performance of integrated approaches for hazard identification of skin sensitizing chemicals. Regul Toxicol Pharmacol 69:371–379. doi:10.1016/j.yrtph.2014.04.018
30. Strickland J, Zang Q, Kleinstreuer N et al (2016) Integrated decision strategies for skin sensitization hazard. J Appl Toxicol 36(9):1150–1162. doi:10.1002/jat.3281
31. Kim S, Thiessen PA, Bolton EE, Bryant SH (2015) PUG-SOAP and PUG-REST: web services for programmatic access to chemical information in PubChem. Nucleic Acids Res 43:W605–W611. doi:10.1093/nar/gkv396
32. NICEATM LLNA database (2013) http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/immunotoxicity/nonanimal/index.html#NICEATM-Murine-Local-Lymph-Node-Assay-LLNA-Database
33. Bienfait B, Ertl P (2013) JSME: a free molecule editor in JavaScript. J Cheminf. doi:10.1186/1758-2946-5-24
34. RDKit: Open-source cheminformatics. http://www.rdkit.org/
35. Yap CW (2011) PaDEL-descriptor: an open source software to calculate molecular descriptors and fingerprints. J Comput Chem 32:1466–1474. doi:10.1002/jcc.21707

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