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A*STAR Institute of Bioengineering and Nanotechnology

PROXTOX-HTS: RAPID AND ACCURATE HIGH-THROUGHPUT PREDICTION OF NEPHROTOXICITY IN HUMANS

The challenge of accurately predicting nephrotoxicity

The human kidney can be damaged by a wide range of xenobiotics, such as industrial chemicals, environmental toxicants and drugs. Drugs with kidney-damaging potential include antibiotics, anti-cancer agents, immunosuppressants, non-steroidal anti-inflammatory drugs, contrast agents, anti-viral compounds and others[1]. Major targets for compound-induced damage are renal proximal tubule cells (PTCs) due to their role in compound transport, metabolism and clearance. Therefore, PTCs are typically applied in kidney-specific *in vitro* toxicity methods[1]. Predictiveness of currently used *in vitro* nephrotoxicity methods for humans is unknown, as these assays are typically tested with only few compounds (<10), which does not allow to determine the predictive performance of such methods[1]. This can be only achieved by testing a statistically robust set of compounds, which comprises both compounds that are known to be toxic as well as compounds that are known to be not toxic for PTCs in humans. Apart from problems with *in vitro* methods, also animal models often do not reliably predict nephrotoxicity in humans. Due to the lack of pre-clinical models with high predictivity, nephrotoxicity is typically detected only late during drug development, during clinical trials or post-marketing [2,3]. Examples of drugs where nephrotoxicity was detected only during post-marketing are tenofovir and COX-2 inhibitors [3,4]. In addition to problems with predictivity, currently used models for assessing nephrotoxicity are often low throughput, inefficient and costly.

Predicting nephrotoxicity with human primary renal cells and human induced pluripotent stem cell-derived renal cells using ProxTox-HTS

Offered by SOLVO Biotechnology in collaboration with researchers at the Institute of Bioengineering and Nanotechnology (Singapore), ProxTox-HTS is the first *in vitro* method developed to predict nephrotoxicity in humans with high accuracy [5-9]. The method uses advanced

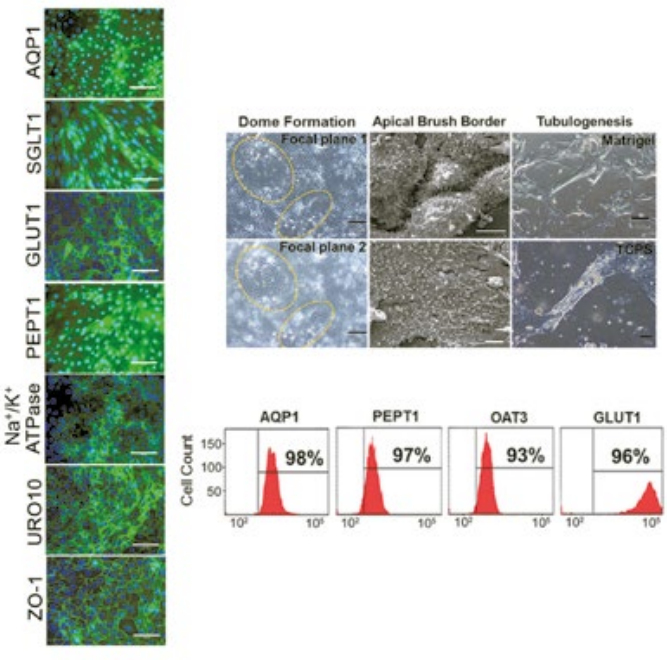

Figure 1 – iPSC-derived PTC-like cells. Upper right: iPSC-derived PTC-like cells form polarized epithelia with an apical brush border similar to PTCs *in vivo*. They show tubulogenesis on various substrates (TCPS: tissue culture polystyrene) and dome formation, which are typical features of PTCs. Scale bars (left to right): 300 µm, 5 µm and 100 µm. Left: Immunostaining (green; cell nuclei: blue) of characteristic PTC markers. PTC markers are expressed throughout the differentiated epithelia generated *in vitro* and display their characteristic subcellular localization. Scale bars: 100 µm.Lower right: percentages of cells expressing PTC markers were determined by flow cytometry. iPSC-derived PTC-like cells have a purity of >90%.
AQP1: aquaporin 1; GLUT1: solute carrier family 2 member 1 (glucose transporter); OAT3: solute carrier family 22 (organic anion transporter), member 8; PEPT1: solute carrier family 15 member 1 (oligopeptide transporter); SGLT1: solute carrier family 5 (sodium/glucose cotransporter), member 1, URO10: urothelial glycoprotein 10, ZO-1: tight junction protein 1. For details see[5].

and physiologically relevant cell models, such as human primary PTCs [7, 9] and human induced pluripotent stem cell (iPSC)-derived PTC-like cells[5, 10]. iPSC-derived PTC-like cells (Fig. 1) can be generated with a robust 1-step protocol and are ready for use after only 8 days of differentiation[5]. The cells have a purity of >90% (Fig. 1). Expression of organic anion transporters is high in iPSC-derived PTC-like cells, and cellular pathways and injury mechanisms are correctly induced in these cells in a compound-specific manner[5].

Using the ProxTox-HTS *in vitro* model for the prediction of nephrotoxicity in humans, comparable predictive performances were obtained with human primary PTCs and iPSC-derived PTC-like cells (Table 1)[5, 7, 8]. Pre-validation was performed with a set of 41 compounds with well-characterized effects on human kidneys (Table 2; for a detailed description of the compound-specific effects on human kidneys see[6]). The predictive performance analysis was based on comparing *in vitro* results to the compound specific effects on PTCs in human kidneys *in vivo* (see Table 2 for compound annotation). The training and test performances (Table 1) were estimated by using machine learning and 10-fold cross validation with 10 random trials[5,8]. The work described here and in the following section (high-throughput prediction) was performed with differentiated and polarized epithelia of primary proximal tubule cells *in vitro* as shown in Figure 1. In human kidneys PTCs are organized into similar polarized epithelia.

PTC MODEL	SENSITIVITY Training / Test, %	SPECIFICITY Training / Test, %	BALANCED ACCURACY Training / Test, %
Primary PTC, Donor 1	100.0 / 92.5	100.0 / 91.5	100.0 / 92.0
Primary PTC, Donor 2	99.9 / 88.5	99.6 / 68.0	99.8 / 78.3
Primary PTC, Donor 3	99.8 / 87.0	99.8 / 64.5	99.8 / 75.8
Primary PTC, Mean (3 Donors)	**99.9 / 89.3**	**99.8 / 74.7**	**99.9 / 82.0**
iPSC-derived PTC-like cells	**99.7 / 89.0**	**100.0 / 85.0**	**99.8 / 87.0**

Table 1 – Predictive performance of the ProxTox-HTS renal *in vitro* model with primary PTCs or iPSC-derived PTC-like cells. Primary PTCs derived from 3 different donors were tested. Training and test performances were estimated by using machine learning and 10-fold cross validation with 10 random trials. Sensitivity was defined as the percentage of nephrotoxicants that were toxic for PTCs in humans (red sector in Table 2) and gave a positive result *in vitro*. Specificity was defined as the percentage of compounds that were not toxic for PTCs in humans (green sector in Table 2) and gave negative results *in vitro*. Balanced accuracy was defined as sensitivity + specificity / 2. For detailed results see [5, 7, 8].

NO.	COMPOUND	ANNOTATION
1	Gentamicin	Nephrotoxicants, directly toxic to PTCs in humans
2	Tobramycin	
3	Rifampicin	
4	Tetracycline	
5	Puromycin	
6	Cephalosporin C	
7	5-Fluorouracil	
8	Cisplatin	
9	Ifosfamide	
10	Paraquat	
11	Arsenic(III) oxide	
12	Bismuth(III) oxide	
13	Cadmium(II) chloride	
14	Copper(II) chloride	
15	Germanium(IV) oxide	
16	Gold(I) chloride	
17	Lead acetate	
18	Potassium dichromate	
19	Tacrolimus	
20	Cyclosporin A	
21	Citrinin	
22	Tenofovir	
23	Vancomycin	Nephrotoxicants, not directly toxic to PTCs in humans
24	Phenacetin	
25	Acetaminophen	
26	Ibuprofen	
27	Furosemide	
28	Lithium Chloride	
29	Lindane	
30	Ethylene glycol	
31	Valacyclovir	
32	Lincomycin	
33	Ciprofloxacin	
34	Ribavirin	Not nephrotoxic
35	Glycine	
36	Dexamethasone	
37	Melatonin	
38	Levodopa	
39	Triiodothyronine	
40	Acarbose	
41	Atorvastatin	

Table 2 – Library of 41 compounds used for pre-validation of ProxTox-HTS. The compounds have well-characterized clinical effects on human kidneys (described in detail in[6]) and were known to be either toxic (red sector) or not toxic (green sector) to PTC in human kidneys *in vivo*. Some compounds (23-33) damage human kidneys by mechanisms that do not involve direct PTC injury, and such compounds would be expected to give negative results in a PTC-based *in vitro* method.

Rapid high-throughput prediction of nephrotoxicity in humans using ProxTox-HTS

In order to achieve rapid and efficient prediction of nephrotoxicity, a robust (high-throughput) screening platform has been established that predicts renal proximal tubule injury in humans with high accuracy[9]. The ProxTox-HTS high-throughput platform is based on a 384-well format and high-content imaging. It has been prevalidated with 44 chemically diverse compounds with well-characterised effects on human kidneys. This library of 44 compounds consisted mainly of the compounds listed in Table 2 and included some additional compounds (details described in[9]).

The high-throughput platform was developed without using pre-defined endpoints. Instead, predictive cellular changes were identified by phenotypic profiling after compound treatment (Fig. 2). Analysis of 129 cellular features was conducted and their changes were compared by machine learning to the known clinical effects of the 44 compounds on human kidneys. Screening was performed with human primary PTCs and HK-2 cells (a widely used immortalized human PTC line) and sets of 4 (primary PTCs) or 5 (HK-2 cells) features that resulted in high predictivity were selected[9]. This is a reference included actin and DNA changes, and DNA damage response.

With respect to predicting PTC toxicity in humans, the test balanced accuracies using ProxTox-HTS were 82% (primary PTCs) and 89% (HK-2 cells), respectively[9]. These data were obtained using relatively short (16 hours) compound exposure, and also compounds that typically lead to chronic kidney injury gave positive results. ProxTox-HTS can therefore be used for the identification of acutely as well as chronically nephrotoxic compounds.

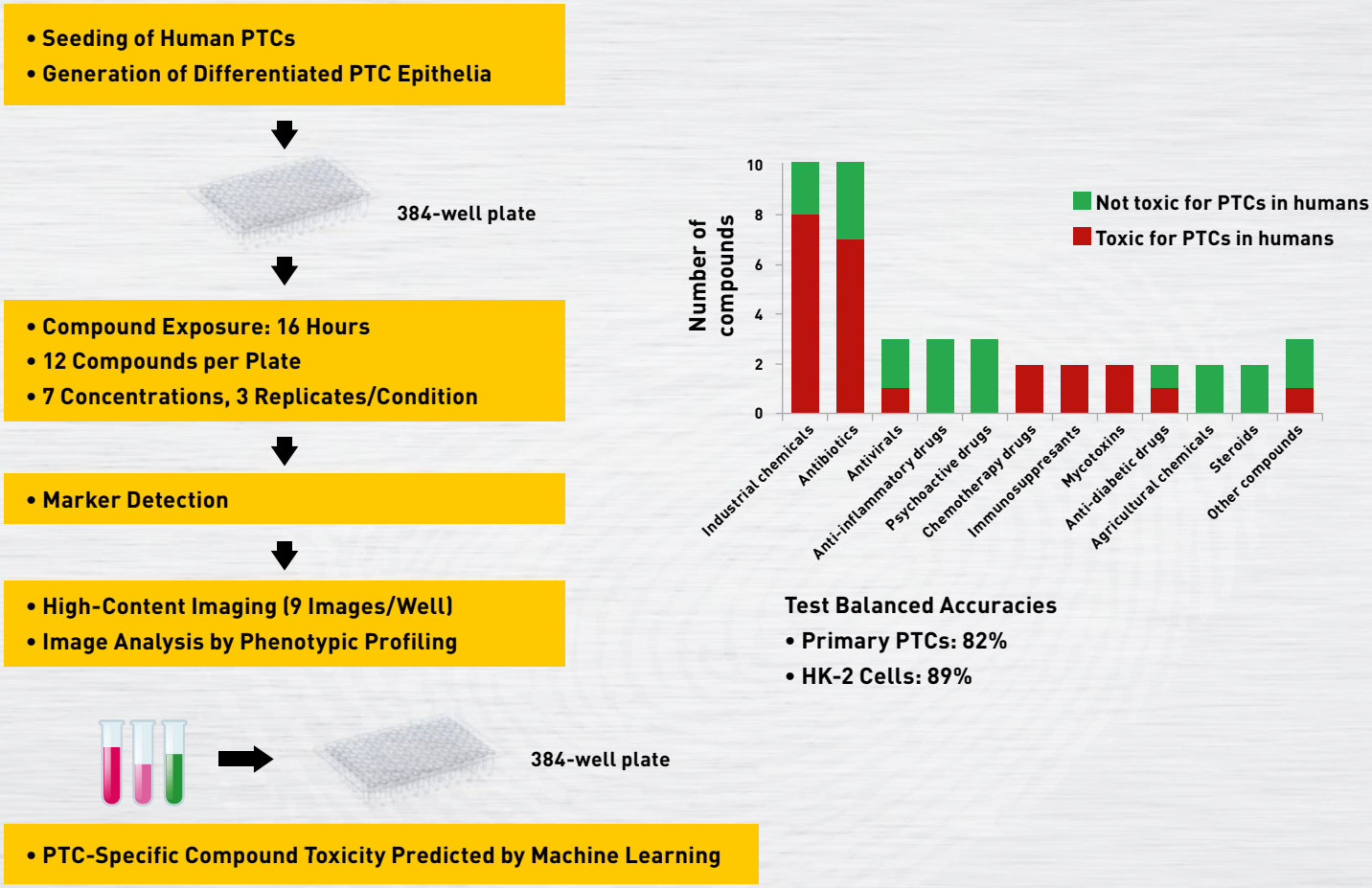

Figure 2 – Flow diagram outlining the ProxTox-HTS high-throughput platform for the rapid prediction of nephrotoxicity (left). Pre-validation and determination of the predictive performance (right) has been performed by using 44 compounds with diverse chemical structures. For details see[9].

ProxTox-HTS award winning new model

ProxTox-HTS represents the only available high-throughput method for efficient and accurate prediction of nephrotoxicity in humans. This innovation has been awarded with the Lush Science Prize 2016. The US Environmental Protection Agency is currently utilizing the platform for the prediction of the human nephrotoxicity of hundreds of ToxCast compounds. The human nephrotoxicity for most of these compounds is unclear due to the previous lack of efficient screening methods for nephrotoxicity assessment.

Contact SOLVO Biotechnology today to learn more about the ProxTox-HTS platform and how you can incorporate the power of cost-effective high-throughput *in vitro* nephrotoxicity screening into your drug discovery, development, and safety toxicology programs.

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