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Cisplatin Induces Cell Death in Rat Adult Kidney Stem/ Progenitor Cell-Derived Kidney Organoids

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ABSTRACT

Background The use of stem/ progenitor cell-derived organoids to evaluate the toxicity of chemical substances has widely increased. Organoids with nephron-like structures (NLS) can be derived from rat adult kidney stem/ progenitor cells (rKS cells) using three-dimensional culture. In this study, we examined the effects of cisplatin, an anticancer drug that induces nephrotoxicity *in vivo*, on rKS cell-derived NLS.

Methods Twelve organoids were simultaneously derived from three-dimensionally cultured rKS cells in Matrigel matrices. The surface area of each organoid was measured using microscopy-based imaging, and the morphological changes of NLS were monitored using an image analysis method. NLS were exposed to cisplatin, and their associated effects were assessed.

Results NLS elongated over time. The surface areas of the 12 organoids were almost constant. Cisplatin exposure induced cell death in NLS and inhibited the increase in the surface area of the organoids.

Conclusion Cisplatin exposure induces damage to NLS derived from rKS cells. Thus, the organoids may be valuable as an *in vitro* model to assess nephrotoxicity.

Key words cell death; cisplatin; kidney organoids; stem cells; three-dimensional culture

The kidney is a target organ for assessing drug toxicity. Conventional evaluation of nephrotoxicity involves urinalysis, blood biochemistry, and histopathology using laboratory animals.[1–3] However, developing an *in vitro* nephrotoxicity assay using cultured cells is crucial to promote animal welfare and substitute animal models.[4]

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Abbreviations: AKI, acute kidney injury; ES cells, embryonic stem cells; FCS, fetal calf serum; iPS cells, induced pluripotent stem cells; NLS, nephron-like structure; PI, propidium iodide; rKS cells, rat kidney stem/progenitor cells

The kidney is a complex organ composed of over 30 cell types.[5, 6] Despite the development of nephrotoxicity assays using kidney-derived cultured cells,[7–10] substituting animal experiments entirely with cultured cells is challenging. Recently, reconstitution of kidney organoids, including nephrons, the basic unit of the kidney, has been achieved using undifferentiated stem cells such as induced pluripotent stem (iPS) cells and embryonic stem (ES) cells.[11, 12] These kidney organoids express kidney markers and may be used as a new model to evaluate drug nephrotoxicity.

Most toxicity data have been generated from rat models, so rat and human-derived kidney organoids must be simultaneously compared to evaluate their suitability to assess nephrotoxicity. Kitamura *et al.* reported that rat kidney stem/progenitor cells (rKS cells) obtained from the S3 segment of renal proximal tubules from the adult rat could be cultured to derive organoids with three-dimensional nephron-like structures (NLS) *in vitro*.[13, 14] Morphological and immunohistochemical analyses revealed that these NLS contain various substructures of the kidney, including glomeruli, proximal tubules, the loop of Henle, and distal tubules.[13, 14] Therefore, we speculated that these organoids could potentially be used to study nephrotoxicity in rats.

Cisplatin is an effective anticancer drug used to treat various cancers; however, its prolonged use for 1–2 weeks results in acute kidney injury (AKI) in 20–30% of the cases.[15] Notably, cisplatin-induced AKI is characterized by the necrosis of proximal tubular cells and their eventual shedding.[15] Although regeneration of the proximal tubule effectively restores renal function, insufficient regeneration may induce proximal tubular atrophy and interstitial fibrosis. Cisplatin also reportedly damages the proximal tubule in human iPS cell-derived kidney organoids.[11, 12]

Based on these observations, we cultured rat kidney organoids and monitored alterations in their growth, size, and morphology over time. We also analyzed the effects of cisplatin on the organoids with nephron-like structures. Collectively, we aimed to investigate whether rat kidney-derived organoids could serve as a kidney

model for assessing drug-induced nephrotoxicity.

MATERIALS AND METHODS

Cell culture and differentiation

The cell line and culture methods previously described by Kitamura *et al.*[13, 14] were used in the present study. Briefly, rKS cells were cultured on a type IV collagen plate (Corning Life Sciences, Kennebunk, ME) and maintained in a 1:1 mixture of conditioned culture supernatant from mouse mesenchymal cells [DMEM (Thermo Fisher Scientific, Waltham, MA) containing 10% Fetal Calf Serum (FCS, Thermo Fisher Scientific)] and modified K1 medium in a humidified atmosphere at 37°C and 5% CO_2. The modified K1 medium comprised a 1:1 mixture of DMEM and Ham's F-12 medium (Thermo Fisher Scientific), supplemented with 10% FCS, 5 μg/mL insulin, 2.75 μg/mL transferrin, 3.35 ng/mL sodium selenious acid (Thermo Fisher Scientific), 50 nM hydrocortisone (Sigma, St. Louis, MO), 25 ng/mL hepatocyte growth factor (Sigma), and 2.5 mM nicotinamide (Sigma).

rKS cell layers were subsequently dissociated using trypsin (Thermo Fisher Scientific) and harvested. Cell aggregates were obtained using the hanging drop method, and these were incubated in a humidified atmosphere at 37°C and 5% CO_2 for approximately 6 h. Each aggregate contained approximately 1.0×10^5 rKS cells. Cell aggregates were then transferred into a "half-Matrigel" solution placed on the filters of Transwell inserts in wells containing differentiation medium. The "half-Matrigel" solution comprised a 1:1 mixture of Matrigel (Corning Life Sciences) and differentiation medium. The differentiation medium contained DMEM/F-12 supplemented with 10% FCS, 250 ng/mL glial cell line-derived neurotrophic factor (GDNF) (R&D Systems, Minneapolis, MN), 250 ng/mL basic fibroblast growth factor (bFGF) (R&D Systems), 250 ng/mL epidermal growth factor (EGF) (R&D Systems), 250 ng/mL bone morphogenetic protein-7 (BMP-7) (R&D Systems), and 250 ng/mL hepatocyte growth factor (HGF) (Sigma). Cell aggregates in the "half-Matrigel" solution were cultured for up to 28 days to yield organoids.

Measurement of the organoid surface area

An All-in-one Fluorescence Microscope (BZ-X700, Keyence, Osaka, Japan) was used to monitor the organoids. To measure the surface area of the organoid, bright-field images were selected every 10 μm in the Z-direction from top to bottom of the organoid. A BZ-X Analyzer (Keyence) was used to synthesize these screenshots and construct a full-focus image. The cell area was selected using the mask function in the full-focus image, and the surface area of the organoid was measured. Twelve organoids were used for surface area measurements.

Drug treatment and toxicity assay

For toxicity assays, organoids cultured in the "half-Matrigel" on day 14 were placed in the differentiation medium supplemented with 0, 10, or 20 μM cisplatin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) up to day 20. To quantify cell death in organoids, the cells were stained with propidium iodide (PI), which was incorporated into the nuclei of the dead cells. On day 14, organoids cultured in the "half-Matrigel" were placed in contact with a differentiation medium supplemented with 0, 10, 20, 25, or 50 μM cisplatin up to day 19 ($n = 4$ for each). On day 19, the medium was removed and replaced with a medium containing PI (final concentration 1 μg/mL). Organoids were monitored, and PI-positive sites were quantified on day 20. The area of PI-positive sites in the organoids was measured using an All-in-one Fluorescence Microscope (BZ-X700, Keyence). A fluorescence filter (TRITC OP-87764, Keyence) with excitation and observation wavelengths of 545 nm and 605 nm, respectively, was used for monitoring the PI uptake using a dedicated software.

Statistical analyses

The significances of observed differences in the results of the measurement of the organoid surface area and the toxicity assay of cisplatin were analyzed using a Student's t-test. Differences showing P values < 0.05 were considered significant. F-tests were used to confirm variances for the measurements.

RESULTS

Chronological observation of NLS formation

The cell aggregates of rKS cells obtained from the hanging drop technique were embedded in the Matrigel in Transwell inserts, and the three-dimensional culture was set up in the differentiation medium containing five growth factors (GDNF, bFGF, EGF, BMP-7, and HGF) (Fig. 1A). Reconstruction of the NLS with time was observed using a stereomicroscope. On days 5 and 7 of 3D culture, cyst and tubule-like structures were observed, respectively. Moreover, the growth of tubules was prominent from days 14–21. Minimal growth was observed from days 21–28 (Fig. 1B).

Measurement of the surface area of simultaneously constructed organoids

To accurately analyze the effects of cisplatin exposure, the prepared organoids must be of optimal quality and

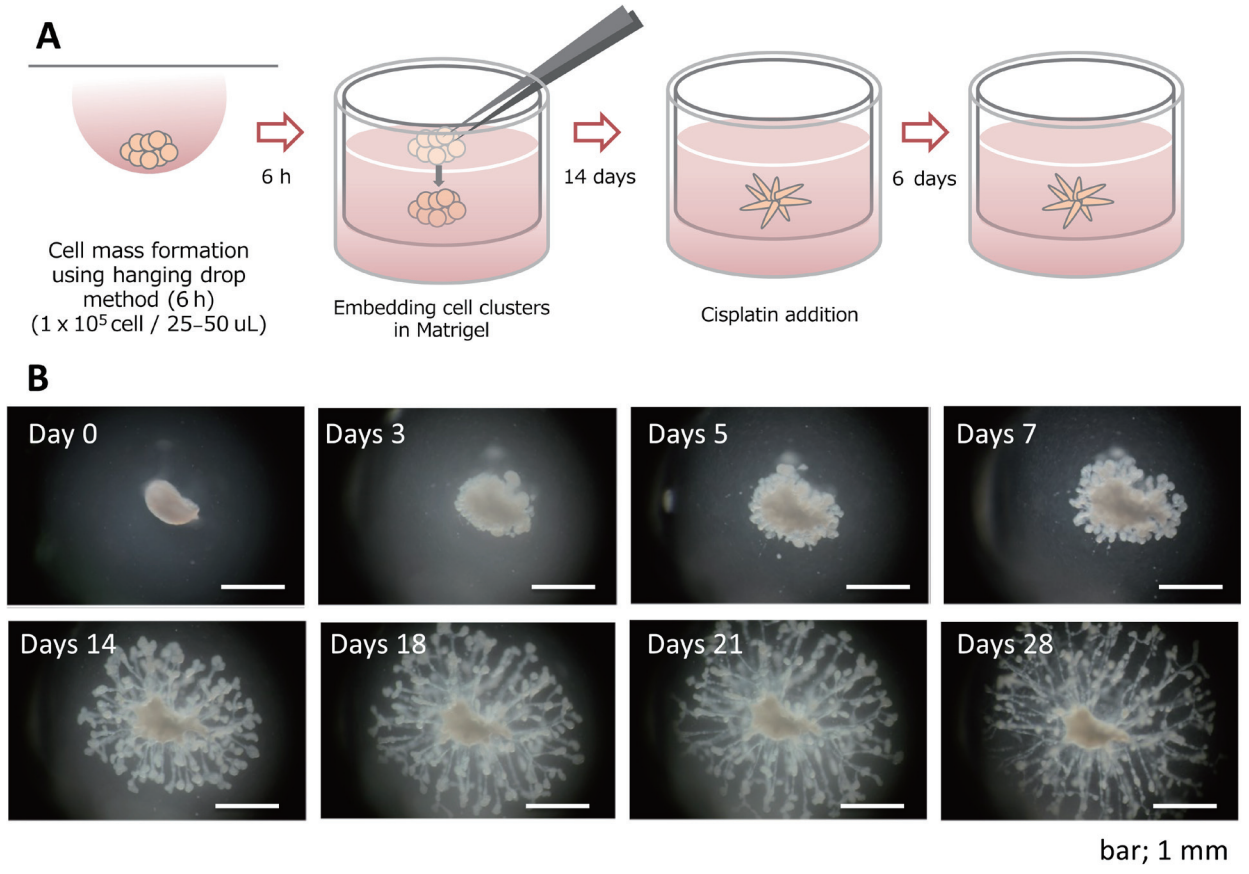

Fig. 1. Morphological observation of nephron-like structures (NLS) over time. **A**) Schematic representation depicting the development of NLS from rKS cells. **B**) Morphology of NLS during 3D culture. The point at which the cell clusters were embedded in Matrigel was set as day 0. Bar = 1 mm.

standard. Twelve organoids were prepared simultaneously, and their shapes and sizes were compared. After two weeks of 3D culture, all rKS cell aggregates differentiated to organoids with NLS (Fig. 2A). Quantitative analysis of organoid size was performed using a microscope with a sectioning function. The difference in surface areas between the 12 organoids was not significant (Fig. 2B). The variation range in the surface area was within ± 10% of the average surface area.

Cisplatin induces cell death in NLS

On day 14 of culture, the NLS were exposed to cisplatin for six days. The results from the PI assay confirmed that cisplatin induced cell death in these organoids. PI staining was observed in the central region of the control (no cisplatin treatment) and cisplatin-treated organoids. In contrast, PI staining in the tubular and cystic structures was observed only in the cisplatin-treated group (Fig. 3A). The PI uptake area increased by six- and seven-fold in the 10 μM and 20 μM cisplatin exposed groups, respectively, compared with that in the control (Figs. 3B and C). PI uptake was significantly higher in the 20 μM cisplatin exposed group than in the 10 μM cisplatin exposed group, suggesting a dose-dependent relationship between the amount of cisplatin and PI uptake. These results indicate that cisplatin induced cell death in the tubular and cystic structures of the organoids.

Cisplatin inhibits the growth of NLS

Time-based observations of NLS formation in the organoids exhibited expansion of the tubular structure from days 14–21 (Fig. 1B). A 1.6-fold increase in the surface area of the four non-treated samples was observed during this period (Fig. 4A). However, on day 20, a 20% reduction in the surface area of NLS was observed in both the 10 μM and 20 μM cisplatin exposed groups compared to with the control (Fig. 4B). These results demonstrate that cisplatin inhibited the growth of NLS in organoids.

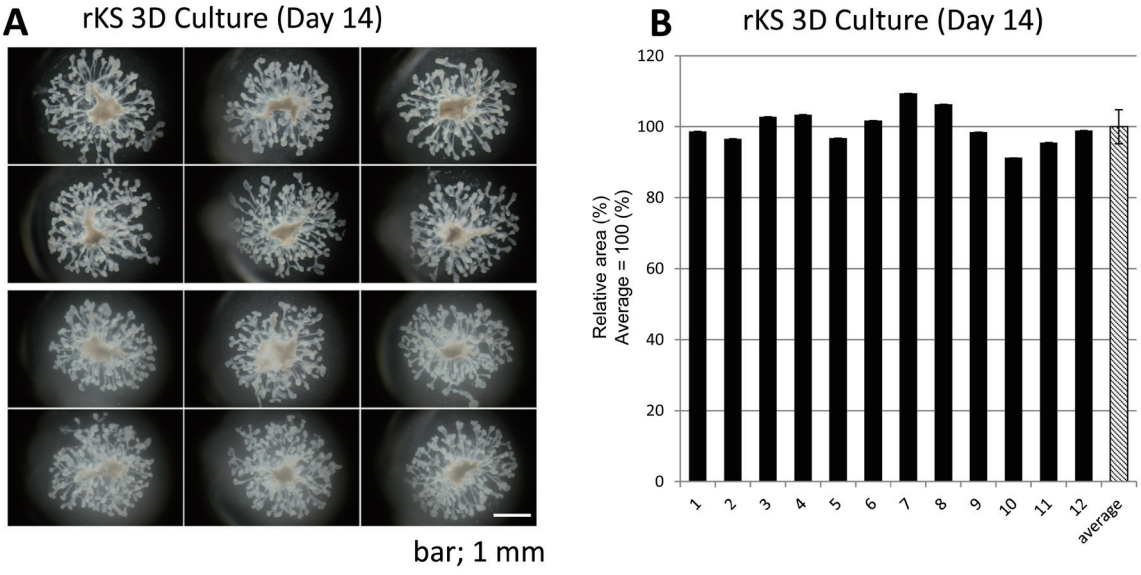

Fig. 2. Simultaneous culture and morphological observation of 12 nephron-like structures (NLS). **A**) Microscopic images of 12 NLS on day 14. Bar = 1 mm. **B**) Surface area of 12 individual NLS relative to the average surface area of NLS.

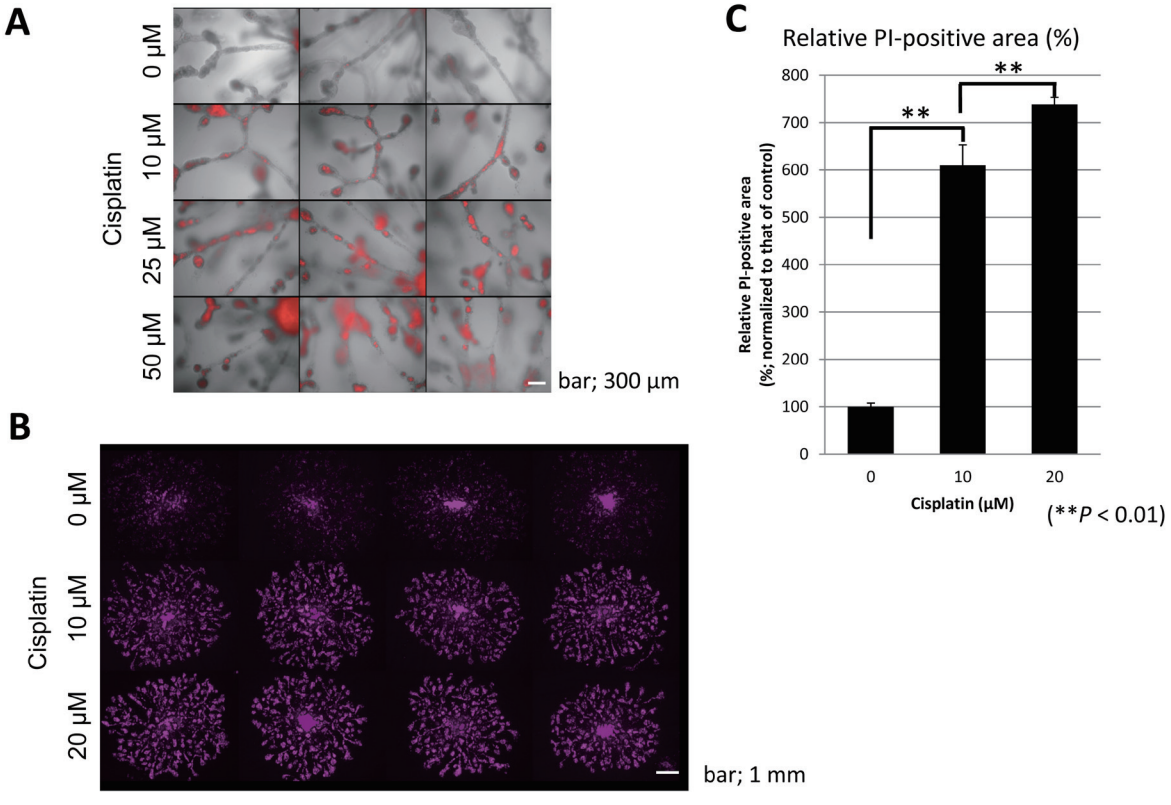

Fig. 3. Cisplatin-induced cell death in NLS. **A**) Fluorescence microscopy image depicting the PI uptake (enlarged view of the tubular structure). Bar = 300 μm. **B**) Fluorescence microscopy image depicting the PI uptake (overview of NLS). Bar = 1 mm. **C**) PI-positive areas in the NLS after cisplatin exposure ($n = 4$ under each concentration). $^{**}P < 0.01$; Student's *t*-test.

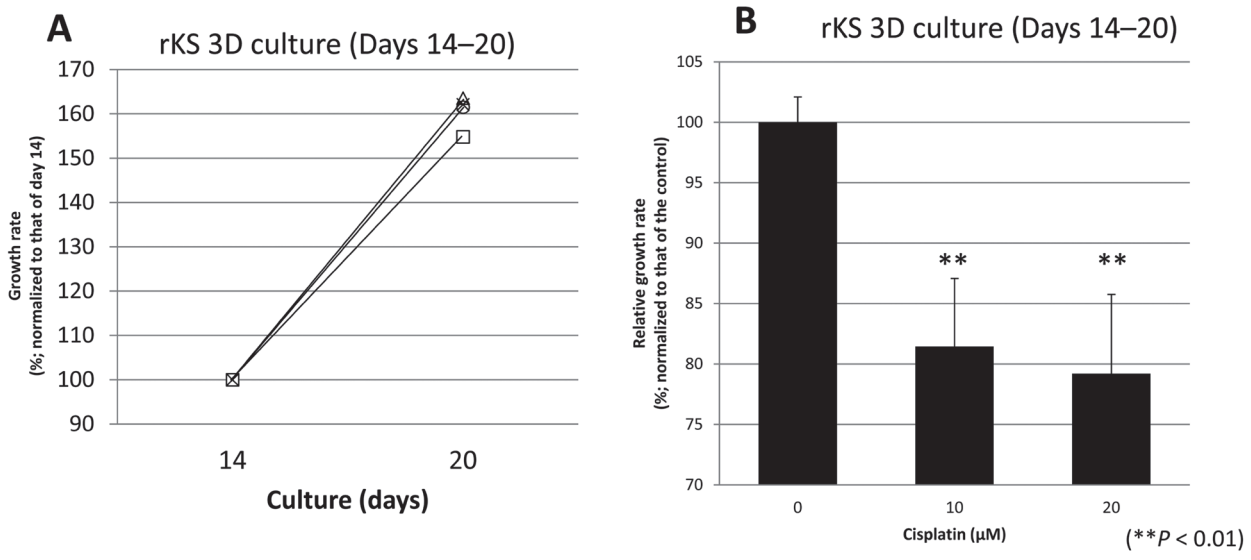

Fig. 4. Cisplatin-induced inhibition of NLS growth. **A**) Growth rate from days 14–20 under cisplatin-free conditions ($n = 4$ for each condition). **B**) Cisplatin-induced inhibition of NLS growth relative to that without cisplatin treatment ($n = 4$ for each condition). $^{**}P < 0.01$; Student's *t*-test.

DISCUSSION

In this study, elongation of NLS was monitored over time under three-dimensional culture, thereby recapitulating the organoid culture reported by Kitamura et al. The surface areas of the 12 reconstituted organoids were similar, with minimal variation among them. Performing cell culture under stable quality control is crucial to ensure the reliability of test results; however, maintaining stable culture conditions remains a challenge. The characteristics and quality of organoids vary substantially depending on the facilities and the human iPS cell line used, leading to poor reproducibility of the *in vitro* models.[16] The findings presented in this study suggest that the 3D cell culture system may be suitable for *in vitro* toxicity assays to maintain quality.

However, the size and morphology of organoids were not compared at various times of culture in this study. This will be further investigated in the follow-up studies.

The organoids used in this study were derived from rKS cells, which are renal stem progenitor cells derived from the S3 segment of the rat proximal tubules. In animal studies using rats, cisplatin reportedly induces cell death in the proximal tubules, especially in the S3 segment.[15] Based on these findings, cisplatin was assumed to be highly cytotoxic to the rat kidney organoid. Concordantly, exposure to cisplatin induced cell death of NLS, and the rate of increase in the surface area of organoids from days 14–20 was suppressed compared with that in the control group. These results suggest that the *in vitro* cisplatin-induced cell death may be similar to *in vivo* AKI. However, further studies are required to determine whether the cell death observed in this study is attributed to cytotoxicity or the induction of proximal tubule-targeted cell death. In a report on rKS cells-derived organoids, cisplatin induced apoptosis and DNA damage in cells constituting the NLS.[17] It is important to further elucidate the extent to which the mechanism of cisplatin nephrotoxicity is mimicked in the organoids through comparative studies from a histopathological or biochemical perspective.

In conclusion, we have validated the application of kidney cell-derived organoids with NLS as a viable model for assessing nephrotoxicity using a well-established anticancer drug. Therefore, our study would serve as a valuable addition to the current trend of developing novel drug screening methods using organoids and microphysiological systems. Our study also contributes to animal welfare by providing a feasible replacement for animal models. We believe that our model may also be used to assess the effects of other nephrotoxic substances on NLS in the future.

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The authors declare no conflict of interest.

REFERENCES

1 Ferguson MA, Vaidya VS, Bonventre JV. Biomarkers of nephrotoxic acute kidney injury. Toxicology. 2008;245:182-93. DOI: 10.1016/j.tox.2007.12.024, PMID: 18294749

2 Griffin BR, Faubel S, Edelstein CL. Biomarkers of Drug-Induced Kidney Toxicity. Ther Drug Monit. 2019;41:213-26. DOI: 10.1097/FTD.0000000000000589, PMID: 30883514

3 Te JA, AbdulHameed MDM, Wallqvist A. Systems toxicology of chemically induced liver and kidney injuries: histopathology-associated gene co-expression modules. J Appl Toxicol. 2016;36:1137-49. DOI: 10.1002/jat.3278, PMID: 26725466

4 Marx U, Andersson TB, Bahinski A, Beilmann M, Beken S, Cassee FR, et al. Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. Altern Anim Exp. 2016;33:272-321. DOI: 10.14573/altex.1603161, PMID: 27180100

5 Saxén L. Organogenesis of the kidney. Cambridge University Press; 1987.

6 Dressler GR. Advances in early kidney specification, development and patterning. Development. 2009;136:3863-74. DOI: 10.1242/dev.034876, PMID: 19906853

7 Heussner AH, Dietrich DR. Primary porcine proximal tubular cells as an alternative to human primary renal cells in vitro: an initial characterization. BMC Cell Biol. 2013;14:55. DOI: 10.1186/1471-2121-14-55, PMID: 24308307

8 Wilmer MJ, Ng CP, Lanz HL, Vulto P, Suter-Dick L, Masereeuw R. Kidney-on-a-chip technology for drug-induced nephrotoxicity screening. Trends Biotechnol. 2016;34:156-70. DOI: 10.1016/j.tibtech.2015.11.001, PMID: 26708346

9 Wieser M, Stadler G, Jennings P, Streubel B, Pfaller W, Ambros P, et al. hTERT alone immortalizes epithelial cells of renal proximal tubules without changing their functional characteristics. Am J Physiol Renal Physiol. 2008;295:F1365-75. DOI: 10.1152/ajprenal.90405.2008, PMID: 18715936

10 Rindler MJ, Chuman LM, Shaffer L, Saier MH Jr. Retention of differentiated properties in an established dog kidney epithelial cell line (MDCK). J Cell Biol. 1979;81:635-48. DOI: 10.1083/jcb.81.3.635, PMID: 222773

11 Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature. 2015;526:564-8. DOI: 10.1038/nature15695, PMID: 26444236

12 Morizane R, Lam AQ, Freedman BS, Kishi S, Valerius MT, Bonventre JV. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. Nat Biotechnol. 2015;33:1193-200. DOI: 10.1038/nbt.3392, PMID: 26458176

13 Kitamura S, Sakurai H, Makino H. Single adult kidney stem/progenitor cells reconstitute three-dimensional nephron structures in vitro. Stem Cells. 2015;33:774-84. DOI: 10.1002/stem.1891, PMID: 25422083

14 Kitamura S, Yamasaki Y, Kinomura M, Sugaya T, Sugiyama H, Maeshima Y, et al. Establishment and characterization of renal progenitor like cells from S3 segment of nephron in rat adult kidney. FASEB J. 2005;19:1789-97. DOI: 10.1096/fj.05-3942com, PMID: 16260649

15 Herrera-Pérez Z, Gretz N, Dweep H. A Comprehensive Review on the Genetic Regulation of Cisplatin-induced Nephrotoxicity. Curr Genomics. 2016;17:279-93. DOI: 10.2174/1389202917666160202220555, PMID: 27252593

16 Pamies D, Bal-Price A, Simeonov A, Tagle D, Allen D, Gerhold D, et al. Good Cell Culture Practice for stem cells and stem-cell-derived models. Toxicol Lett. 2017;280:S80-1. DOI: 10.1016/j.toxlet.2017.07.196, PMID: 27554434

17 Ueno S, Kokura K, Kuromi Y, Osaki M, Okada F, Kitamura S, et al. Kidney organoid derived from renal tissue stem cells is a useful tool for histopathological assessment of nephrotoxicity in a cisplatin-induced acute renal tubular injury model. J Toxicol Pathol. 2022;35:333-43. DOI: 10.1293/tox.2022-0006, PMID: 36406172