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P-417

Development of the nephrotoxicity evaluation system by nucleic acid drugs using 3D-cultured human renal proximal tubule epithelial cells

3次元培養ヒト近位尿細管上皮細胞を用いた核酸医薬品による腎毒性評価系の構築 評価化合物の拡充とバイオマーカー探索

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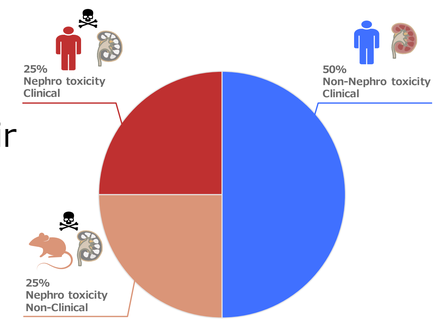
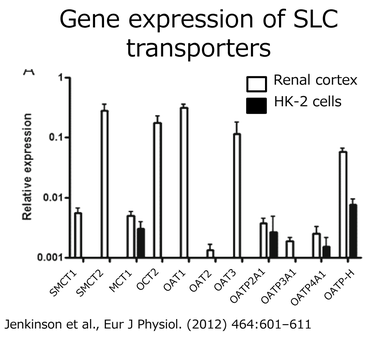
Introduction

In recent years, the development of new modality drugs has been progressing.
Among them, nucleic acid drugs are high specificity, but thrombocytopenia, complement activation, hepatotoxicity and nephrotoxicity have been reported in many clinical trials.
The cost of synthesizing nucleic acid drugs is significantly higher than that of small molecules, and their high specificity makes it difficult to detect toxicity in animal studies, so in silico analysis and in vitro studies using human cells are being conducted. Currently, studies on the detection of nephrotoxicity have not progressed sufficiently due to the lack of kidney cells with well-maintained renal function.
In this study, we attempted to develop the evaluation of nephrotoxicity by nucleic acid drugs using 3D-cultured human renal proximal tubule epithelial cells.



(1) Nephrotoxicity rate of nucleic acid drug (Clinical)



Jenkinson et al., Eur J Physiol. (2012) 464:601–611

(2) Function of commercially available kidney cells

Conclusion

1. プロテオーム測定により、2Dと3D-RPTECの機能を比較

- 3D-RPTECでは、生体の近位尿細管のような嫌気性代謝に関連するタンパク発現が増加
- 3D-RPTECでは、細胞分裂に関連するタンパク発現が減少
- 2Dと3Dではトランスポーターの発現が大きく異なり、3D-RPTECは核酸医薬品の評価に適していると考えられる、エンドサイトーシス関連のタンパク発現が増加していた。

2. バイオマーカーやHCAによって核酸医薬品の腎毒性を評価可能

- ATP測定では核酸医薬品の毒性評価するために曝露期間が必要
- バイオマーカー評価やHCAは、高感度に腎毒性評価が可能
- バイオマーカー評価やHCAは、核酸医薬品の毒性機序の解明に有用

問い合わせ先

日機装株式会社
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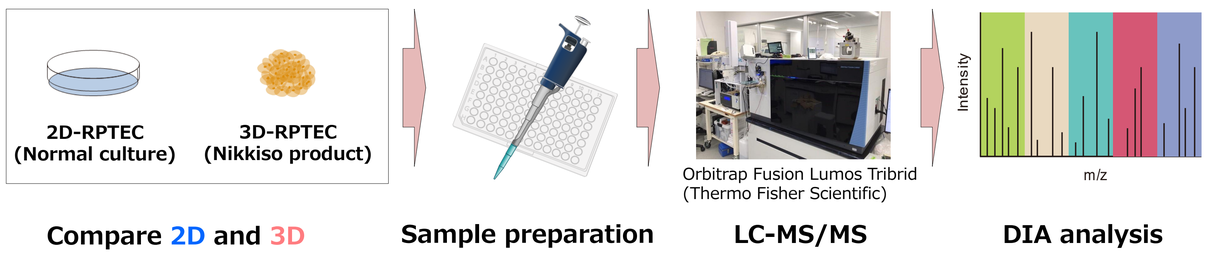
https://www.nikkiso.co.jp/products/medical/3drptec/

1. Features of 3D-RPTEC

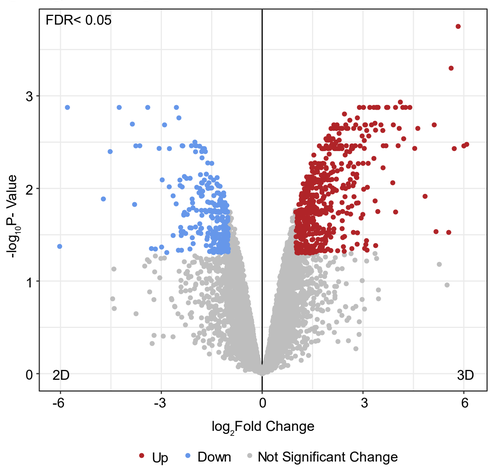
3D-RPTEC® : RPTECs were cultured as spheroids in ultra-low attachment 96-well culture plate. The medium was changed once every 2-3 days.3D-RPTEC® can be available from Nikkiso.

(Fig.1-A) Proteome analysis methods

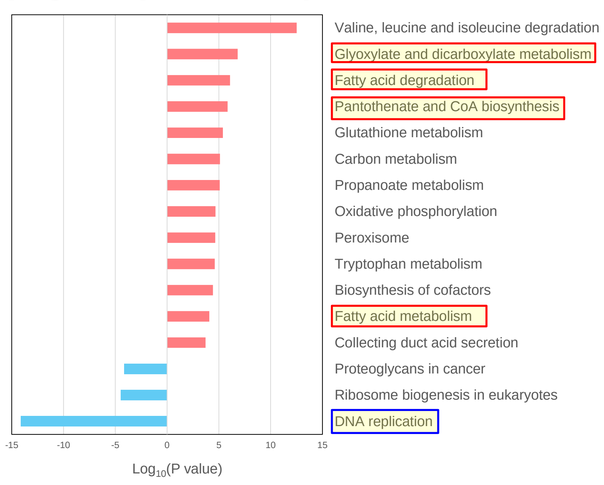


- After harvesting cells from both 2D and 3D RPTEC cultures, proteins were extracted following a series of preparatory steps, including reduction, alkylation, enzymatic digestion, and desalting.
- The extracted peptides were analyzed using LC-MS/MS.
- Data acquisition was performed using the Data Independent Acquisition (DIA) method.

(Fig.1-B) Volcano plot

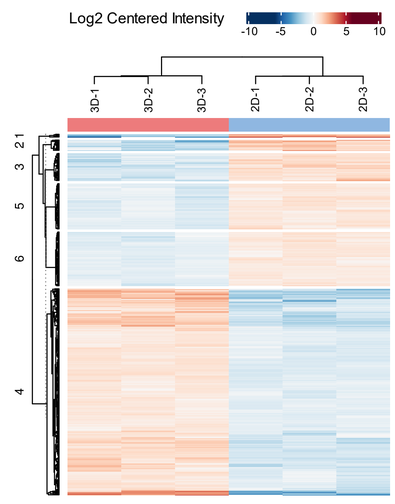


(Fig.1-C) GO analysis

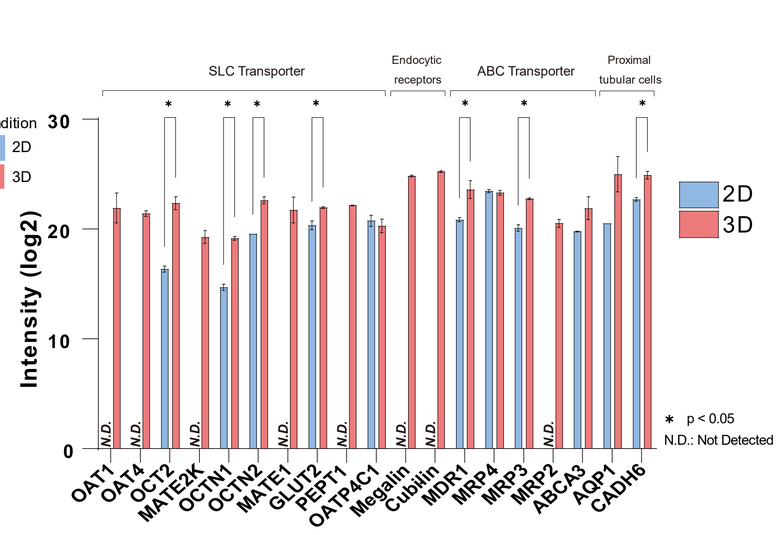


- The results of the volcano plot (Fig. 1-B), we identified and analyzed proteins that were significantly upregulated or downregulated in 3D-RPTEC compared to 2D-RPTEC.
- 3D-RPTEC showed increased expression of proteins involved in anaerobic metabolism, such as fatty acid and pyruvate metabolism, resembling the metabolic profile of *in vivo* RPTEC.
- Conversely, functions related to cell division, including DNA synthesis, were found to be decreased (Fig. 1-C).

(Fig.1-D) Cluster analysis



(Fig.1-F) Transporter expression



- Proteomic analysis revealed marked differences in protein expression profiles between 2D and 3D cultured RPTEC (Fig.1-D).
- Focused analysis on transporter proteins demonstrated that the expression of solute carrier (SLC) transporters and ATP-binding cassette (ABC) transporters were significantly increased in 3D-RPTEC (Fig.1-F).
- The increased expression levels of SLC and ABC transporters under 3D culture conditions are likely due to the enhanced physiological relevance of the model, as three-dimensional culturing more closely mimics the *in vivo* functions of RPTEC.

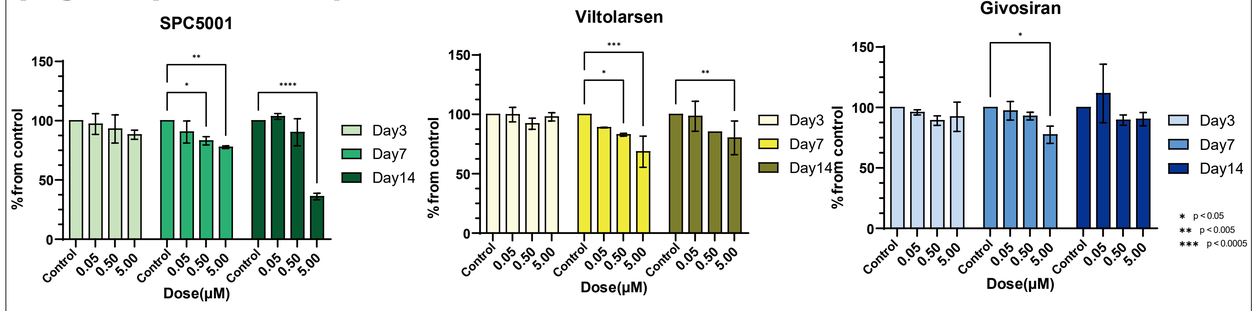
2. Toxicological Assay Result

Test Compounds	Type	Cat.No.	MW (g/mol)	Modification	Manufacturer
SPC5001	Antisence Oligo	None	4689.85	PS, LNA, 5mC	Ajinomoto Bio-Pharma Services.Inc
Viltolarsen	Antisence Oligo	HY-132586A	7386.42	PMO	MedChemExpress
Givosiran	siRNA	HY-132610	16300.6	PS, 2’-Ome, 2’-F	MedChemExpress

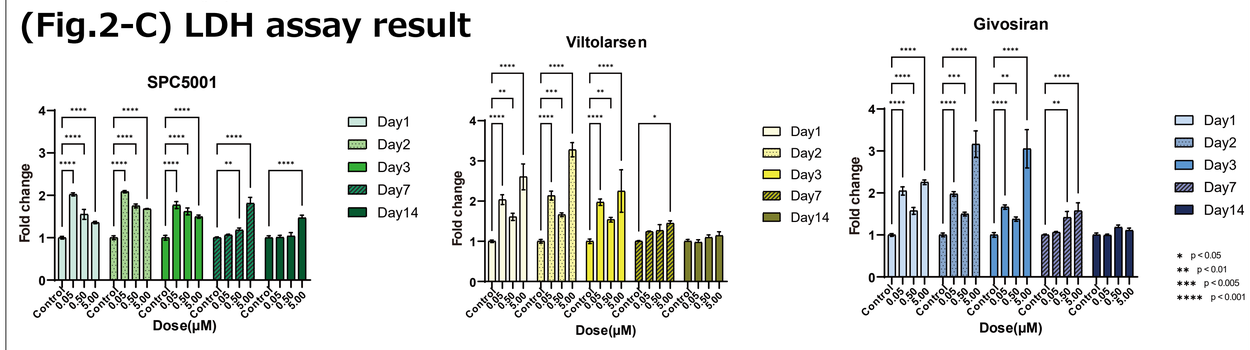
(Table.2-A) Nucleic acid drugs

(Fig.2-A) ATP, LDH and Biomarker assay methods

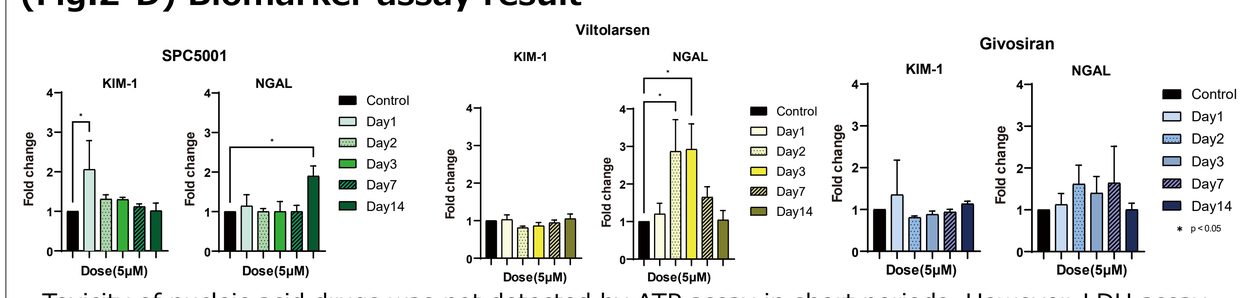
(Fig.2-B) ATP assay result



(Fig.2-C) LDH assay result



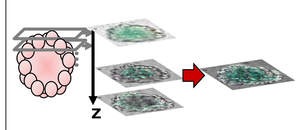
(Fig.2-D) Biomarker assay result

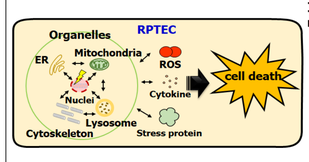


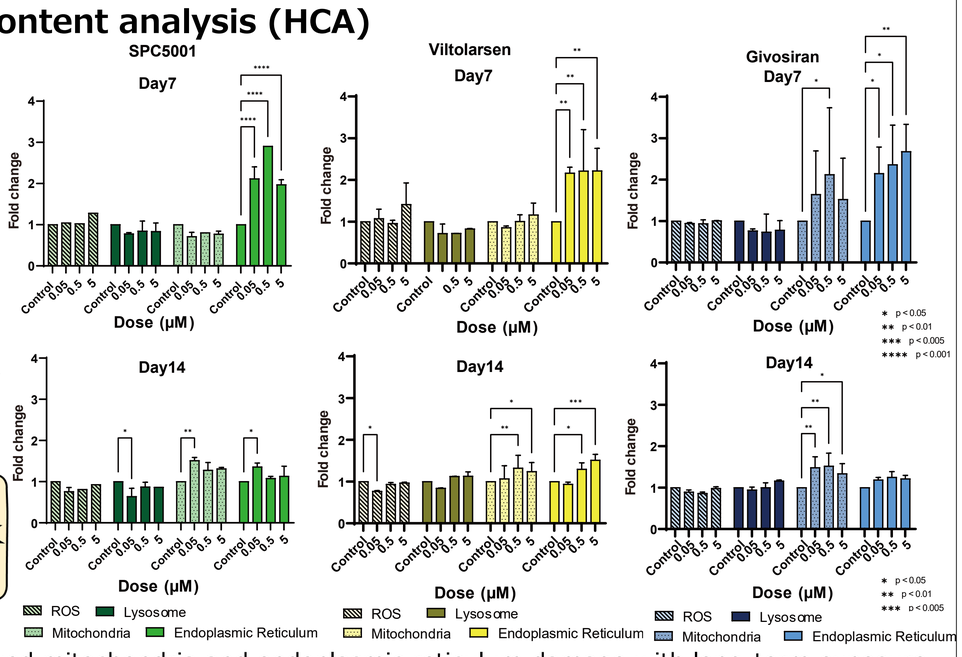
- Toxicity of nucleic acid drugs was not detected by ATP assay in short periods. However, LDH assay was detected the toxicity in short periods(Fig.2-B and 2-C).
- Toxicity of viltolarsen was detected by NGAL assay(Fig.2-D). Biomarker assessment enables highly sensitive evaluation of nephrotoxicity.

(Fig.2-E) High content analysis (HCA)

Confocal Quantitative Image Cytometer, CQ1(Yokogawa Electric Corporation)







The result of HCA showed mitochondria and endoplasmic reticulum damage with long-term exposure of nucleic acid drugs (one and two weeks).

COI Disclosure Information

I have no COI regarding this presentation.
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Organization: Nikkiso Co. Ltd

Acknowledgement

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References

(1) Jenkinson et al., Eur J Physiol. (2012) 464:601–611
(2) C Weiland et al., Toxicology in Vitro (2007)21:466–491