第７回臨床検査学ランチョンセミナー

教室紹介とトランスレーショナルリサーチ

（基礎と臨床の橋渡し研究）

疾患モデル科学　長尾　静子 センター長

日時：７月17日(火)12：20～12：50

場所：１号館１階 学生ホール

教室紹介

http://www.fujita-hu.ac.jp/CAMHD/LAS/member/member.html

藤田保健衛生大学

疾患モデル科学

Laboratory Animal Science

長尾静子

研究立案や
まとめ役

釘田雅則

培養細胞を用いた解析
と分子遺伝学的解析

吉原大輔

疾患モデル動物を用いた解析
と臨床検査的解析

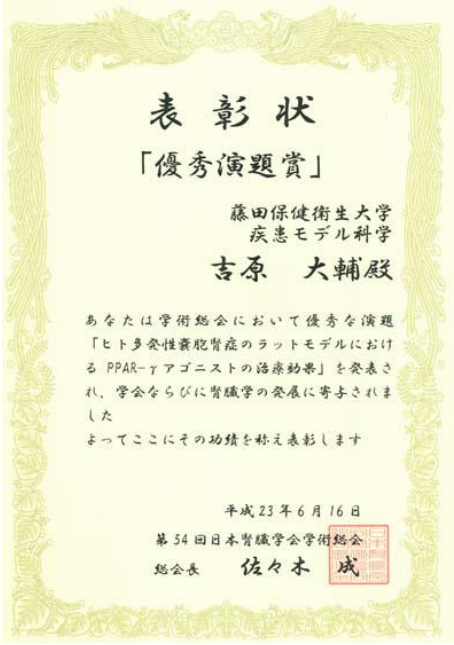
疾患モデル教育研究センター
医学部1号館B3

どんな研究をしているの？

病気の発症機序の解析と治療に向けた研究

日本腎臓学会で
優秀演題賞を頂きました！

第54回日本腎臓学会



表彰状

「優秀演題賞」

藤田保健衛生大学
疾患モデル科学
吉原　大輔殿

あなたは学術総会において優秀な演題
「ヒト多発性嚢胞腎症のラットモデルにおける PPAR-γアゴニストの治療効果」を発表され、学会ならびに腎臓学の発展に寄与されました
よってここにその功績を称え表彰します

平成23年6月16日
第54回日本腎臓学会学術総会
総会長　佐々木　成

2011

第55回日本腎臓学会

表彰状

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疾患モデル教育研究センター
吉原　大輔殿

あなたは学術総会において優秀な演題
「ラット多発性嚢胞腎における PPARγアゴニスト投与の治療効果に関する網羅的遺伝子発現プロファイリング」を発表され、学会ならびに腎臓学の発展に寄与されました
よってここにその功績を称え表彰します

平成24年6月2日
第55回日本腎臓学会学術総会
総会長　富田　公夫

2012

研究成果は、欧米の科学研究雑誌に掲載されました！

PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease

Daisuke Yoshihara, Hiroki Kurahashi, Miwa Morita, Masanori Kugita, Yoshiyuki Hiki, Harold M. Aukema, Tamio Yamaguchi, James P. Calvet, Darren P. Wallace and Shizuko Nagao

Am J Physiol Renal Physiol 300:F465-F474, 2011. First published 8 December 2010; doi:10.1152/ajprenal.00460.2010

Research Article

Global Gene Expression Profiling in PPAR-γ Agonist-Treated Kidneys in an Orthologous Rat Model of Human Autosomal Recessive Polycystic Kidney Disease

Daisuke Yoshihara,[1,2] Masanori Kugita,[1] Tamio Yamaguchi,[1,3] Harold M. Aukema,[3] Hiroki Kurahashi,[2] Miwa Morita,[1] Yoshiyuki Hiki,[4] James P. Calvet,[5] Darren P. Wallace,[5] Takafumi Toyohara,[6] Takaaki Abe,[6] and Shizuko Nagao[1]

研究成果は、国際腎臓学会でも報告

PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease

Daisuke Yoshihara, Hiroki Kurahashi, Miwa Morita, Masanori Kugita, Yoshiyuki Hiki, Harold M. Aukema, Tamio Yamaguchi, James P. Calvet, Darren P. Wallace, Shizuko Nagao

Education and Research Center of Animal Models for Human Diseases, Fujita Health University, Toyoake, Aichi, Japan
Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada
The Kidney Institute, University of Kansas Medical Center, Kansas City, KS, USA

INTRODUCTION AND AIMS

In autosomal recessive polycystic kidney disease (**ARPKD**), progressive enlargement of fluid-filled cysts is due to aberrant proliferation of tubule epithelial cells and transepithelial fluid secretion leading to extensive nephron loss and interstitial fibrosis. Congenital hepatic fibrosis associated with biliary cysts/dilatations is the most common extrarenal manifestation in ARPKD, and can lead to massive liver enlargement. Peroxisome proliferator-activated receptor-γ (**PPAR-γ**), a member of the ligand-dependent nuclear receptor superfamily, is expressed in a variety of tissues, including the kidneys and liver, and plays important roles in cell proliferation, fibrosis and inflammation. **In the current study, we determined that pioglitazone (PIO), a PPAR-γ agonist, decreases polycystic kidney and liver disease progression in the PCK rat, an orthologous model of human ARPKD.**

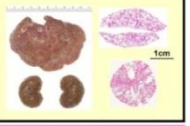
Method

birth — 4 weeks — Pioglitazone (PIO) 10 mg/Kg/day — 20 weeks

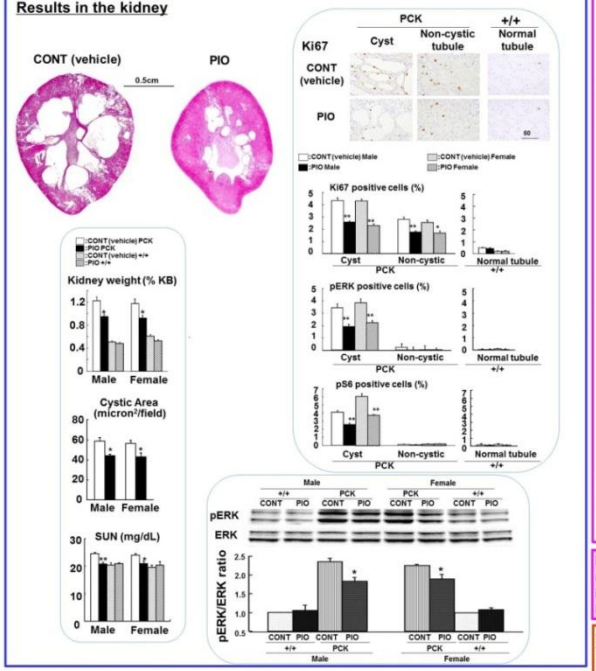
PCK rat:
Orthologous model of human ARPKD polycystic kidney and liver disease

Pioglitazone(PIO)
: PPAR-γ agonist
(well-known roles and effects)
glucose homeostasis
anti-proliferative effect
anti-fibrotic effect

Kidney weight (% of body weight)
Renal cystic area SUN
Liver weight (% of body weight)
Liver cystic area Fibrotic index
Blood sugar Serum AST
Total bilirubin levels
Ki67 ERK S6 TGF-β

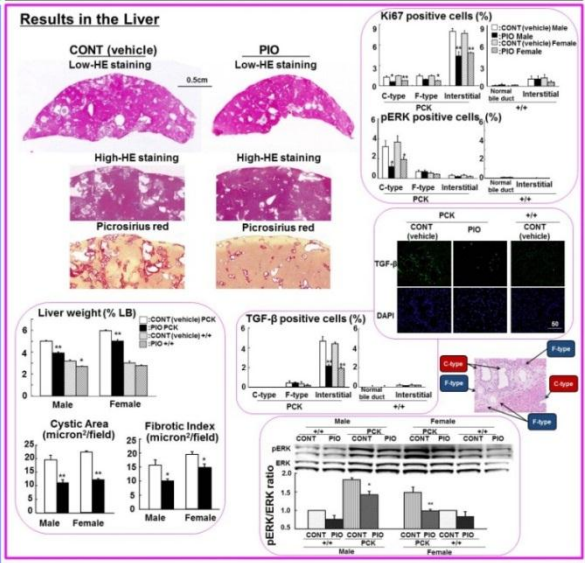


Results in the kidney



Daily treatment with 10 mg/kg PIO for 16 weeks decreased kidney weight (%KB), renal cystic area, SUN and the number of Ki67-, pERK1/2- and pS6-positive cells in the kidney.
Body weight, food and water intake, fibrotic index and the number of TGF-β of gender-matched PCK and +/+ rats did not differ at 20 weeks of age and was unaffected by treatment with PIO.

Results in the Liver

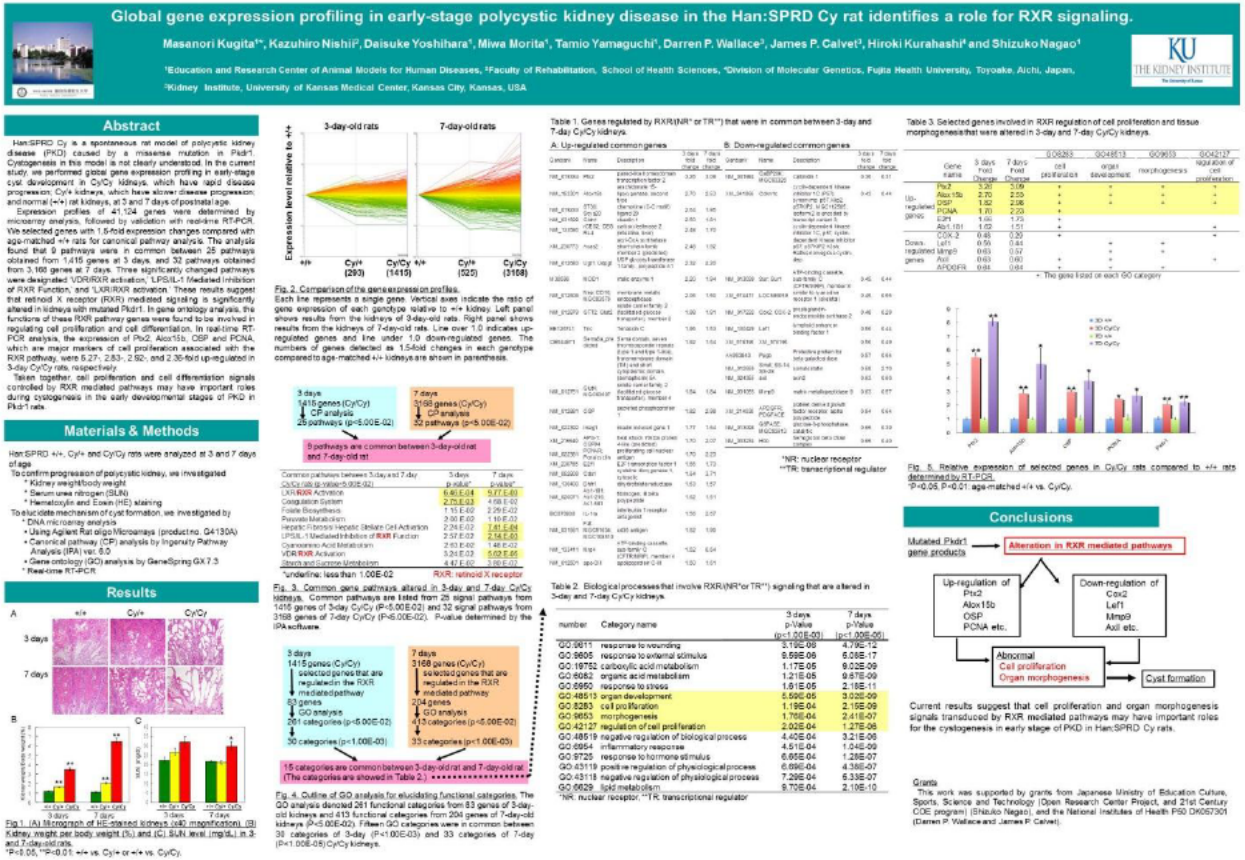


PIO-treatment decreased liver weight (%LB), liver cystic area, fibrotic index, and the number of Ki67-, pERK1/2-, pERK5- and TGF-β-positive cells in the liver.
Blood sugar levels, serum AST, total bilirubin levels and the number of pS6 of gender-matched PCK and +/+ rats did not differ at 20 weeks of age and was unaffected by treatment with PIO.

CONCLUSIONS

The PPAR-γ agonist PIO ameliorates polycystic kidney disease in association with inhibition of the ERK and mTOR signaling pathways, and fibrocystic liver disease in association with inhibition of the ERK and TGF-β signaling pathways in PCK rats, an orthologous model of human ARPKD. These data support the use of PPAR-γ agonists as therapeutic agents for the treatment of polycystic kidney and liver disease in ARPKD patients.

別の研究成果を、米国腎臓学会に報告



Global gene expression profiling in early-stage polycystic kidney disease in the Han:SPRD Cy rat identifies a role for RXR signaling.

Masanori Kugita[1*], Kazuhiro Nishii[2], Daisuke Yoshihara[1], Miwa Morita[1], Tamio Yamaguchi[1], Darren P. Wallace[3], James P. Calvet[3], Hiroki Kurahashi[1] and Shizuko Nagao[1]

[1]Education and Research Center of Animal Models for Human Diseases, [2]Faculty of Rehabilitation, School of Health Sciences, [1]Division of Molecular Genetics, Fujita Health University, Toyoake, Aichi, Japan,

[3]Kidney Institute, University of Kansas Medical Center, Kansas City, Kansas, USA

Abstract

Han:SPRD Cy is a spontaneous rat model of polycystic kidney disease (PKD) caused by a missense mutation in Pkdr1. Cystogenesis in this model is not clearly understood. In the current study, we performed global gene expression profiling in early-stage cyst development in Cy/Cy kidneys, which have rapid disease progression; Cy/+ kidneys, which have slower disease progression; and normal (+/+) rat kidneys, at 3 and 7 days of postnatal age.

Expression profiles of 41,124 genes were determined by microarray analysis, followed by validation with real-time RT-PCR. We selected genes with 1.5-fold expression changes compared with age-matched +/+ rats for canonical pathway analysis. The analysis found that 9 pathways were in common between 25 pathways obtained from 1,415 genes at 3 days, and 32 pathways obtained from 3,168 genes at 7 days. Three significantly changed pathways were designated 'VDR/RXR activation,' 'LPS/IL-1 Mediated Inhibition of RXR Function,' and 'LXR/RXR activation.' These results suggest that retinoid X receptor (RXR) mediated signaling is significantly altered in kidneys with mutated Pkdr1. In gene ontology analysis, the functions of these RXR pathway genes were found to be involved in regulating cell proliferation and cell differentiation. In real-time RT-PCR analysis, the expressions of Pkd2, Alox15b, OSP and PCNA, which are major markers of cell proliferation associated with the RXR pathway, were 6.27-, 2.83-, 2.92-, and 2.36-fold up-regulated in 3-day Cy/Cy rats, respectively.

Taken together, cell proliferation and cell differentiation signals controlled by RXR mediated pathways may have important roles during cystogenesis in the early developmental stages of PKD in Pkdr1 rats.

Materials & Methods

Han:SPRD +/+, Cy/+ and Cy/Cy rats were analyzed at 3 and 7 days of age.

To confirm progression of polycystic kidney, we investigated
- * Kidney weight/body weight
- * Serum urea nitrogen (SUN)
- * Hematoxylin and Eosin (HE) staining

To elucidate mechanism of cyst formation, we investigated by
- * DNA microarray analysis
- • Using Agilent Rat oligo Microarrays (product no. G4130A)
- • Canonical pathway (CP) analysis by Ingenuity Pathway Analysis (IPA) ver. 6.0
- • Gene ontology (GO) analysis by GeneSpring GX 7.3
- * Real-time RT-PCR

Results

Fig 1. (A) Micrograph of HE-stained kidneys (x40 magnification). (B) Kidney weight per body weight (%) and (C) SUN level (mg/dL) in 3- and 7-day-old rats.
*P<0.05, **P<0.01 +/+ vs. Cy/+ or +/+ vs. Cy/Cy.

Fig. 2. Comparison of the gene expression profiles.
Each line represents a single gene. Vertical axes indicate the ratio of gene expression of each genotype relative to +/+ kidney. Left panel shows results from the kidneys of 3-day-old rats. Right panel shows results from the kidneys of 7-day-old rats. Line over 1.0 indicates up-regulated genes and line under 1.0 down-regulated genes. The numbers of genes detected as 1.5-fold changes in each genotype compared to age-matched +/+ kidneys are shown in parenthesis.

Fig. 3. Common gene pathways altered in 3-day and 7-day Cy/Cy kidneys. Common pathways are listed from 25 signal pathways from 1415 genes of 3-day Cy/Cy (P<5.00E-02) and 32 signal pathways from 3168 genes of 7-day Cy/Cy (P<5.00E-02). P-value determined by the IPA software.

Fig. 4. Outline of GO analysis for elucidating functional categories. The GO analysis denoted 261 functional categories from 83 genes of 3-day-old kidneys and 413 functional categories from 204 genes of 7-day-old kidneys (P<5.00E-02). Fifteen GO categories were in common between 30 categories of 3-day (P<1.00E-03) and 33 categories of 7-day (P<1.00E-05) Cy/Cy kidneys.

Table 1. Genes regulated by RXR(NR* or TR**) that were in common between 3-day and 7-day Cy/Cy kidneys.

A: Up-regulated common genes

Gene Symbol	Name	Description	3 days fold change	7 days fold change
Hsd3b5		hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5	3.05	3.08
Alox15b	Alox15b	lipoxygenase, arachidonic type	2.70	2.23
Slc22a2	Slc22a2	solute carrier family 22 (organic cation transporter), member 2	2.64	1.65
Cyp4b1	Cyp4b1	cytochrome P450, family 4, subfamily b, polypeptide 1	2.60	1.61
Cldn2	Cldn2	claudin 2	2.58	1.70
Hsd17b2	Hsd17b2	hydroxysteroid (17-beta) dehydrogenase 2	2.40	1.52
Ugt1a6	Ugt1a6	UDP glucuronosyltransferase 1 family, polypeptide A6	2.32	2.20
Mod1	Mod1	malic enzyme 1	2.23	1.64
Slc22a5	Slc22a5	solute carrier family 22 (organic cation/carnitine transporter), member 5	2.06	1.60
Slc2a2	Slc2a2	solute carrier family 2 (facilitated glucose transporter), member 2	1.99	1.91
Tkt	Tkt	transketolase	1.95	1.53
Serpina_pre	Serpina_pre	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	1.82	1.54
Cyp2c23	Cyp2c23	cytochrome P450, family 2, subfamily c, polypeptide 23	1.54	1.54
Cdh1	Cdh1	cadherin 1	1.52	2.08
Hsd3b1	Hsd3b1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	1.77	1.64
Mpv17	Mpv17	MPV17 mitochondrial membrane protein	1.70	2.07
Pon3	Pon3	paraoxonase 3	1.70	2.23
Cdh1	Cdh1	cadherin 1	1.64	2.71
Aldh1b1	Aldh1b1	aldehyde dehydrogenase 1 family, member B1	1.62	1.57
Aldh1a1	Aldh1a1	aldehyde dehydrogenase 1 family, member A1	1.62	1.61
Ttr	Ttr	transthyretin	1.50	1.57
Kcnj16	Kcnj16	potassium inwardly-rectifying channel, subfamily J, member 16	1.62	1.58
Ugt1a1	Ugt1a1	UDP glucuronosyltransferase 1 family, polypeptide A1	1.62	1.64
Slc2a1	Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1	1.62	1.61

B: Down-regulated common genes

Gene Symbol	Name	Description	3 days fold change	7 days fold change
Ctsh	Ctsh	cathepsin H	0.36	0.31
Ccnd1	Ccnd1	cyclin D1	0.43	0.44
Slc1a3	Slc1a3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	0.46	0.49
Lef1	Lef1	lymphoid enhancer binding factor 1	0.56	0.44
Ccnd2	Ccnd2	cyclin D2	0.46	0.29
Ccnd2	Ccnd2	cyclin D2	0.56	0.44
Ctsl	Ctsl	cathepsin L	0.57	0.64
Ctsl	Ctsl	cathepsin L	0.58	0.70
Axin2	Axin2	axin 2	0.62	0.63
Mmp9	Mmp9	matrix metallopeptidase 9	0.63	0.57
Cox2	Cox2	cytochrome c oxidase subunit VIIa polypeptide 2 like	0.64	0.64
Ccnd1	Ccnd1	cyclin D1	0.66	0.59
Mmp2	Mmp2	matrix metallopeptidase 2	0.66	0.59

*NR: nuclear receptor
**TR: transcriptional regulator

Table 2. Biological processes that involve RXR(NR*or TR**) signaling that are altered in 3-day and 7-day Cy/Cy kidneys.

number	Category name	3 days p-Value (p<1.00E-03)	7 days p-Value (p<1.00E-05)
GO:0011	response to wounding	3.19E-09	4.79E-12
GO:9605	response to external stimulus	9.59E-09	6.09E-17
GO:19752	carboxylic acid metabolism	1.17E-05	9.02E-09
GO:6082	organic acid metabolism	1.21E-05	9.87E-09
GO:9950	response to stress	1.61E-05	2.18E-11
GO:48513	organ development	5.59E-05	3.02E-09
GO:8283	cell proliferation	1.19E-04	2.15E-09
GO:9653	morphogenesis	1.76E-04	2.41E-07
GO:42127	regulation of cell proliferation	2.02E-04	1.27E-06
GO:48519	negative regulation of biological process	4.40E-04	3.21E-06
GO:6954	inflammatory response	4.51E-04	1.04E-09
GO:9725	response to hormone stimulus	6.95E-04	1.28E-07
GO:43119	positive regulation of physiological process	6.69E-04	4.38E-07
GO:43118	negative regulation of physiological process	7.29E-04	6.33E-07
GO:6629	lipid metabolism	9.70E-04	2.10E-10

*NR: nuclear receptor, **TR: transcriptional regulator

Table 3. Selected genes involved in RXR regulation of cell proliferation and tissue morphogenesis that were altered in 3-day and 7-day Cy/Cy kidneys.

	Gene name	3 days Fold Change	7 days Fold Change	GO:8283 cell proliferation	GO:48513 organ development	GO:9653 morphogenesis	GO:42127 regulation of cell proliferation
Up-regulated genes	Ptx2	3.20	3.09	+	+	+	+
	Alox15b	2.70	2.23	+	+	+	+
	OSP	1.82	2.06	+	+	+	+
	PCNA	1.70	2.03	+			
	EZH	1.66	1.73	+			
	Abi1-1B1	1.62	1.51	+			+
	COX-2	0.45	0.20	+			+
Down-regulated genes	Lef1	0.56	0.44		+	+	
	Mmp9	0.63	0.57		+	+	
	Axil	0.63	0.60	+	+	+	+
	APOBFR	0.64	0.64	+	+	+	

+: The gene listed on each GO category

Fig. 5. Relative expression of selected genes in Cy/Cy rats compared to +/+ rats determined by RT-PCR.
*P<0.05, P<0.01: age-matched +/+ vs. Cy/Cy.

Conclusions

Current results suggest that cell proliferation and organ morphogenesis signals transduced by RXR mediated pathways may have important roles for the cystogenesis in early stage of PKD in Han:SPRD Cy rats.

Grants
This work was supported by grants from Japanese Ministry of Education Culture, Sports, Science and Technology (Open Research Center Project, and 21st Century COE program) (Shizuko Nagao), and the National Institutes of Health P50 DK057301 (Darren P. Wallace and James P. Calvet).

欧米科学雑誌にも掲載

Global gene expression profiling in early-stage polycystic kidney disease in the Han:SPRD Cy rat identifies a role for RXR signaling

Masanori Kugita, Kazuhiro Nishii, Miwa Morita, Daisuke Yoshihara, Hiroe Kowa-Sugiyama, Kouji Yamada, Tamio Yamaguchi, Darren P. Wallace, James P. Calvet, Hiroki Kurahashi and Shizuko Nagao

Am J Physiol Renal Physiol 300:F177-F188, 2011. First published 6 October 2010; doi:10.1152/ajprenal.00470.2010

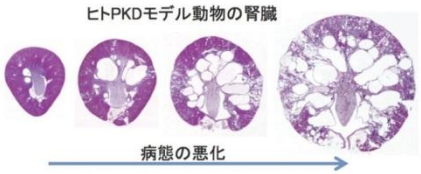
中部TLOニュース「CTLOTodayNo.12」

東北大学、慶應義塾大学と特許を共同出願

多発性嚢胞腎症（PKD）の早期診断・治療マーカーの開発

腎臓をはじめとする全身の諸臓器に多数の嚢胞が発生する多発性嚢胞腎症（PKD）は、人口1,000人に1人の患者が存在し、遺伝性疾患として極めて高い発症率を示します。患者の半数が慢性腎臓病（CKD）から末期腎不全（ESRD）に陥るため、人工透析や腎移植が必要となるばかりではなく、心血管疾患の発症リスクも高くなります。

そこで私共の研究グループでは、東北大学阿部高明教授および慶応義塾大学曽我朋義教授と共に、キャピラリー電気泳動—質量分析計を用いて、藤田保健衛生大学で維持しているPKD疾患モデル動物の血中尿毒症物質を明らかにし、PKDに固有な診断・治療マーカーを発見しました。この新しいマーカーを用いることで、本疾患の治療薬の開発にも大きく貢献することができます。



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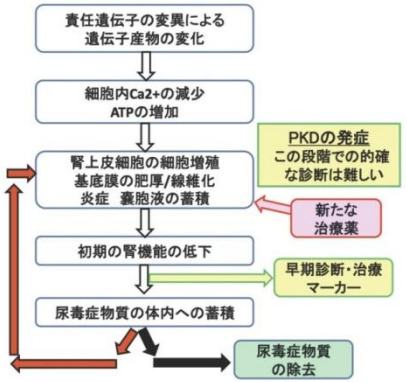
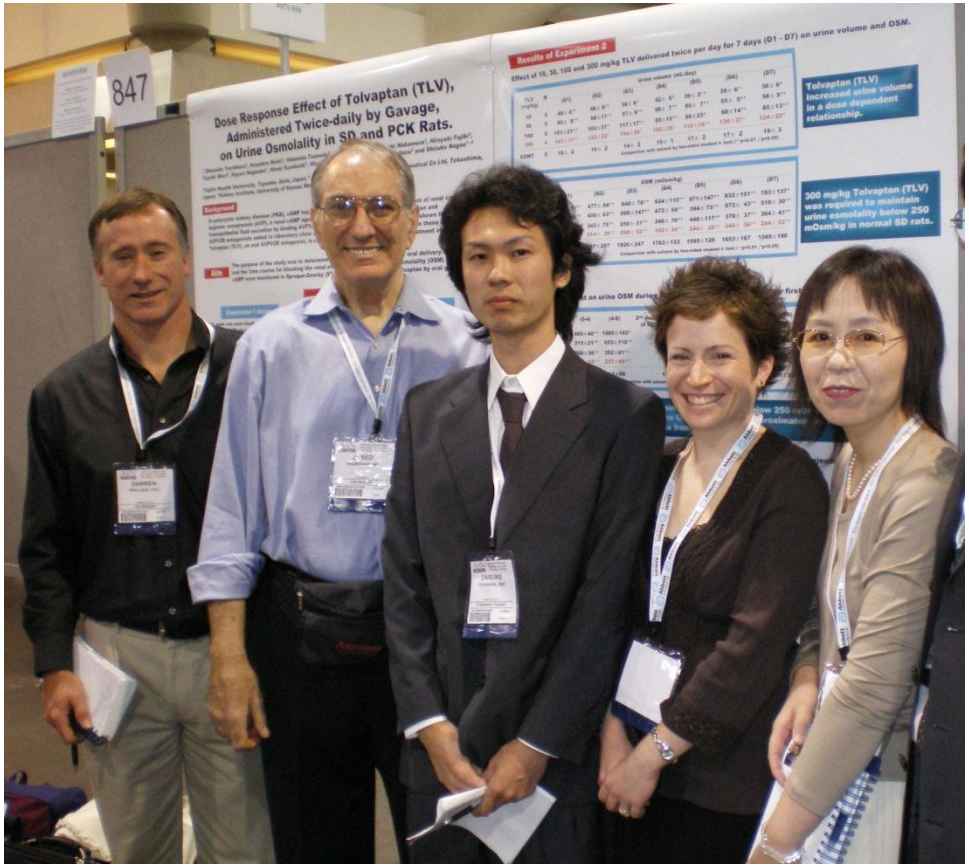
疾患モデル教育研究センター
センター長
長尾 静子



PKDの内科的治療に向けて

[開発者のメッセージ]

PKDの患者様にとって、早期の診断・治療マーカーや有効な治療薬が少ないことは大きな負担となっております。新たなマーカーの発見によって、早期の治療開始を可能にすると共に、新たな治療薬開発の迅速化に繋げたいと考えます。



吉原先生、カンザス大学腎臓研究所に留学
米国腎臓学会での発表デビュー！
（保健学研究科在籍中）

Graduate Seminar Series - Human Nutritional Sciences

Date: Tuesday, November 15, 2011
Time: 3:00 PM - 5:00 PM
Location: Room 207 Human Ecology Building

The Department of Human Nutritional Sciences invites you to a talk by Dr. Shizuko Nagao. The presentation is entitled "Therapeutic approaches in animal models of polycystic kidney disease: Effect of PPAR-gamma agonists on disease progression"
Dr. Nagao is the Director, Education and Research Centre of Animal Models for Human Diseases and an Associate Professor in Fujita Health University

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For more information, contact:
Glenda Parsons
Office Assistant
Human Ecology
gparson@ms.umanitoba.ca
Phone: (204) 474-8508
Fax: (204) 474-7592

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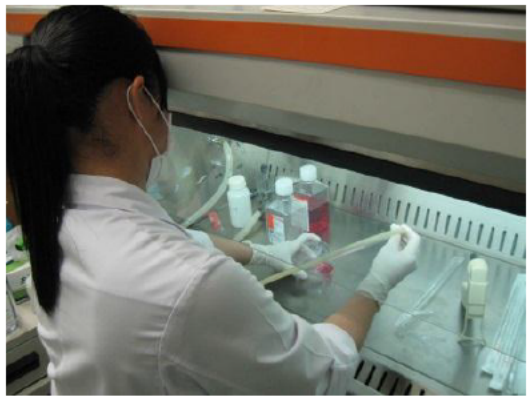
Questions or comments?

カナダ　マニトバ大学　Aukema博士
共同研究の打ち合わせと講演会

カナダ　マニトバ大学　講演会の様子

トランスレーショナルリサーチとは
（基礎と臨床の橋渡し研究）

培養細胞



メカニズム

前臨床試験

効果と毒性

臨床試験

臨床応用

基礎研究から臨床治験へ

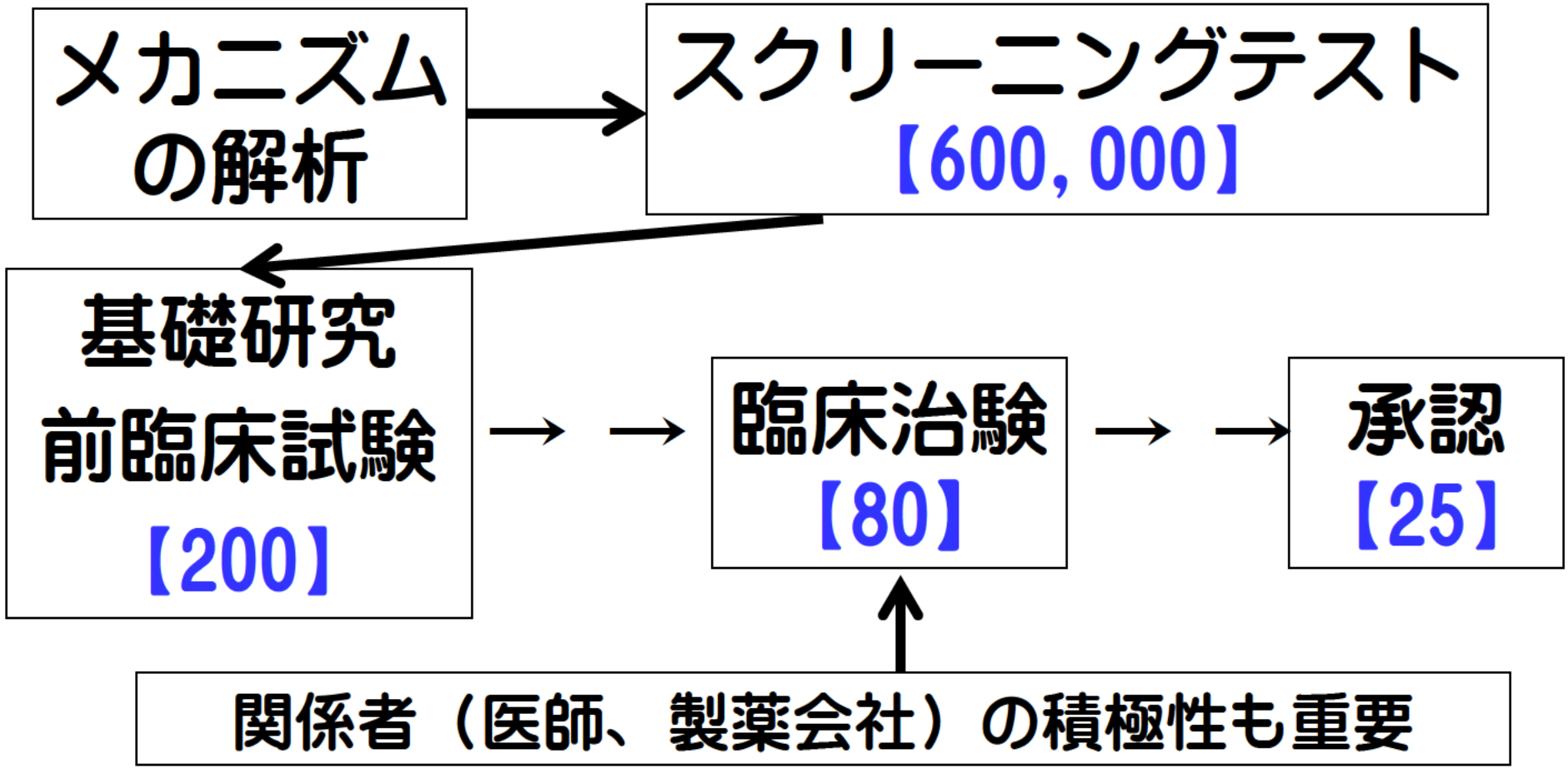
開発期間　　：9～17年

開発費　　：500億円

化合物→新薬　　：24,000分の１

日本製薬工業協会HPより

基礎研究から臨床治験へ



日本製薬工業協会HPより

どんな病気の研究をしているの？

多発性嚢胞腎症（PKD）

腎臓（100％）肝臓（90％）
その他の臓器に多数の嚢胞が発生

臨床症状：40歳
発症率：1000人 - 2000人に1人
慢性腎不全：患者さんの約半数
治療方法：現在は対症療法、透析、移植

囊胞腎

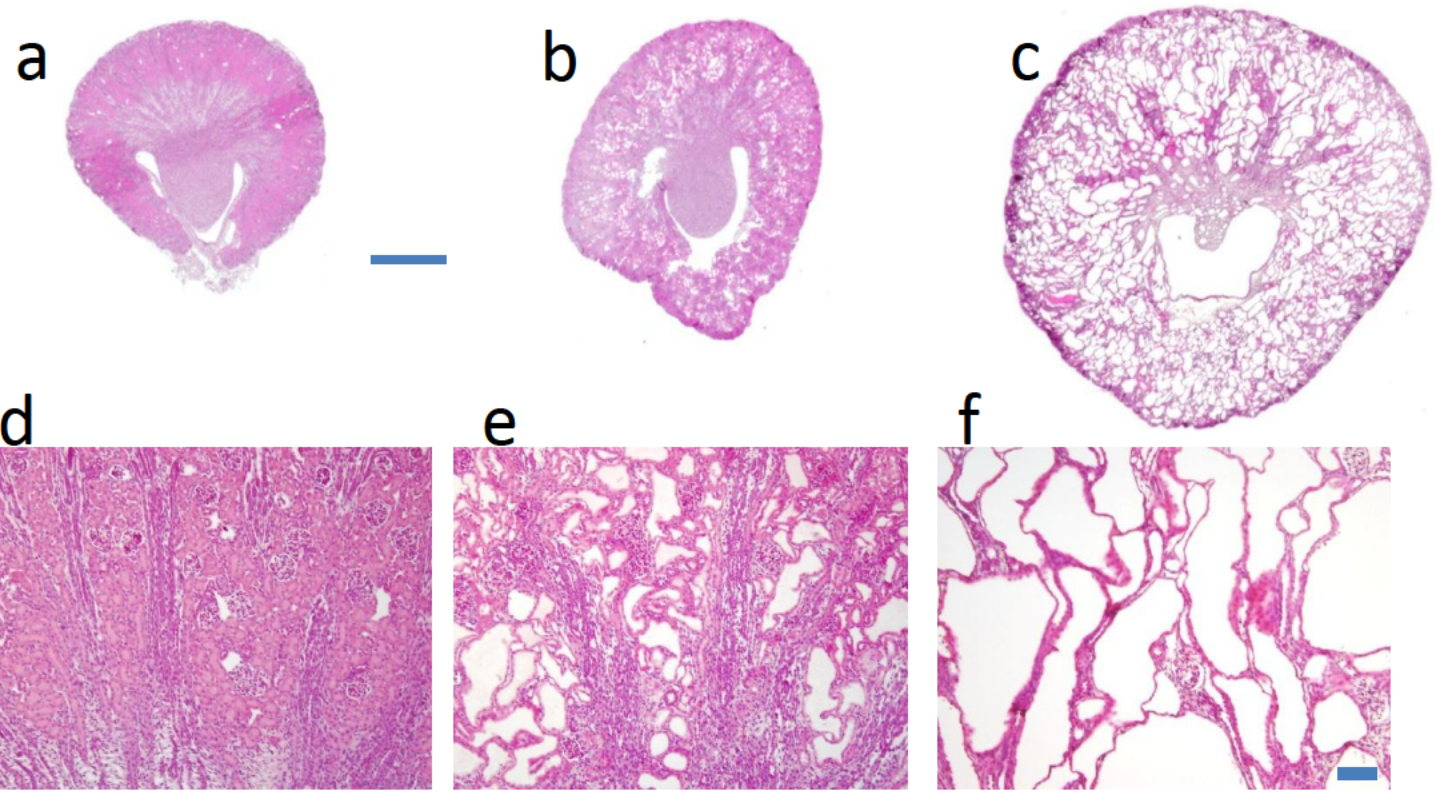
正常腎

囊胞肝

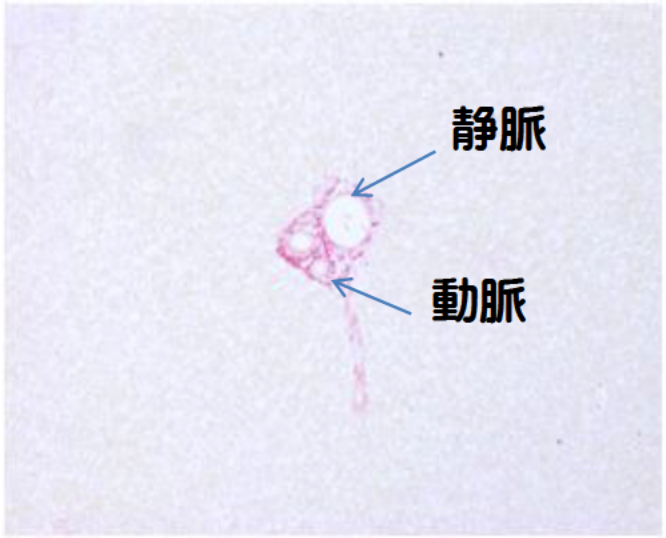
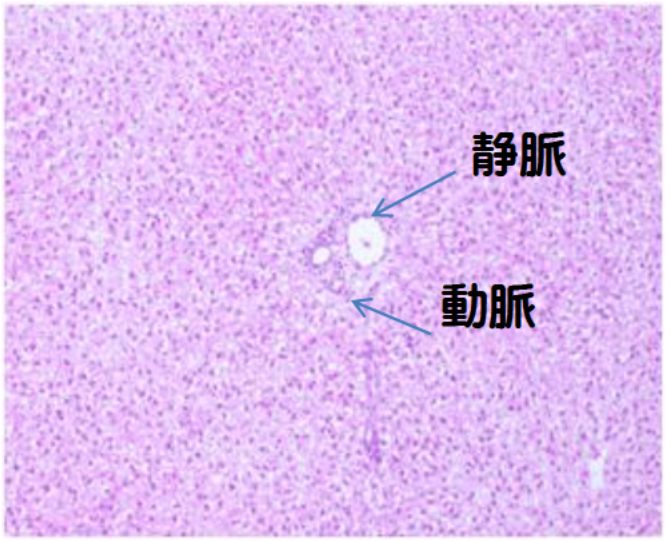
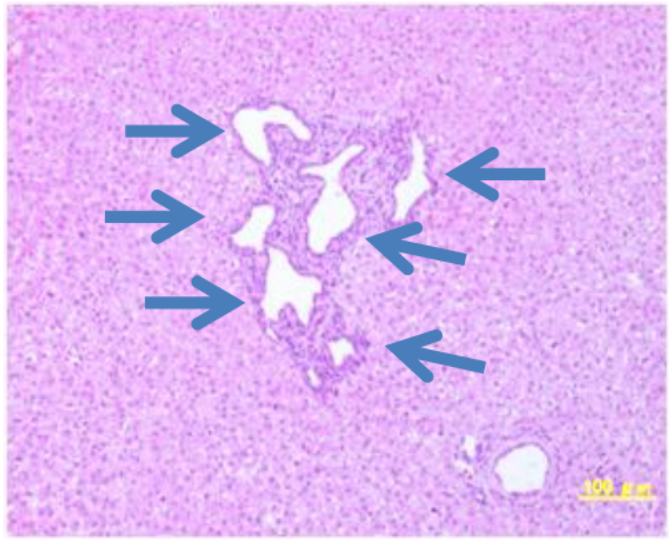
正常肝

腎臓

PKDが進行



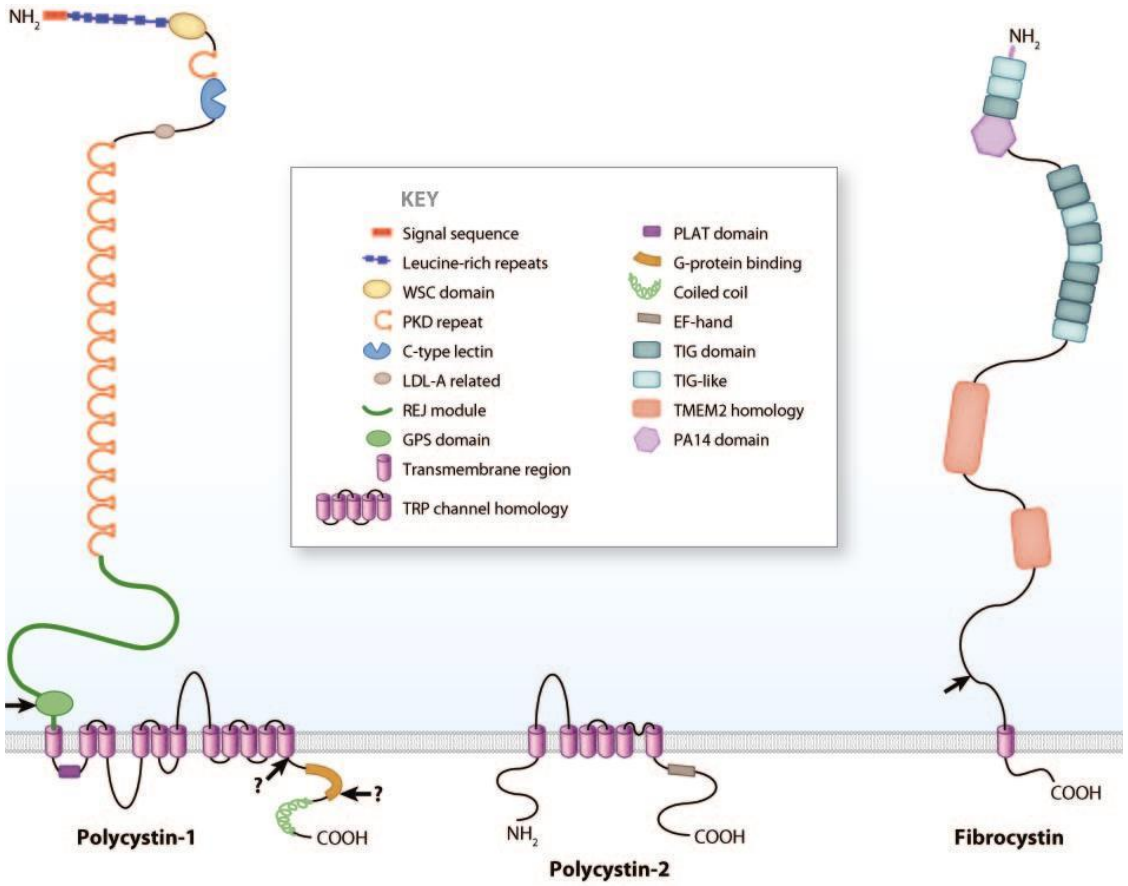
肝臓

	正常	嚢胞
特殊染色		
H & E 染色		

病気になってしまうメカニズムは、
PKD細胞の中の情報伝達経路の異常？

情報伝達経路とは？
　受容体→蛋白質の相互作用
　　あるいは酵素の活性化
　　↓
　　特異的な細胞機能
　　細胞増殖の促進/抑制
　　アポトーシスの促進/抑制

情報伝達経路の異常は、責任遺伝子の変異による蛋白質の機能異常？

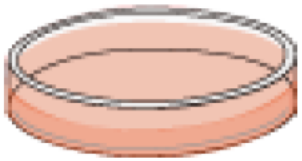


Harris PC, Torres VE. 2009.
Annu. Rev. Med. 60:321–37

どんな解析をするの？

培養細胞

核酸
トランスフェクション



培養細胞

作動薬
拮抗薬

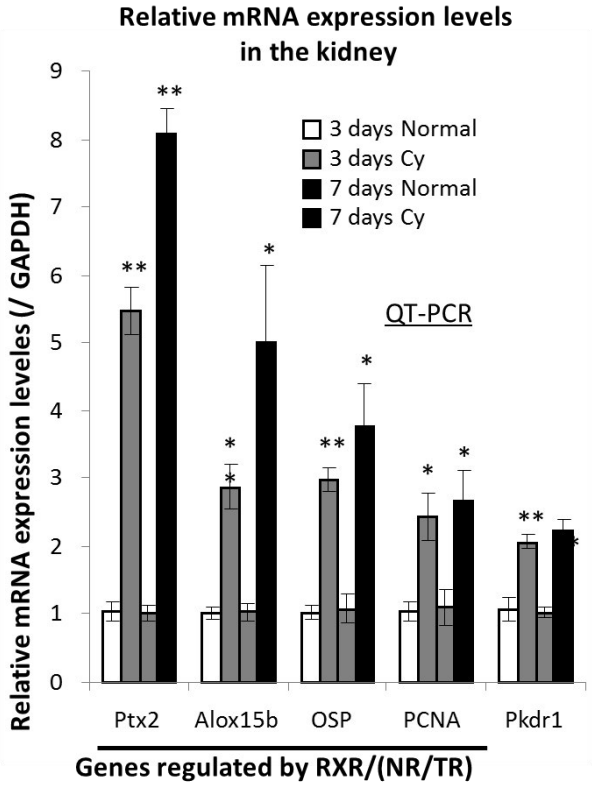
治療薬

DNAアレイ解析（RNA）　定量PCR解析（RNA）

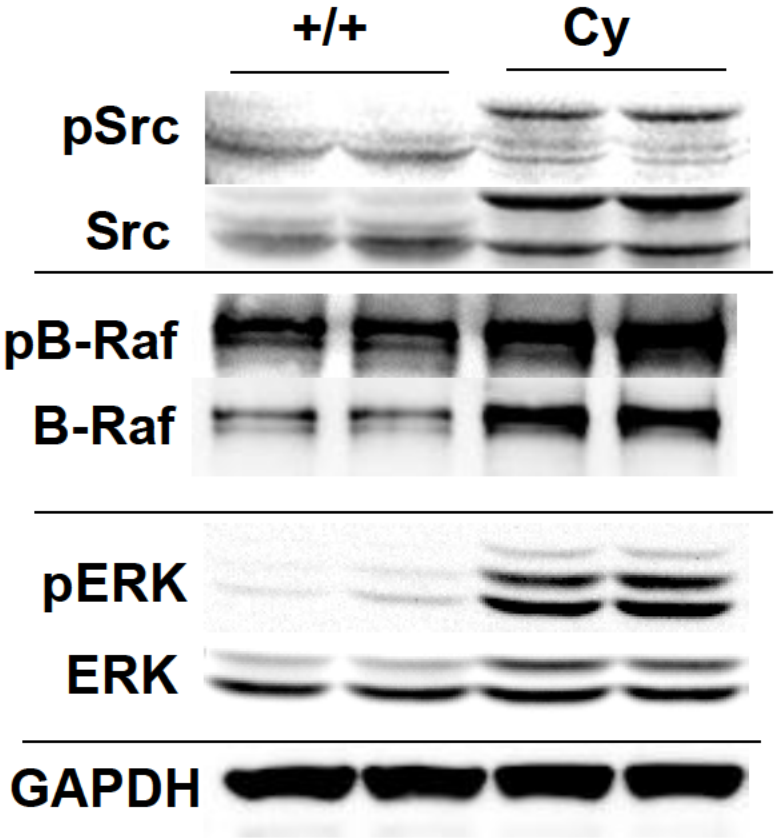
Biological process of genes regulated by RXR/(NR/TR) at 3 days and 7 days in Cy/Cy kidneys.

Category was listed in order of 3 days P-value.

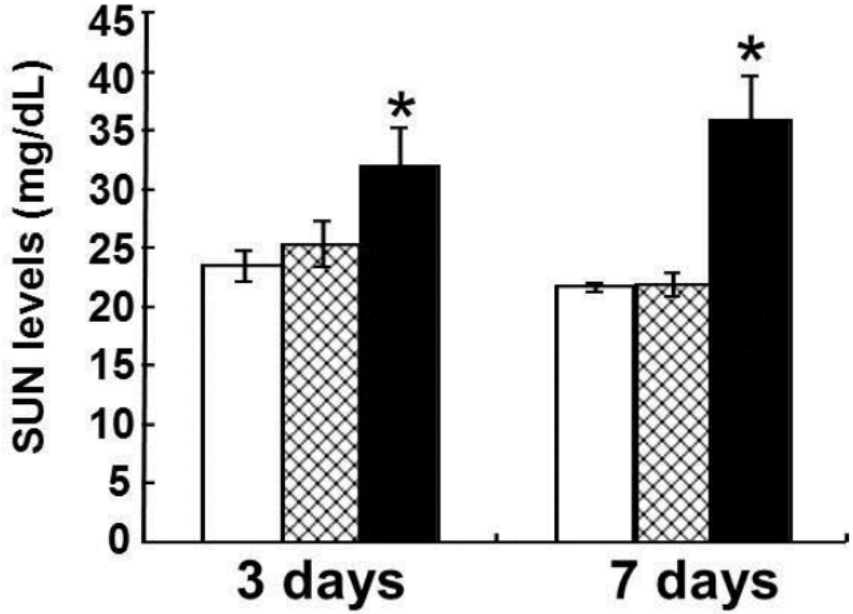
number	Category name	p-Value (p < 1.00E-03) 3 days	p-Value (p < 1.00E-05) 7 days
GO:9611	response to wounding	3.19E-06	4.79E-12
GO:9605	response to external stimulus	9.59E-06	5.08E-17
GO:19752	carboxylic acid metabolism	1.17E-05	9.02E-09
GO:6082	organic acid metabolism	1.21E-05	9.67E-09
GO:6950	response to stress	1.61E-05	2.18E-11
GO:48513	**organ development**	**5.59E-05**	**3.02E-09**
GO:8283	**cell proliferation**	**1.19E-04**	**2.15E-09**
GO:9653	**morphogenesis**	**1.76E-04**	**2.41E-07**
GO:42127	**regulation of cell proliferation**	**2.02E-04**	**1.27E-06**
GO:48519	negative regulation of biological process	4.40E-04	3.21E-06
GO:6954	inflammatory response	4.51E-04	1.04E-09
GO:9725	response to hormone stimulus	6.65E-04	1.28E-07
GO:43119	positive regulation of physiological process	6.69E-04	4.38E-07
GO:43118	negative regulation of physiological process	7.29E-04	5.33E-07
GO:6629	lipid metabolism	9.70E-04	2.10E-10



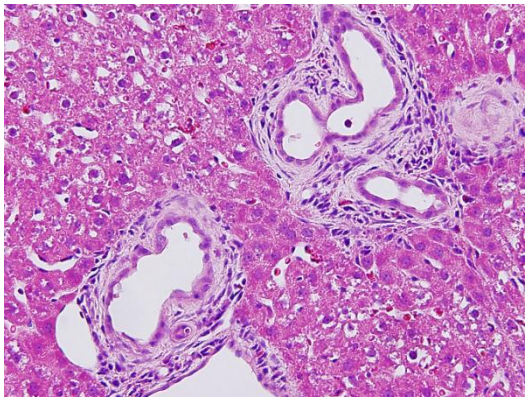
ウェスタンブロット解析
（蛋白質）



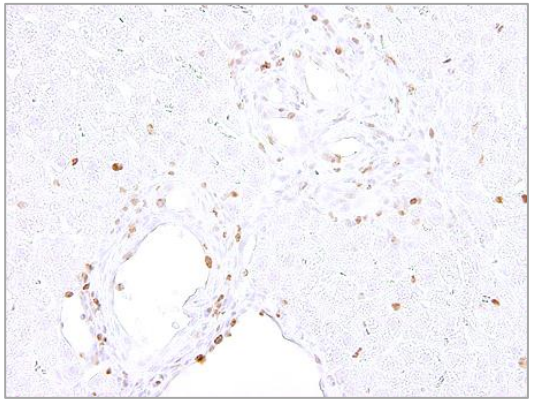
臨床化学的解析
（腎機能、肝機能）

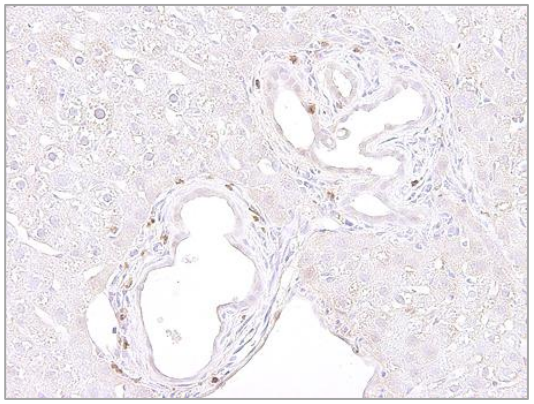


Ｈ＆Ｅ染色



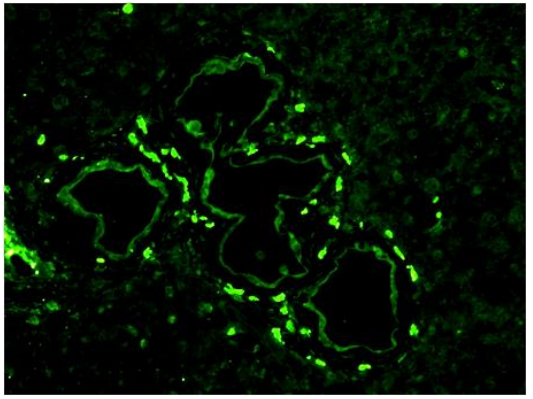
免疫組織化学染色

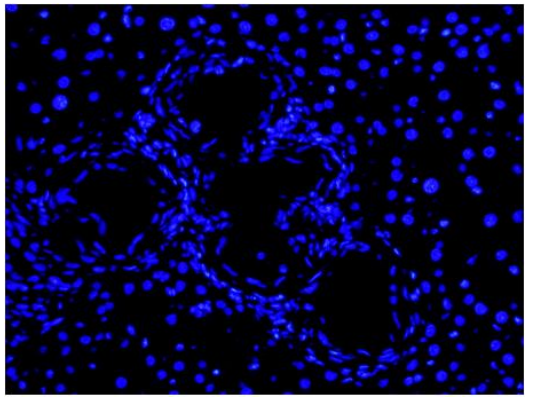




茶色（DAB）
抗体が反応した部分

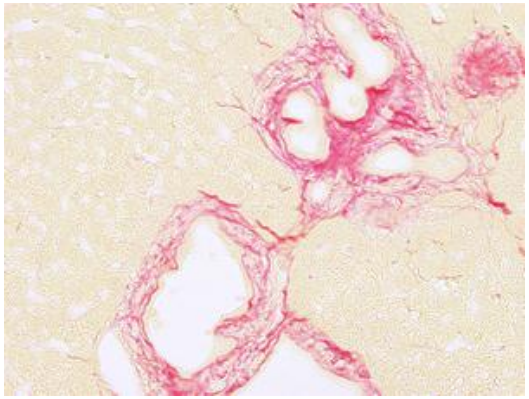
蛍光染色



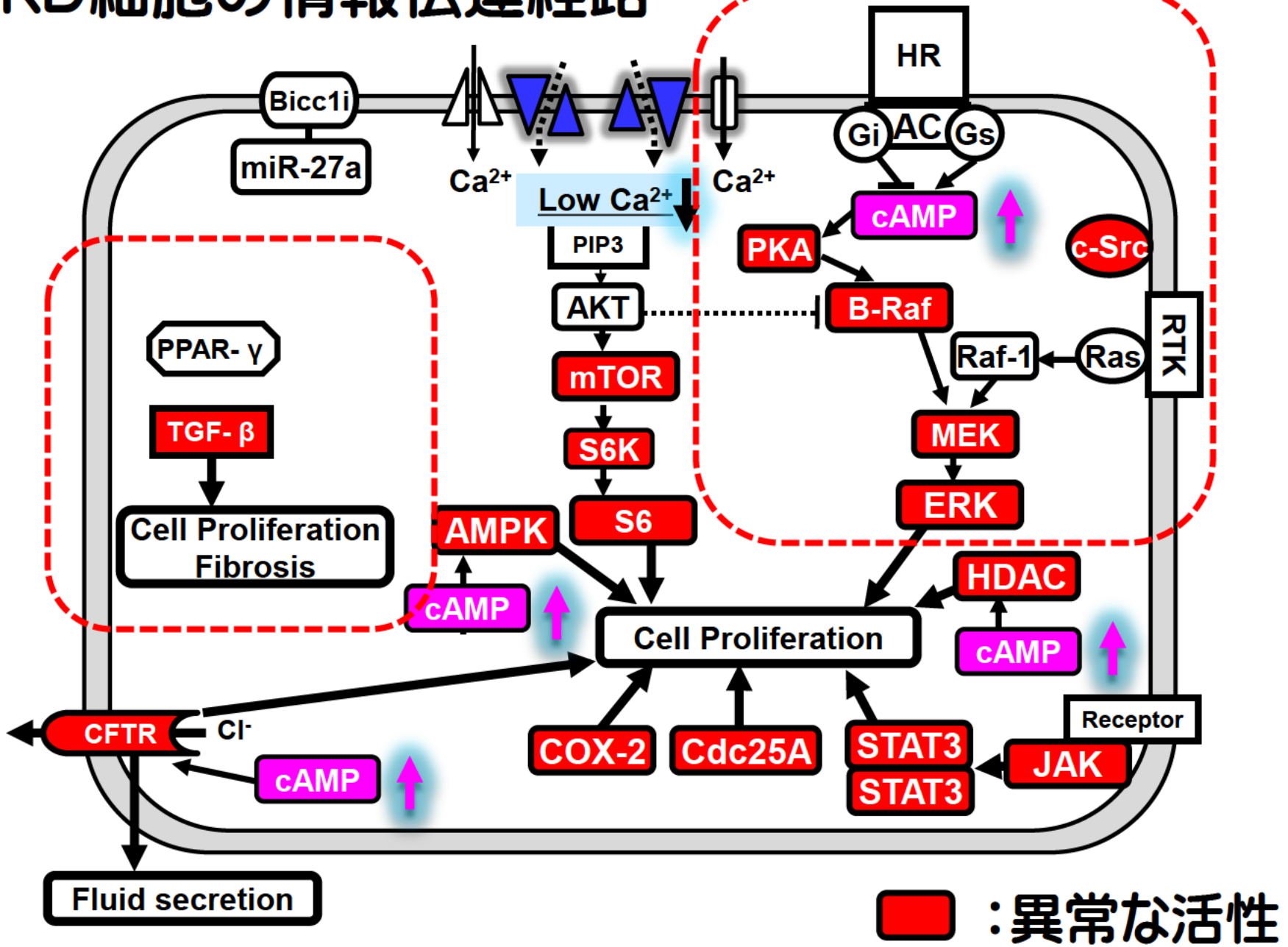


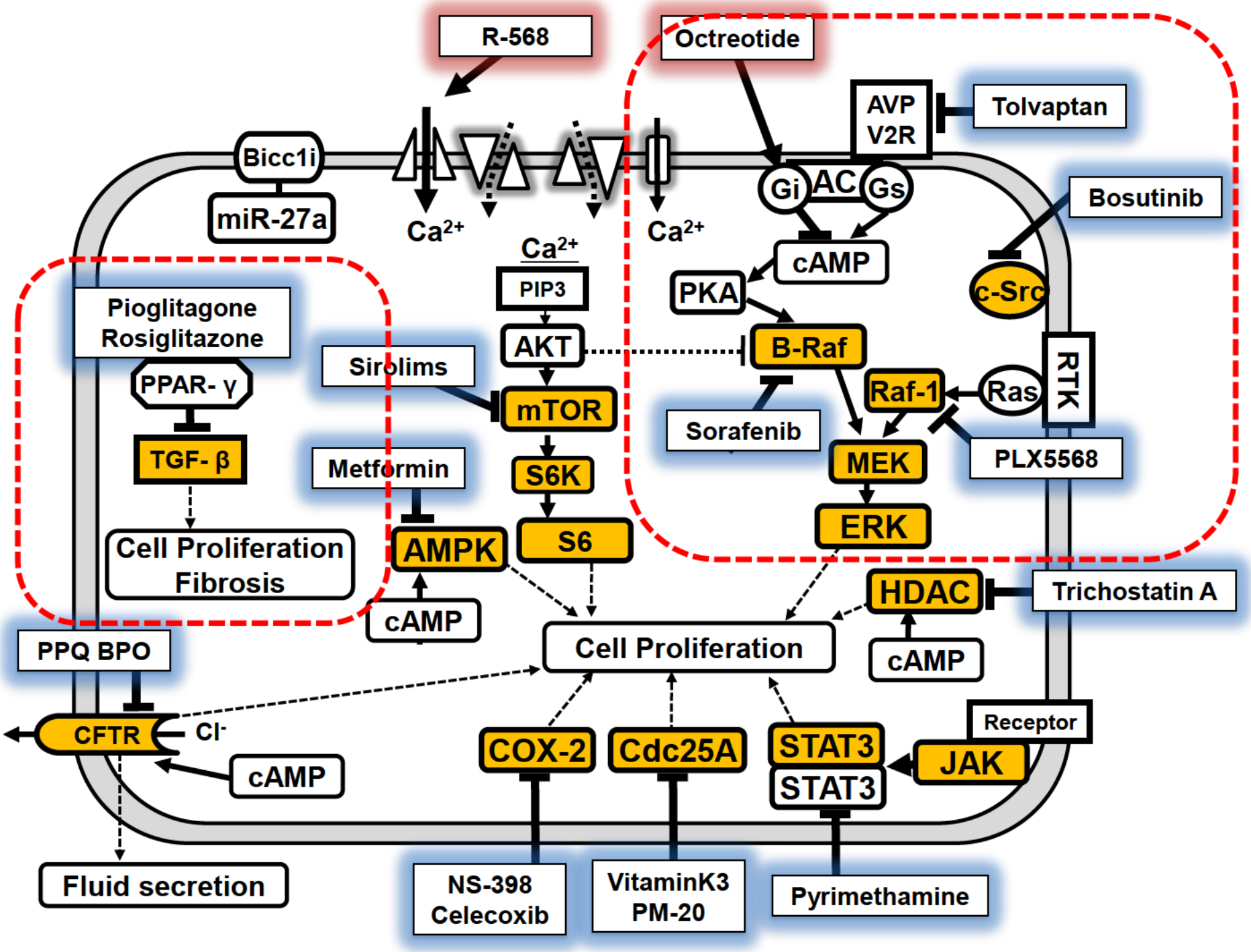
緑色（アレクサ488）
抗体が反応した部分

特殊染色



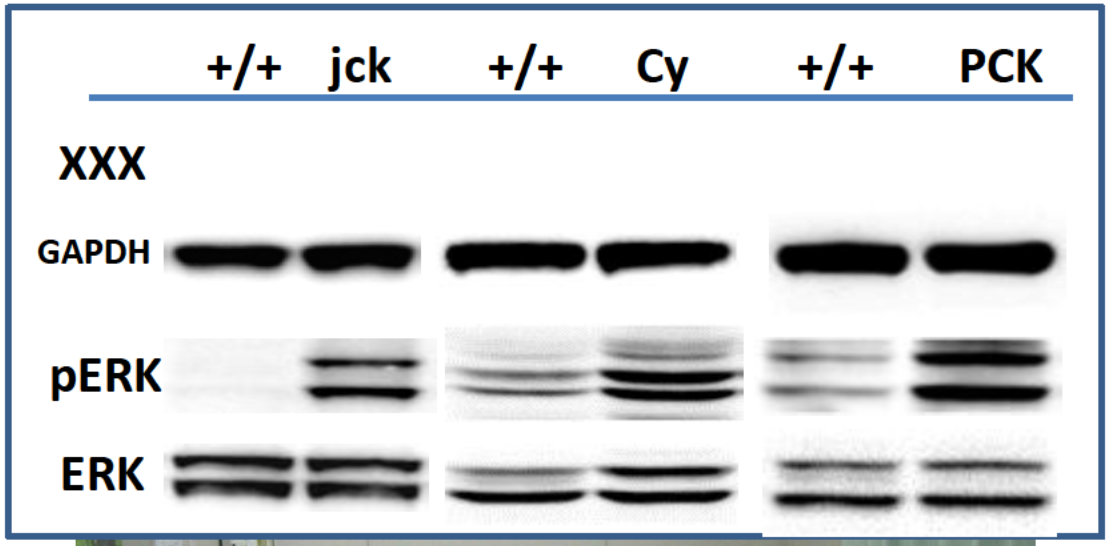
PKD細胞の情報伝達経路

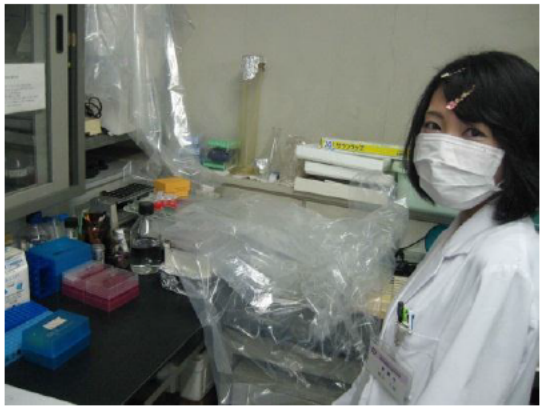



R-568
Octreotide
AVP
V2R
Tolvaptan
Bicc1i
miR-27a
Ca2+
Ca2+
Ca2+
Gi
AC
Gs
Bosutinib
cAMP
PKA
c-Src
PIP3
AKT
B-Raf
RTK
Ras
Raf-1
Sirolims
mTOR
Sorafenib
MEK
PLX5568
Pioglitagone
Rosiglitazone
PPAR- γ
TGF- β
Metformin
S6K
S6
ERK
Cell Proliferation
Fibrosis
AMPK
HDAC
Trichostatin A
cAMP
cAMP
PPQ BPO
Cell Proliferation
Receptor
CFTR
Cl-
cAMP
COX-2
Cdc25A
STAT3
STAT3
JAK
Fluid secretion
NS-398
Celecoxib
VitaminK3
PM-20
Pyrimethamine

新発見！

PKDモデル動物におけるXXXとpERKの発現増加





質問