

# 目 次

I. 総括研究報告書	
再生医療実用化に向けた細胞組織加工医薬品の安全性・品質等 の確保に関する基盤技術開発研究	1
山口 照英	
II. 分担研究報告書	
1. 細胞組織加工医薬品のウイルス安全性評価技術の開発	219
内田恵理子	
2. 細胞組織利用医薬品の同一性・安定性評価手法の開発に関する研究	241
鈴木 和博	
3. 細胞組織加工医薬品の同等性評価技術の開発	287
川崎 ナナ	
4. 細胞組織加工医薬品の免疫学的安全性評価に関する基盤技術開発	315
川端 健二	
5. 細胞組織加工医薬品の造腫瘍性・異常増殖性評価技術の開発に関する研究	339
佐藤 陽治	
6. 細胞・組織加工医薬品等の品質評価技術の開発に関する研究	363
佐藤 陽治	
7. 細胞組織加工医薬品の特性解析・品質管理に関する研究	399
石井 明子	
8. 人工多能性幹細胞の品質・安全性に関する研究	425
山中 伸弥	
9. 細胞特性・品質解析技術としての糖鎖解析技術の開発	431
早川 堯夫	
III. 研究成果の刊行に関する一覧表	459
IV. 研究成果の刊行物・別刷	475

研究成果の発表に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	頁
Kawabata K., Inamura M., Mizuguchi H.	Efficient hepatic differentiation from human iPS cells by gene transfer.		Liver Stem Cells: Methods and Protocols	Humana Press (part of the Springer publishing group)	米国	印刷中	
内田恵理子	第8章 細胞基材のマイコプラズマ試験	山口照英	先端バイオ医薬品の評価技術	シーエムシー出版	東京	2010	pp151-167
内田恵理子	20. バイオテクノロジー応用医薬品/生物起源由来医薬品の製造に用いる細胞基材に対するマイコプラズマ否定試験	(財)日本公定書協会	日本薬局方技術情報 2010 追補 (JPTI2010) 第15改正第一追補/第二追補対応	(株)じほう	東京	2010	pp85-91
川崎ナナ, 石井明子, 荒戸照世, 山口照英	抗体医薬品の構造及び品質特性解析 抗体医薬品製造の留意点～承認申請をふまえて～			サイエンス&テクノロジー社	東京	2009	p.119-132
Kawasaki N, Itoh S, Yamaguchi T.	LC/MS of oligosaccharides	Naoyuki Taniguchi	<i>Glycoscience Lab Manual.</i>	Springer	東京	2008	
Itoh S, Takakura D, Kawasaki N, Yamaguchi T.	Glycosylation analysis using LC/MS and LC/MSn.	John Walker	<i>The Protein Protocols Handbook. Third</i>	Humana Press	米国	2009	

	Site-specific glycosylation analysis of a glycoprotein.		<i>Edition.</i>				
--	---	--	-----------------	--	--	--	--

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	頁	出版年
Ken Nishimura, Masayuki Sano, Manami Ohtaka, Birei Furuta, Yoko Umemura, Yoshiro Nakajima, Yuzuru Ikehara, Toshihiro Kobayashi, Hiroaki Segawa, Satoko Takayasu, Hideyuki Sato, Kaori Motomura, Eriko Uchida, Toshie Kanayasu-Toyoda , Makoto Asashima, Hiromitsu Nakauchi, Teruhide Yamaguchi and Mahito Nakanishi	Development of defective and persistent sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming,	<i>J Biol Chem.</i>	<b>286</b>	4760-71	2011
Yamaguchi T, Suzuki T, Arai H, Tanabe S, Atomi Y.	Continuous mild heat stress induces differentiation of mammalian myoblasts, shifting fiber-type from fast to slow.	<i>Am J Physiol Cell Physiol.</i>	<b>298</b>	C140 -C148	2010

Tashiro K., Kawabata K., Inamura M., Takayama K., Furukawa N., Sakurai F., Katayama K., Hayakawa H., Furue-Kusuda M., Mizuguchi H.	Adenovirus vector-mediated efficient transduction into human embryonic and induced pluripotent stem cells.	<i>Cell Reprogram.</i>	<b>12</b>	501-507	2010
Inamura M., Kawabata K., Takayama K., Tashiro K., Sakurai F., Katayama K., Toyoda M., Akutsu H., Miyagawa Y., Okita H., Kiyokawa N., Umezawa A., Hayakawa T., Furue MK., Mizuguchi H.	Efficient generation of hepatoblasts from human ES cells and iPS cells by transient overexpression of homeobox gene HEX.	<i>Mol Ther.</i>	<b>19</b>	400-407	2011
Jin MH, Yokoyama U, Sato Y, Shioda A, Jiao Q, Ishikawa Y, Minamisawa S.	DNA microarray profiling identified a new role of growth hormone in vascular remodeling of rat ductus arteriosus.	<i>J Physiol Sci.</i>	<b>61</b>	167-79	2011
Nishida M, Suda R, Nagamatsu Y, Tanabe S, Onohara N, Nakaya M,	Pertussis toxin upregulates angiotensin type 1 receptors through Toll-like receptor 4-mediated Rac activation.	<i>J Biol Chem.</i>	<b>285</b>	15268 -77	2010

Kanaho Y, Shibata T, Uchida K, Sumimoto H, Sato Y, Kurose H.					
Yamada K, Hyodo S, Kinoshita M, Hayakawa T, Takehi K.	Hyphenated technique for releasing and MALDI MS analysis of O-glycans in mucin-type glycoprotein samples.	<i>Anal Chem.</i>	<b>82(17)</b>	7436 -7443.	2010
Kinoshita M, Kakoi N, Matsuno YK, Hayakawa T, Takehi K.	Determination of sulfate ester content in sulfated oligo- and poly-saccharides by capillary electrophoresis with indirect UV detection.	<i>Biomed Chromatogr.</i>	<b>25(5)</b>	588-93	2011
小木 美恵子, 石 丸 幸大, 西脇 基晃, 宮脇 英 明, 内田 恵理 子, 得永 嘉昭	遺伝子導入用インパルス応力波 素子開発のための実験的検討	電子情報通信 学会技術研究 報告	<b>110 (366)</b>	31-34	2011
奥田晴宏, 川崎 ナナ, 内田恵理 子, 山本美智子, 宮田直樹	薬の名前 ステムを知れば薬がわ かる 第50回	<i>Pharm Tech Japan</i>	<b>26(10)</b>	1927 -1936	2010
鈴木孝昌	日本の体外診断用医薬品の規制 をめぐる動向～DNA チップを用い た遺伝子型判定装置に関する評 価指標の策定	<i>PHARMSTAGE</i>	7月号	1-2	2010
川端健二, 田代 克久, 水口裕之	iPS 細胞への遺伝子導入を用いた 分化誘導の最適化	<i>薬学雑誌</i>	<b>130</b>	1527 -1534	2010
佐藤 陽治	再生医療・細胞治療の規制等に 関する欧米の動向—臨床応用に 関する規制当局の支援の比較—	<i>ヒューマン サイエンス</i>	<b>22(2)</b>	28-32	2011
佐藤 陽治	再生医療・細胞治療の規制に関 する国際動向	<i>PHARMSTAGE</i>	<b>10(12)</b>	1-2	2011
佐藤 陽治, 鈴木 和博, 早川 堯夫	EU における細胞・組織加工製品 の規制動向	<i>医薬品・医療 機器レギュラト</i>	<b>42</b>	142-8	2011

		リーサイエンス			
西田 基宏, 齋木 翔太, 北島 直 幸, 仲矢 道雄, 佐藤 陽治, 黒瀬 等	TRPC チャンネルのリン酸化による心 血管機能制御	<i>YAKUGAKU ZASSHI</i>	<b>130</b>	1427-33	2010
石井明子, 川崎 ナナ	バイオ治験薬の品質安全性確保	ファームテクジ ヤパン	<b>16</b>	69-80	2010
Watanabe T, Tanaka G, Hamada S, Namiki C, Suzuki T, Nakajima M, Furihata C.	Dose-dependent alterations in gene expression in mouse liver induced by diethylnitrosamine and ethylnitrosourea and determined by quantitative real-time PCR.	<i>Mutat Res.</i>	<b>673</b>	9-20	2009
Yamaguchi T, Suzuki T, Arai H, Tanabe S, Atomi Y.	Continuous mild heat stress induces differentiation of mammalian myoblasts, shifting fiber type from fast to slow.	<i>Am. J. Physiol. Cell Physiol.</i>	<b>298</b>	C140 -C148	2010
Noritaka Hashii, Nana Kawasaki, Satsuki Itoh, Yukari Nakajima, Akira Harazono, Toru Kawanishi, Teruhide Yamaguchi	Identification of glycoproteins carrying a target glycan-motif by liquid chromatography/multiple-stage mass spectrometry. Identification of Lewis x-glycoproteins in mouse kidney.	<i>J. Proteome Res.</i>	<b>8</b>	3415 -3429	2009
Kawasaki N, Itoh S, Hashii N, Takakura, D, Qin Y, Xiaoyu H, Yamaguchi T.	The significance of glycosylation analysis in development of biopharmaceuticals	<i>Biol. Pharm. Bull.</i>	<b>32 (5)</b>	796-800	2009

Noritaka Hashii, Nana Kawasaki, Satsuki Itoh, Yukari Nakajima, Toru Kawanishi, Teruhide Yamaguchi	Alteration of N-glycosylation in the kidney of systemic lupus erythematosus model mouse. Relative quantification of N-glycans by using isotope tagging method.	<i>Immunology</i>	<b>126(3)</b>	336-345	2009
Tashiro K., Inamura M., Kawabata K., Sakurai F., Yamanishi K., Mizuguchi H.	Efficient adipocyte and osteoblast differentiation from mouse induced pluripotent stem cells by adenoviral transduction.	<i>Stem Cells.</i>	<b>27</b>	1802 -1811	2009
Tashiro K, Kondo A, Kawabata K, Sakurai H, Sakurai F, Yamanishi K, Hayakawa T, Mizuguchi H.	Efficient osteoblast differentiation from mouse bone marrow stromal cells with polylysine-modified adenovirus vectors.	<i>Biochem. Biophys. Res. Commun.</i>	<b>379</b>	127-132	2009
Sakamoto K, Hiraiwa M, Saito M, Nakahara T, Sato Y, Nagao T, Ishii K.	Protective effect of all-trans retinoic acid on NMDA-induced neuronal cell death in rat retina.	<i>Eur J Pharmacol.</i>	<b>635 (1-3)</b>	56-61	2010
Nishida M, Watanabe K, Sato Y, Nakaya M, Kitajima N, Ide T, Inoue R, Kurose H.	Phosphorylation of TRPC6 channels at Thr69 is required for anti-hypertrophic effects of phosphodiesterase 5 inhibition.	<i>J Biol Chem.</i>	<b>285(17)</b>	13244 -53	2010
Fujishita K, Ozawa T, Shibata K, Tanabe S, Sato Y, Okuda T, Maeda S,	Grape seed extract (GSE) acting on astrocytes reveals neuronal protection against oxidative stress via interleukin-6-mediated mechanisms.	<i>Cell Mol Neurobiol.</i>	<b>29</b>	1121-9	2009

Koizumi S.					
Takuo Suzuki, Akiko Ishii-Watabe, Minoru Tada, Tetsu Kobayashi, Toshie Kanayasu-Toyoda , Toru Kawanishi, and Teruhide Yamaguchi	Importance of FcRn in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1: A comparative study of the affinity of monoclonal antibodies and Fc-fusion proteins to human FcRn.	<i>J Immunol.</i>	<b>184(4)</b>	1968-76	2010
Kakoi N, Kinoshita M, Kawasaki N, Yamaguchi T, Hayakawa T, Kakehi K.	Capillary electrophoresis analysis of contaminants in heparin sodium for the Japanese pharmacopoeia purity test.	<i>Yakugaku Zasshi.</i>	<b>129(10)</b>	1255 -1264	2009
Yamada K, Watanabe S, Kita S, Kinoshita M, Hayakawa T, Kakehi K.	Determination of Tn antigen released from cultured cancer cells by capillary electrophoresis.	<i>Anal Biochem.</i>	<b>396(1)</b>	161-163	2010
Kinoshita M, Ohta H, Higaki K, Kojima Y, Urashima T, Nakajima K, Suzuki M, Kovacs KM, Lydersen C, Hayakawa T, Kakehi K.	Structural characterization of multibranched oligosaccharides from seal milk by a combination of off-line high-performance liquid chromatography-matrix-assisted laser desorption / ionization-time-of-flight mass spectrometry and sequential exoglycosidase digestion.	<i>Anal Biochem.</i>	<b>388(2)</b>	242-253	2009
Yamada K, Kinoshita M, Hayakawa T, Nakaya S, Kakehi K.	Comparative studies on the structural features of O-glycans between leukemia and epithelial cell lines.	<i>J Proteome Res.</i>	<b>8(2)</b>	521-37	2009

Tanabe S, Sato Y, Suzuki K.	Characteristics of stem cells based on expression profile of molecular markers.	<i>Res. Adv. in Biochemistry.</i>		1-8	2009
内田恵理子, 山口照英	医薬品のウイルス安全性確保:核酸増幅検査(NAT)によるC型肝炎ウイルス検出の評価とNATによる高感度検出のためのウイルス濃縮法の開発	YAKUGAKU ZASSHI	130 (2)	163-169	2010
宮田直樹, 川崎ナナ, 内田恵理子, 蜂須賀暁子	平成19年度「日本薬局方の試験法に関する研究」研究報告:日本薬局方の名称関連項目の科学的整備に関する研究	医薬品研究	40	587-598	2009
山口照英, 内田恵理子	核酸医薬品の開発動向とその品質・安全性確保	Pharmstage	9 (2)	1-5	2009
川崎ナナ	糖鎖関連医薬品の開発と分析化学	ぶんせき	421(1)	17-22	2010
佐藤陽治	ヒト幹細胞からの肝細胞分化誘導とその創薬非臨床試験への応用	実験医学(増刊)	28	334-338	2010
西田基宏, 佐藤陽治, 仲矢道雄, 黒瀬等	Gタンパク質共役型受容体-TRPCチャネルタンパク複合体形成による心肥大シグナル制御	日本薬理学雑誌	134	131-6	2009
佐藤陽治	ヒト細胞・組織加工医薬品などの安全性確保	医学のあゆみ	229	893-896	2009
山口照英, 石井明子	早期臨床開発段階でのバイオ医薬品の品質・安全性確保	臨床評価	36	611-627	2009
Nishimaki-Mogami T, Tamemihon N, Sato Y, Okuhira K, Sai K, Kagechika H, Shudo K, Abe-Dohmae S, Yokoyama S, Ohno Y, Inoue K, Sawada J.	The RXR agonists PA024 and HX630 have different abilities to activate LXR/RXR and to induce ABCA1 expression in macrophage cell lines.	<i>Biochem Pharmacol.</i>	76	1006-13	2008

Itoh S, Hachisuka A, Kawasaki N, Hashii N, Teshima R, Hayakawa T, Kawanishi T, Yamaguchi T.	Glycosylation analysis of IgLON family proteins in rat brain by liquid chromatography and multiple-stage mass spectrometry.	<i>Biochemistry</i>	<b>47</b>	10132-54	2008
Sano K, Asahi M, Yanagibashi M, Hashii N, Itoh S, Kawasaki N, Ogawa H.	Glycosylation and ligand-binding activities of rat plasma fibronectin during liver regeneration after partial hepatectomy.	<i>Carbohydr Res.</i>	<b>343</b>	2329-35	2008
Aiba K, Nedorezov T, Piao Y, Nishiyama A, Matoba R, Sharova LV, Sharov AA, Yamanaka S, Niwa H, Ko MS.	Defining developmental potency and cell lineage trajectories by expression profiling of differentiating mouse embryonic stem cells.	<i>DNA Res.</i>	<b>16(1)</b>	73-80	2009
Nishida M, Sato Y, Uemura A, Narita Y, Saito H, Nakaya M, Ide T, Suzuki K, Inoue K, Nagao T, Kurose H.	P2Y6 receptor-G $\alpha$ 12/13 signaling in cardiomyocytes triggers pressure overload-induced cardiac fibrosis.	<i>EMBO J.</i>	<b>27</b>	3104-15	2008
Mukai N, Akahori T, Komaki M, Qin Li, Kanayasu-Toyoda T, Ishii-Watabe A, Kobayashi A., Yamaguchi T,	A comparison of the tube forming potentials of early and late endothelial progenitor cells.	<i>Exp Cell Res.</i>	<b>314</b>	430-440	2008

Abe M, Amagasa T, Morita I.					
Sakurai F, Nakamura SI, Akitomo K, Shibata H, Terao K, Kawabata K, Hayakawa T, Mizuguchi H.	Adenovirus serotype 35 vector-mediated transduction following direct administration into organs of nonhuman primates.	<i>Gene Ther.</i>	<b>16(2)</b>	297-302	2009
Kizuka Y, Kobayashi K, Kakuda S, Nakajima Y, Itoh S, Kawasaki N, Oka S.	Laminin-1 is a novel carrier glycoprotein for the nonsulfated HNK-1 epitope in mouse kidney.	<i>Glycobiology</i>	<b>18</b>	331-38	2008
Haghighi K, Chen G, Sato Y, Fan GC, He S, Kolokathis F, Pater L, Paraskevaidis I, Jones WK, Dorn GW 2nd, Kremastinos DT, Kranias EG.	A human phospholamban promoter polymorphism in dilated cardiomyopathy alters transcriptional regulation by glucocorticoids.	<i>Hum Mutat.</i>	<b>29</b>	640-7	2008
Hashii N, Kawasaki N, Itoh S, Nakajima Y, Kawanishi T, Yamaguchi T.	Alteration of N-glycosylation in the kidney in a mouse model of systemic lupus erythematosus: relative quantification of N-glycans using an isotope-tagging method.	<i>Immunology.</i>	<b>126</b>	336-45	2008
Tanabe S, Sato Y, Suzuki T, Suzuki K, Nagao T, Yamaguchi T.	Gene expression profiling of human mesenchymal stem cells for identification of novel markers in early- and late-stage cell culture.	<i>J Biochem.</i>	<b>144</b>	399-408	2008

Urayama S, Harada Y, Nakagawa Y, Ban S, Akasaka M, Kawasaki N, Sawada H.	Ascidian Sperm Glycosylphosphatidylinositol-anch ored CRISP-like Protein as a Binding Partner for an Allorecognizable Sperm Receptor on the Vitelline Coat.	<i>J Biol Chem.</i>	<b>283</b>	21725 -33	2008
Harazono A, Kawasaki N, Itoh S, Hashii N, Matsuiishi-Nakaji ma Y, Kawanishi T, Yamaguchi T.	Simultaneous glycosylation analysis of human serum glycoproteins by high-performance liquid chromatography/tandem mass spectrometry.	<i>J Chromatogr B Analyt Technol Biomed Life Sci.</i>	<b>869</b>	20-30	2008
Suzuki T, Tamehiro N, Sato Y, Kobayashi T, Ishii-Watabe A, Shinozaki Y, Nishimaki-Moga mi T, Hashimoto T, Asakawa Y, Inoue K, Ohno Y, Yamaguchi T, Kawanishi T.	The novel compounds that activate farnesoid x receptor: the diversity of their effects on gene expression.	<i>J Pharmacol Sci.</i>	<b>107</b>	285-94	2008
Harashima M, Harada K, Ito Y, Hyuga M, Seki T, Ariga T, Yamaguchi T, Niimi S.	Annexin A3 Expression Increases in Hepatocytes and is Regulated by Hepatocyte Growth Factor in Rat Liver Regeneration,	<i>J Biochem.</i>	<b>143</b>	537-45	2008
Watanabe K, Hyuga S, Hyuga M, Sekiguchi A, Endo M, Tsuda T, Oikawa T, Yamaguchi T, Hanawa T.	Unkeito, a traditional Kampo formula, exhibits a selective estrogen receptor modulator-like activity. - Prevention of osteoporosis in ovariectomized mice –	<i>J Trad Med.</i>	<b>26(1)</b>	18-24	2009

Kawasaki N, Itoh S, Yamaguchi T.	LC/MSn for glycoproteome analysis: Glycosylation analysis and peptide sequencing of glycopeptides.	<i>Methods Mol Biol.</i>	<b>534</b>	1-10	2009
Sakurai F, Nakamura S, Akitomo K, Shibata H, Terao K, Kawabata K, Hayakawa T, Mizuguchi H.	Transduction properties of adenovirus serotype 35 vectors after intravenous administration into nonhuman primates.	<i>Mol Ther.</i>	<b>16</b>	726-33	2008
Watanabe T, Tanaka G, Hamada S, Namiki C, Suzuki T, Nakajima M, Furihata C.	Dose-dependent alterations in gene expression in mouse liver induced by diethylnitrosamine and ethylnitrosourea and determined by quantitative real-time PCR.	<i>Mutat Res.</i>	<b>673</b>	9-20	2009
Kiyonaka S, Kato K, Nishida M, Mio K, Numaga T, Sawaguchi Y, Yoshida T, Wakamori M, Mori E, Numata T, Ishii M, Takemoto H, Ojida A, Watanabe K, Uemura A, Kurose H, Morii T, Kobayashi T, Sato Y, Sato C, Hamachi I, Mori Y.	Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound.	<i>Proc Natl Acad Sci USA.</i>	<b>106(13)</b>	5400 -5405	2009

Okita K, Nakagawa M, Hong H, Ichisaka T, Yamanaka S.	Generation of mouse induced pluripotent stem cells without viral vectors.	<i>Science.</i>	<b>322</b>	949-53	2008
Kawasaki N, Itoh S, Hashii N, Harazono A, Takakura D, Yamaguchi T.	Mass spectrometry for analysis of carbohydrate heterogeneity in characterization and evaluation of glycoprotein products.	<i>Trends Glycosci Glycotech.</i>	<b>20</b>	97-116	2008
Satoh K, Iwata-Takakura A, Yoshikawa A, Gotanda Y, Tanaka T, Yamaguchi T, Mizoguchi H.	A new method of concentrating hepatitis B virus (HBV) DNA and HBV surface antigen: an application of the method to the detection of occult HBV infection.	<i>Vox Sanguinis.</i>	<b>95</b>	174-80	2008
原園 景, 川崎ナ ナ, 伊藤さつき, 小林 哲, 石川リ カ, 高井俊紀, 古 賀明子, 岡本寿 美子, 山口秀人, 濱詰康樹, 佐藤 貴之, 窪田雅之, 掛樋一晃, 木下 充弘, 島 圭介, 山田真希, 山口 照英	質量分析法を用いたペプチド及び タンパク質性医薬品の確認試験法 に関する研究	<i>医薬品研究</i>	<b>39(10)</b>	627-46	2008
掛樋一晃, 木下 充弘, 橋井則貴, 川崎ナナ, 寺尾 敏光, 河合健蔵, 余田 光, 山口照 英	ヘパリンナトリウム純度試験に関す る研究(第3報)キャピラリー電気泳 動法によるヘパリンナトリウム不純 物の分析	<i>医薬品研究</i>	<b>39(11)</b>	713-20	2008

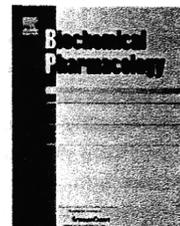
川崎ナナ, 橋井則貴, 杉本直樹, 高倉大輔, 秦艶, 細山沙織, 戸井田敏彦, 山口照英	ヘパリン純度試験に関する研究(4) 合成過硫酸化コンドロイチン硫酸の日局標準品としての適用性の評価	医薬品研究	39(11)	721-29	2008
橋井則貴, 川崎ナナ, 高倉大輔, 伊藤さつき, 川原信夫, 正田卓司, 杉本直樹, 薮島由二, 品川麻衣, 榛葉信久, 宮田一義, 塚本秀樹, 千秋和久, 長谷川泰介, 河合健蔵, 余田 光, 木下充弘, 掛樋一晃, 合田幸広, 奥田晴宏, 棚元憲一, 山口照英	ヘパリン純度試験に関する研究 (第1報) 1H-NMR によるヘパリンナトリウム純度試験に関する研究	医薬品研究	39	651-59	2008
橋井則貴, 川崎ナナ, 高倉大輔, 伊藤さつき, 福原潔, 品川麻衣, 榛葉信久, 有村雅敏, 辰巳昌史, 奥田晴宏, 山口照英	ヘパリン純度試験に関する研究 (第2報) 1H-NMR によるヘパリンカルシウム純度試験に関する研究	医薬品研究	39	660-64	2008
川崎ナナ, 石井明子, 山口照英	糖鎖と生物薬品	Journal Applied Glycoscience.			印刷中
内田恵理子, 川崎ナナ, 宮田直樹	薬の名前 ステムを知られば薬がわかる 第24回	Pharm Tech Japan.	24	1605-11	2008

蜂須賀暁子, 川崎ナナ, 内田恵理子, 宮田直樹	薬の名前 ステムを知れば薬がわかる 第28回	Pharm Tech Japan.	24	2515-23	2008
川崎ナナ, 内田恵理子, 宮田直樹	薬の名前 ステムを知れば薬がわかる 第21回	Pharm Tech Japan.	24	651-6	2008
山口照英, 石井明子	細胞・組織加工医薬品の品質と安全性確保への提言	PHARMASTAGE	7	1-6	2008
新見伸吾, 原島瑞, 日向昌司, 山口照英, 早川堯夫	癌に対する抗血管新生療法の現状と展望(その1)	医薬品研究	39	1-37	2008
新見伸吾, 原島瑞, 日向昌司, 山口照英, 早川堯夫	癌に対する抗血管新生療法の現状と展望(その2)	医薬品研究	39(6)	359-87	2008
川崎ナナ, 橋井則貴, 山口照英	糖鎖異常の網羅的解析	蛋白質核酸酵素増刊号「糖鎖情報の独自性と普遍性」	53	1690-96	2008
山口照英, 石井明子	早期臨床開発段階でのバイオ医薬品の品質・安全性確保	臨床評価	36	611-27	2009



ELSEVIER

available at [www.sciencedirect.com](http://www.sciencedirect.com)

journal homepage: [www.elsevier.com/locate/biochempharm](http://www.elsevier.com/locate/biochempharm)

## The RXR agonists PA024 and HX630 have different abilities to activate LXR/RXR and to induce ABCA1 expression in macrophage cell lines

Tomoko Nishimaki-Mogami<sup>a,\*</sup>, Norimasa Tamehiro<sup>a</sup>, Yoji Sato<sup>a</sup>, Kei-ichiro Okuhira<sup>a</sup>, Kimie Sai<sup>a</sup>, Hiroyuki Kagechika<sup>b</sup>, Koichi Shudo<sup>d</sup>, Sumiko Abe-Dohmae<sup>c</sup>, Shinji Yokoyama<sup>c</sup>, Yasuo Ohno<sup>a</sup>, Kazuhide Inoue<sup>a,1</sup>, Jun-ichi Sawada<sup>a</sup>

<sup>a</sup> National Institute of Health Sciences, Tokyo, Japan

<sup>b</sup> School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan

<sup>c</sup> Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

<sup>d</sup> Itsu Research Institute, Tokyo, Japan

### ARTICLE INFO

#### Article history:

Received 24 June 2008

Accepted 4 August 2008

#### Keywords:

Retinoid X receptor modulator  
Peroxisome proliferator-activated receptor  $\gamma$   
Liver X receptor  
ABCA1  
HDL

### ABSTRACT

Release of cellular cholesterol by ATP-binding cassette transporter (ABC)A1 and apolipoproteins is a major source of plasma high-density lipoprotein (HDL). Expression of ABC transporter A1 (ABCA1) is directly stimulated by liver X receptor (LXR)/retinoid X receptor (RXR) activation. We evaluated the abilities of two RXR agonists, PA024 and HX630, to increase ABCA1 expression. In differentiated THP-1 cells, the two agonists efficiently enhanced ABCA1 mRNA expression and apoA-I-dependent cellular cholesterol release. However, in RAW264 cells and undifferentiated THP-1 cells, PA024 was highly effective while HX630 was inactive in increasing ABCA1 mRNA. In parallel, the two agonists had different abilities to activate ABCA1 promoter in an LXR-responsive-element (LXRE)-dependent manner and to directly stimulate LXR $\alpha$ /RXR transactivation. The ability of HX630 to enhance ABCA1 expression was correlated closely with the cellular PPAR $\gamma$  mRNA level. Moreover, HX630 was able to activate PPAR $\gamma$ /RXR. Transfection of PPAR $\gamma$  in RAW264 cells induced HX630-mediated activation of LXRE-dependent transcription and ABCA1 promoter, suggesting the ability of HX630 to activate PPAR $\gamma$ -LXR-ABCA1 pathway. We conclude that RXR agonist PA024 and HX630 have different abilities to activate LXR/RXR, and that the cell-type-dependent effect of HX630 on ABCA1 expression and HDL generation is closely associated with this defect.

© 2008 Elsevier Inc. All rights reserved.

### 1. Introduction

ABC transporter A1 (ABCA1) mediates and rate-limits biogenesis of high-density lipoprotein (HDL) from helical apolipo-

protein acceptors, such as apoA-I, and cellular cholesterol and phospholipids [1]. Mutations in the ABCA1 gene cause Tangier disease and other genetic HDL deficiencies [2–4]. Conversely, overexpression of ABCA1 in mice resulted in a mild elevation

\* Corresponding author at: Division of Biosignaling, National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan. Tel.: +81 3 3700 9478; fax: +81 3 3707 6950.

E-mail address: [mogami@nihs.go.jp](mailto:mogami@nihs.go.jp) (T. Nishimaki-Mogami).

<sup>1</sup> Present address: Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan.

0006-2952/\$ – see front matter © 2008 Elsevier Inc. All rights reserved.

doi:10.1016/j.bcp.2008.08.005

of HDL cholesterol [5,6] and has been shown to protect animals from atherosclerosis [7,8]. Plasma HDL levels are inversely related to the risk of atherosclerotic cardiovascular disease [9], presumably because HDL functions to remove excess cholesterol from peripheral tissues and transport it to the liver for conversion to bile acids [10]. Accordingly, therapies that increase ABCA1 expression are a promising strategy for preventing and treating atherogenesis.

Cellular expression of ABCA1 is highly regulated. Loading cholesterol into macrophages and fibroblasts resulted in enhanced transcription of the ABCA1 gene by the reaction mediated by the oxysterol-activated liver X receptor (LXR) [11-14]. ABCA1 expression can also be increased by PPAR $\alpha$  or PPAR $\gamma$  activators [15].

Retinoid X receptor (RXR) is a member of the nuclear receptor superfamily and forms heterodimers with a number of other receptors. RXR heterodimers, such as the peroxisome proliferator-activated receptor (PPAR)/RXR, LXR/RXR and the farnesoid X receptor (FXR)/RXR, can be activated by agonists for both RXR and the partner receptors and are classified as permissive heterodimers [16-18]. The thyroid hormone receptor/RXR or vitamin D receptor/RXR are not activated by RXR agonists and termed as non-permissive heterodimers [16-18]. A natural RXR agonist, 9-cis-retinoic acid, and synthetic RXR-selective ligands (retinoids) have been shown to increase ABCA1 expression in macrophages [12,19,20].

In the present study, we evaluated the ability of two RXR agonists, PA024 and HX630, to induce ABCA1 expression in macrophage cell lines. Both PA024 and HX630 have been developed as RXR-selective agonists and are inactive alone in the HL-60 differentiation assay but strongly enhance the activity of low concentration of RAR-selective agonist AM80 [21]. However, their effects on the other RXR heterodimers are unknown. We found that PA024 potently induces ABCA1 expression in all cell models examined. However, HX630 failed to induce ABCA1 expression in RAW264 cells and undifferentiated THP-1 cells, and this defect was closely associated with the lack of ability to activate LXR/RXR. Instead, HX630 was able to activate PPAR $\gamma$ /RXR and induce ABCA1 expression in differentiated THP-1 cells. Our data also suggest the ability of HX630 to stimulate the PPAR $\gamma$ -LXR-ABCA1 pathway.

## 2. Materials and methods

### 2.1. Materials

22(R)-hydroxycholesterol and phorbol 12-myristate 13-acetate (PMA) was obtained from Sigma; troglitazone from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA, USA) HX630, PA024, and Am80 were prepared as described previously [21-23].

### 2.2. Cell culture and real time quantitative RT-PCRs

RAW264 cells were obtained from the Riken Gene Bank (Tsukuba, Japan) and maintained in Dulbecco's modified Eagle's medium (DMEM)/F-12 (1:1) containing 10% fetal calf serum. Cells were incubated for 24 h in serum-free medium containing 0.1% BSA in the presence or absence of 22(R)-hydroxycholes-

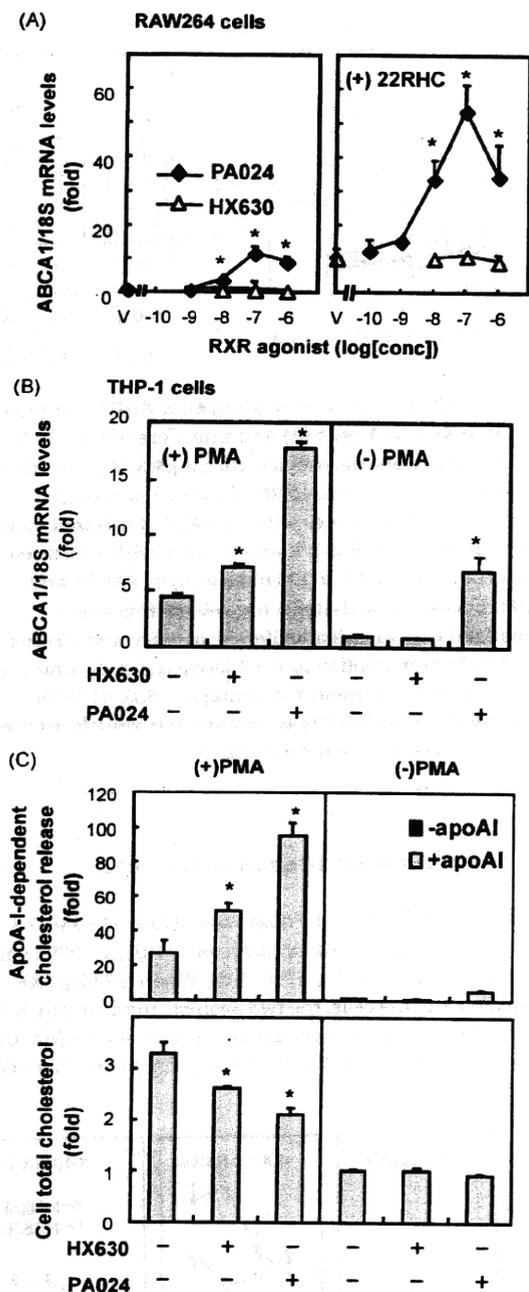
terol (2  $\mu$ g/ml) and RXR agonists. THP-1 cells were maintained in RPMI 1640 medium containing 10% fetal calf serum. Cells were treated with RXR agonists in serum-free medium containing 0.2% BSA. Differentiation of THP-1 cells into macrophages was induced by treatment of the cells with 100 nM PMA for 48 h. RXR agonists were added to the medium during the last 24 h. Cells were harvested and total RNA was extracted using an RNeasy Mini Kit (Qiagen). The RNA samples were treated with DNase according to the manufacturer's protocol (Qiagen). Relative expression levels of mRNA were determined using a TaqMan one-step RT-PCR Master Mix Reagent Kit and an ABI Prism 7700 sequence detection system (Applied Biosystems). Primer/probe sequences used were as follows: human PPAR $\gamma$  forward primer, 5'-AGGCGAGGGCGATCTTG-3', reverse primer, 5'-CCCATCAT-TAAGGAATTCATGTCAT-3', probe, 5'-FAM-CAGGAAAGACAA-CAGACAAATCACCATTTCGT-TAMRA3'. The primer/probe sequences for mouse ABCA1 [24], human ABCA1, human ABCG1, and human LXR $\alpha$  [25] were the same as described previously. Expression data were normalized to 18S rRNA levels, and presented as the fold difference of treated cells against untreated cells.

### 2.3. Plasmid constructs

Plasmids for a LXRE-driven luciferase reporter and a PPAR response element (PPRE)-driven luciferase reporter (pPPRE-tk-Luc) and were constructed by inserting the cDNAs containing two copies of LXRE $\alpha$  and LXRE $\beta$  from the sterol response element binding protein-1c promoter and two copies of PPRE from acyl-CoA oxidase, respectively, upstream from the thymidine kinase (tk) promoter. cDNAs encoding full-length human RXR $\alpha$ , LXR $\alpha$ , LXR $\beta$ , or PPAR $\gamma$  were PCR-cloned and inserted into mammalian expression vector pcDNA3.1 (Invitrogen). A mouse peripheral-type ABCA1 promoter (-1238/+57 of exon 1)-luciferase vector (pABCA1-Luc) has been described previously [24]. A mouse ABCA1 promoter construct containing mutation in LXRE (pABCA1:mutLXRE-Luc) was prepared as described previously [26].

### 2.4. Transient transfections and reporter gene assays

RAW264 cells were transfected with 1.0  $\mu$ g of pABCA1-Luc or pABCA1:mutLXRE-Luc and 0.1  $\mu$ g of Renilla luciferase vector (phRL-TK) (Promega) with SuperFect (Qiagen) in 24-well plates. For LXR activation studies, 1  $\mu$ g of pLXRE-tk-Luc, 50 ng each of pcDNA3.1-LXR and pcDNA3.1-RXR $\alpha$ , and 0.7  $\mu$ g of pSV- $\beta$ -galactosidase control vector (Promega) were used. For the assay of PPAR $\gamma$  activation, cells were transfected with 1.3  $\mu$ g of pPPRE-tk-Luc and 0.1  $\mu$ g of Renilla luciferase vector (phRL-TK) (Promega) in the presence or absence of 50 ng each of pcDNA3.1-LXR and pcDNA3.1-RXR $\alpha$ . An empty pcDNA3.1 expression vector was used to maintain equal amounts of DNA for each transfection. Three hours after transfection, cells were exposed to RXR agonists in the medium containing 10% FCS for 24 h. Undifferentiated THP-1 cells were electroporatically transfected with 0.4  $\mu$ g of pABCA1-Luc or empty vector and 0.1  $\mu$ g of phRL-TK using the Nucleofector transfection system (Amaxa Inc., Gaithersburg, MD) according to the manufacturer's protocol. Four hours after transfection, cells were exposed to RXR agonists in the medium containing 0.2%



**Fig. 1** – Effects of PA024 and HX630 on ABCA1 mRNA expression and cholesterol efflux in RAW264 cells, PMA-differentiated, and undifferentiated THP-1 cells. (A) RAW264 cells were treated for 24 h with PA024 or HX630 in the absence or presence of 22(R)-hydroxycholesterol (22RHC, 2  $\mu$ g/ml). ABCA1 mRNA levels were measured with quantitative real-time RT-PCR analysis, standardized against 18S rRNA levels, and expressed as fold induction relative to the vehicle-treated cells (V, taken as 1). (B) PMA-differentiated or undifferentiated THP-1 cells were treated for 24 h with 100 nM PA024 or HX630. ABCA1 mRNA/18S rRNA levels were expressed as fold induction relative to the vehicle-treated undifferentiated cells. (C) PA024 and HX630 enhance apoA-I mediated cholesterol efflux and

FCS for 24 h. Luciferase and  $\beta$ -galactosidase activities were determined in cell lysates. Firefly luciferase activity was normalized to either that of Renilla luciferase or  $\beta$ -galactosidase for each well.

### 2.5. Coactivator association assay using fluorescence polarization

The assay was performed as described previously [27]. Briefly, TAMRA-labeled peptide (100 nM, with amino acid sequence ILRKLQE) was incubated for 1 h with purified GST-fused human PPAR $\gamma$  LBD (1.5  $\mu$ M) and ligands in 100  $\mu$ l of buffer (10 mM HEPES, 150 mM NaCl, 2 mM MgCl<sub>2</sub>, 5 mM DTT at pH 7.9) in a black polypropylene 96-well plate on a shaker. Ligand-dependent recruitment of the coactivator peptide was measured as increases in fluorescence polarization with a Fusion $\alpha$ -FP (PerkinElmer Life Science).

### 2.6. Measurement of lipid efflux to apolipoprotein A-I (apoA-I)

The differentiated or undifferentiated THP-1 cells were incubated in the presence or absence of 10  $\mu$ g/ml of apoA-I in RPMI 1640 containing 0.2% BSA for 24 h. Lipid was extracted from the medium and the cells with chloroform/methanol (2:1, v/v) and hexane/isopropanol (3:2, v/v), respectively, and cholesterol and choline-phospholipid were determined by enzymatic methods specific for each lipid [28].

### 2.7. Statistical analysis

Data were analyzed by ANOVA followed by the Student-Newman-Keuls method. Statistical significance was established at the  $P < 0.05$  level.

## 3. Results

### 3.1. RXR agonist PA024 and HX630 show different abilities to induce ABCA1 expression

We tested the ability of RXR agonist PA024 and HX630 to induce ABCA1 expression in murine macrophage-like cell line, RAW264. Treatment of RAW264 cells with PA024 markedly induced ABCA1 mRNA expression, which peaked at 100 nM, whereas HX630 up to 1  $\mu$ M had no effect (Fig. 1A left). Expression of ABCA1 can be stimulated by oxysterol-activated

decrease the cellular cholesterol level in differentiated THP-1 cells. PMA-differentiated or undifferentiated THP-1 cells were treated for 24 h with 100 nM PA024 or HX630 in the presence or absence of apoA-I (15  $\mu$ g/ml). ApoA-I-dependent release of cholesterol into the medium and cellular total cholesterol (2.69  $\pm$  0.77  $\mu$ g/mg protein and 15.31  $\pm$  0.39  $\mu$ g/mg protein, respectively, in control cells) were expressed as fold induction relative to the vehicle-treated undifferentiated cells. The values represent the average  $\pm$  S.D. from three experiments. Significantly different from vehicle-treated cells (\*).

LXR [11,13,14]. The addition of 22(R)-hydroxycholesterol to the medium increased ABCA1 expression and strongly enhanced the effect of PA024 (Fig. 1A right). This combination had a greater than additive effect, whereas HX630 again had no effect.

The effect of two RXR agonists on ABCA1 expression was also studied in a human monocytic leukemia cell line, THP-1 (Fig. 1B). PA024 (100 nM) increased the ABCA1 mRNA level both in PMA-differentiated and undifferentiated cells (by 4- and 7-fold, respectively). In contrast, HX630 (100 nM) did not affect the ABCA1 mRNA level in undifferentiated cells. However, the same concentration of HX630 did raise the ABCA1 mRNA level in PMA-differentiated cells by 1.6-fold.

ABCA1 has been shown to play a critical role in the assembly of HDL from apoA-I and cellular cholesterol and phospholipids. We examined the effect of the two RXR agonists on apoA-I-mediated cholesterol release (HDL production) in THP-1 cells (Fig. 1C). HX630 and PA024 at 100 nM increased the amount of cholesterol released into the medium in the presence of apoA-I (by 2- and 3.4-fold, respectively) in differentiated cells, and this increase was accompanied by a decrease in the cellular total cholesterol level. In contrast, the same concentration of HX630 had no effect in undifferentiated cells.

### 3.2. PA024 but not HX630 activates LXR/RXR and ABCA1 promoter

To determine whether the different abilities of the two RXR agonists in ABCA1 mRNA induction were resulted from different abilities in ABCA1 gene transcription, we examined their effects on ABCA1 promoter activity. An ABCA1 promoter (–1238/+57 of exon 1)-luciferase construct was transfected into RAW264 cells. As shown in Fig. 2A, PA024 markedly increased the ABCA1 promoter transcription, and the increase caused by PA024 was lost when a mutation was introduced into LXRE in the promoter. In contrast, HX630 up to 1  $\mu$ M had no effect on the promoter activity. Similarly, when the ABCA1 promoter was transfected into undifferentiated THP-1 cells, PA024 augmented the promoter activity in an LXRE-dependent manner, but HX630 had no effect (Fig. 2B). These findings indicate that PA024-induced activation of ABCA1 promoter was mediated by LXRE. We intended to examine the promoter activity in PMA-differentiated THP-1 cells. However, transfection of the plasmid into the differentiated THP-1 cells was unsuccessful. In addition, cells harboring transfected DNA did not undergo differentiation with PMA.

The possibility that PA024 but not HX630 activates LXR/RXR was tested using a reporter assay. Transfection of the LXRE-driven luciferase-reporter vector alone into RAW264 cells yielded substantial luciferase activity, indicating transcriptional activation by endogenous receptor(s), and this endogenous LXRs-mediated activity was increased by PA024, but not HX630 (Fig. 3 left). Co-transfection of LXR $\alpha$  and RXR $\alpha$  expression vectors enhanced the effect of PA024, whereas HX630 again had no effect (Fig. 3 middle). Expressions of LXR $\beta$  and RXR $\alpha$  augmented luciferase transcription in the vehicle-treated control (Fig. 3 right). However, the response elicited by PA024 was small and similar to that in cells without LXR/RXR plasmids (Fig. 3 left). HX630 again had no effect.

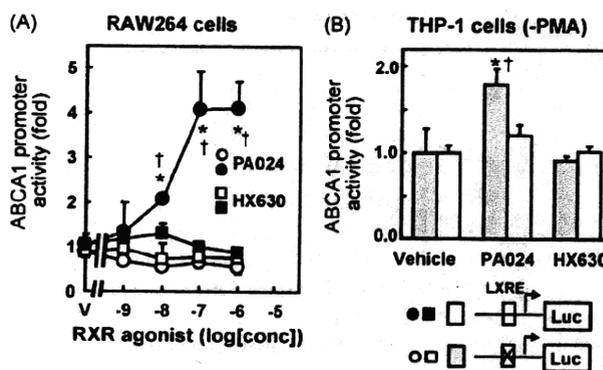


Fig. 2 – PA024 but not HX630 augments ABCA1 promoter activity in RAW264 cells (A) and undifferentiated THP-1 cells (B). Cells were transfected with a pABCA1-Luc (closed symbols) or pABCA1:mutLXRE-Luc (open symbols) reporter plasmid together with a pRL-TK internal control as described in Section 2.4, and treated with indicated concentrations (for A) or 100 nM (for B) of PA024 and HX630. Luciferase activity in the cell extract was normalized using Renilla luciferase activity and expressed as fold induction relative to vehicle-treated cells (indicated as V). The data represent the average  $\pm$  S.D. of three experiments. Significantly different from vehicle-treated cells (\*) or LXRE-mutated promoter (†).

### 3.3. HX630 and PA024 activate PPAR $\gamma$ /RXR

The ability of HX630 and PA024 to activate PPAR $\gamma$ /RXR was tested in RAW264 cells transfected with a PPRE-driven luciferase-reporter vector (Fig. 4A). When PPAR $\gamma$ /RXR was transfected into the cells, the two agonists markedly increased luciferase activity. RXR-homodimer has been shown to activate transcription of the DR-1 element [29]. Upon transfection of

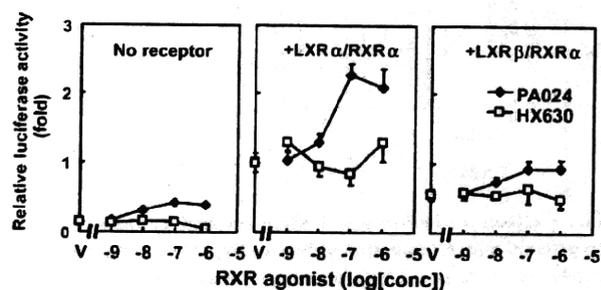


Fig. 3 – PA024 but not HX630 augments LXR $\alpha$ /RXR transactivation. RAW264 cells were transfected with LXREx4-tk-Luc reporter plasmid together with a  $\beta$ -gal internal control in the absence (A) or presence of expression plasmids for human LXR $\alpha$  and RXR $\alpha$  (B) or LXR $\beta$  and RXR $\alpha$  (C). The cells were treated with the indicated concentrations of PA024 or HX630. Luciferase activity in the cell extract was normalized using  $\beta$ -gal and expressed as fold induction relative to vehicle-treated cells (indicated as V). The data represent the average  $\pm$  S.D. of three incubations.