JSSX Award

医薬品個別適正化を目指した薬物動態関連遺伝子の発現調節機構解明とヒトでの機能評価

Pharmacogenomic *in vitro* and *in vivo* human studies for establishment of appropriate individualized drug therapy

Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University, Japan

九州大学大学院薬学研究院薬物動態学分野

家入　一郎

Ichiro Ieiri

December, 11, 2019

Tsukuba International Congress Center, Ibaraki

例1E

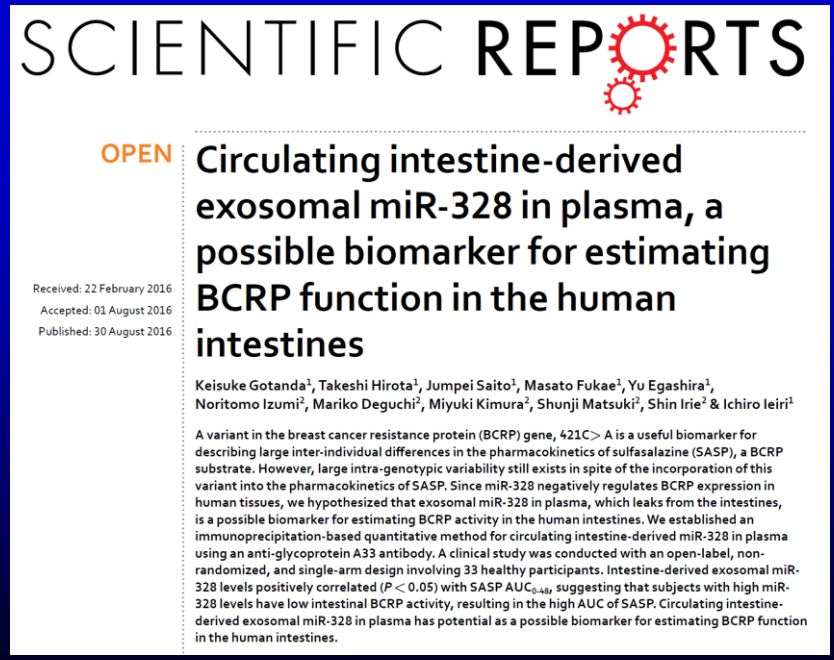
34th JSSX Annual Meeting
COI disclosure information

Author：Ichiro Ieiri

Corroboration study: Daiichi-Sankyo, Taiho Yakuhin

List of our previous human studies toward to PGx based individual drug therapy

Target gene	Drug/Substrate	Type of Study	
cytocrome P450 2C19 (CYP2C19)	omeprazole	healthy volunteers	Clin Pharmacol Ther 1996;59:647
		patients with UL	Br J Clin Pharmacol 1999;47:115
	lansoprazole rabeprazole	healthy volunteers	Eur J Clin Pharmacol 2001;57:485
	phenytoin	healthy volunteers	Br J Clin Pharmacol 1997;43:441
	phenobarbital	epileptic patients	Eur J Clin Pharmacol 2000;55:821 The Drug Monit 2001;23:115
	phenytoin		Ther Drug Monit 2000;22:230
	ticlopidine		Pharmacogenet Genomics 2005;15:851
CYP2C18 and CYP2C19	-	epileptic patients	Pharmacogenetices 1998;8:87
CYP2C9	phenytoin	epileptic patients	Epilepsia 1998;39:1317
	diclofenac	healthy volunteers	Eur J Clin Pharmacol 2000;56:65
CYP2C9 and anti-coagulant related genes	warfarin	patients with warafrin therapy	Blood 2004;103:2630 Blood 2004;103:3055
CYP2C9 and VKORC1			Eur J Clin Pharmacol 2006;62:881 Pharmacogenet & Genomics 2006;16:101
CYP2D6	dextromethorphan	psychatric patients	Pharmacopsychiatry 2003;36:187
	fluvoxamine alprazolam	patients with psychosomatic disease	Eur J Clin Pharmacol 2009;65:699
CYP3A4	midazolam	healthy volunteers	Hum Mol Genet 2004;13:2959
UGT2B7 and MOR1	morphine	cancer patients	Drug Metab Dispos 2003;31:677
ABCB1 (MDR1, Multidrug resistance 1)	-	healthy volunteers	J Pharmacol Exp Therapeutic 2001;297:1137
	digoxin		Clin Pharmacol Ther 2002;72:209
	tacrolimus	patients with liver transplantation	Transplantation 2002;74:571
	^{11}C-verapamil	healthy volunteers	J Nuclear Med 2006;47:1427
ABCC2 (MRP2, multidrug resistance protein 2)	-	a patient with Dubin-Johnson syndrome	Drug Metab Pharmacokinet 2009;24:464
	isoflavonoids	healthy volunteers	Pharmacogenet Genomics 2012;22:344
ABCC3 (MRP3)	4-methylumbelliferone	healthy volunteers	Drug Metab Pharmacokinet 2011;26:347
SLCO1B1 (OATP1B1, organic anion tansporting polypeptide 1B1)	pravastatin, valsartan, temocapril	healthy volunteers	Clin Pharmacol Ther 2006;79:427
	pravastatin olmesartan		J Hum Genet 2008;53:899
	-		Drug Metab Pharmacokinet 2019, in press
SLCO1B1 and SLC22A3 (OCT3, organic cation transporter 3)	pravastatin		Clin Pharmacol Ther 2003;73:554
SLCO1B1, UGT1A1 and ABCC2	bilirubin		Hepatology Res 2004;30:91
SLCO1B1 and UGT1A1	irinorecan	cancer patients	Cancer Chemother Pharmacol 2009;63:1165 The Drug Monit 2007;29:666
SLCO1B1 and ABCG2	pitavastatin	healthy volunteers	Clin Pharmacol Ther 2007;82:541
SLCO1B3, UGT1A1 and 1A3	telmisartan		Pharmacogenet Genomics 2011;21:495
SLCO2B1	celiprolol		J Clin Pharmacol 2012;52:1078
	atenolol		Br J Clin Pharmacol 2012;75:172
SLC22A1 (OCT1) and SLC22A2 (OCT2)	metformin	DM patients	J Hum Genet 2007;52:117
ABCG2 (BCRP, breast cancer resistance protein)	-	healthy volunteers	Drug Metab Dispos 2005;33:94
	curcumin		Br J Pharmacol 2012;166:1793
ABCG2 and NAT2	sulfasaladine		Clin Pharmacol Ther 2008;84:95
5-HT transporter and CYP2D6	paroxetine	patients with paroxetine	Int J Neuropsychopharmaol 2008;11:261 J Clin Psychopharmacol 2010;30:11
4 CYPs and OATPs	cocktails	healthy volunteers	Int J Clin Pharmacol Ther 2012;50:689
CYP3A4, ABCB1 and SLCO1B1	ritonavir saquinavir		J Clin Pharmacol 2013;53:654
RFC1 (Reduced folate carrier 1)	methtrexate	RA patients	Drug Metab Pharmacokinet 2013;28:1
Multiple genes (DMET, DNA chip)	-	healthy volunteers	Pharmacogenet Genomics 2014;24:477
ABCG2 and circulating miR-328	sulfasaladine		Sci Rep 2016 Aug 30;6:32299.



SCIENTIFIC REPORTS

OPEN

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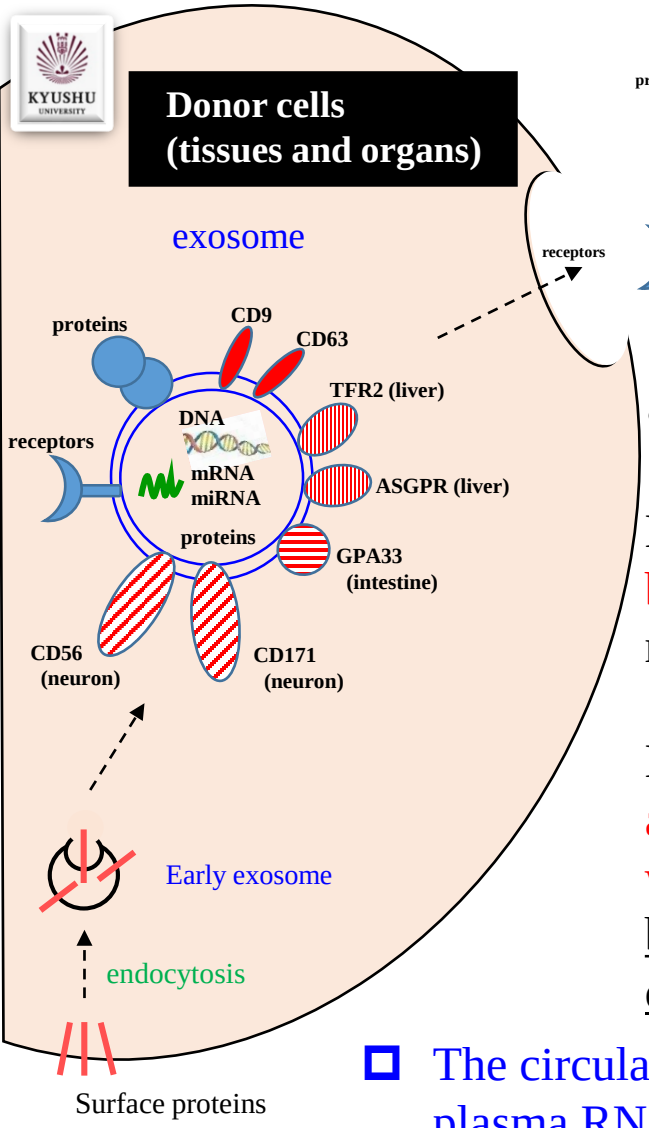
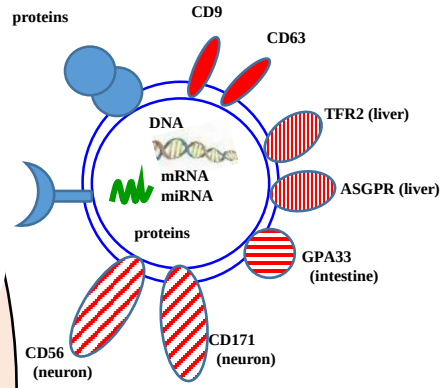
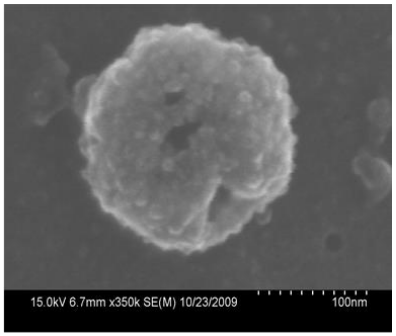
Circulating intestine-derived exosomal miR-328 in plasma, a possible biomarker for estimating BCRP function in the human intestines

Keisuke Gotanda[1], Takeshi Hirota[1], Jumpei Saito[1], Masato Fukae[1], Yu Egashira[1], Noritomo Izumi[2], Mariko Deguchi[2], Miyuki Kimura[2], Shunji Matsuki[2], Shin Irie[2] & Ichiro Ieiri[1]

A variant in the breast cancer resistance protein (BCRP) gene, 421C> A is a useful biomarker for describing large inter-individual differences in the pharmacokinetics of sulfasalazine (SASP), a BCRP substrate. However, large intra-genotypic variability still exists in spite of the incorporation of this variant into the pharmacokinetics of SASP. Since miR-328 negatively regulates BCRP expression in human tissues, we hypothesized that exosomal miR-328 in plasma, which leaks from the intestines, is a possible biomarker for estimating BCRP activity in the human intestines. We established an immunoprecipitation-based quantitative method for circulating intestine-derived miR-328 in plasma using an anti-glycoprotein A33 antibody. A clinical study was conducted with an open-label, non-randomized, and single-arm design involving 33 healthy participants. Intestine-derived exosomal miR-328 levels positively correlated ($P < 0.05$) with SASP $AUC_{0\text{-}48}$, suggesting that subjects with high miR-328 levels have low intestinal BCRP activity, resulting in the high AUC of SASP. Circulating intestine-derived exosomal miR-328 in plasma has potential as a possible biomarker for estimating BCRP function in the human intestines.







Urine
Plasma

Exosomes are small membrane vesicles that are secreted into body fluids such as blood, urine, breast milk, and saliva from many tissues or organs such as liver and intestine.

Exosomes contain functional proteins, lipids, mRNAs, DNAs, and miRNA, which are derived from the tissue or organ from which they originate. These materials are useful as biomarkers in body fluids for estimation of functional state of that tissue or organ.

- The circulating exosomal RNAs (mRNA and miRNA) are protected from plasma RNase by exosome.
- Tissue or cell specific surface proteins are also added in the exosomal membrane.
- Therefore,tissue specific exosomes can be obtained by immunoprecipitation techniaues.

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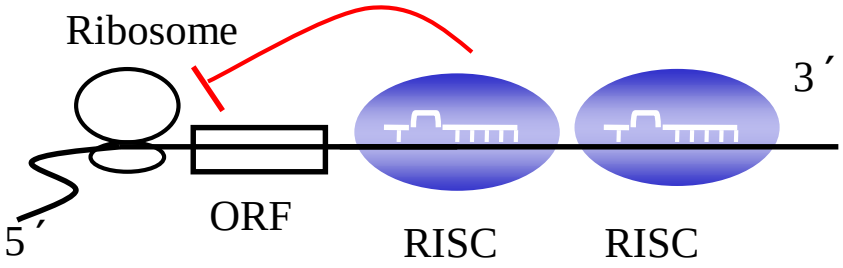
What is microRNA ?

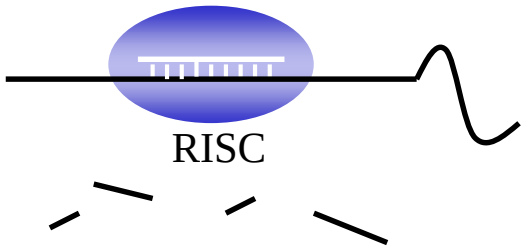
- MicroRNA (miRNA) is a small noncoding RNA, approximately 20-25 nucleotides in length, which binds the 3'-untranslated region (3'-UTR) of mRNA leading to inhibition of translation of mRNA or degradation of mRNA, which depends on sequence complementarity, sequence similarity between miRNA and 3'-UTR of mRNA

- MicroRNAs involve cellular proliferation, differentiation, and apoptosis in metazoans, humans, mice and other organisms

Translational repression

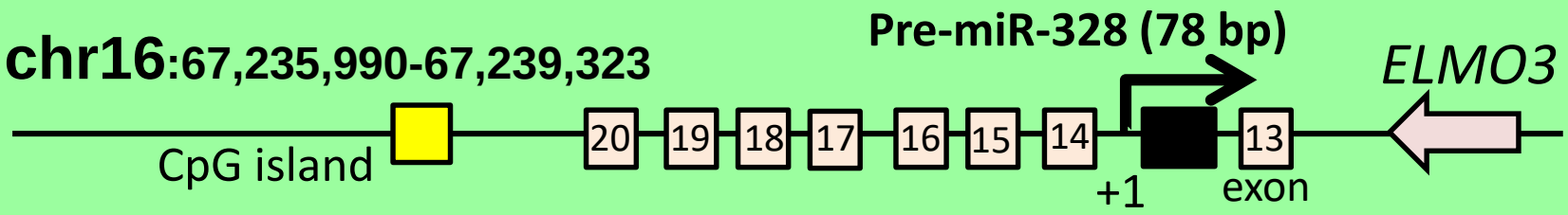


mRNA cleavage



hsa-miR-328

```
uggaguggggggcaggaggggcucagggagaaagugc   3' region of ABCG2 gene
||  ||||||||||||  |||||  |||||  |  ||
guccc-u gccuucccgucucucccg  guccc  c-gacaua   Mature-hsa-miR-328
```

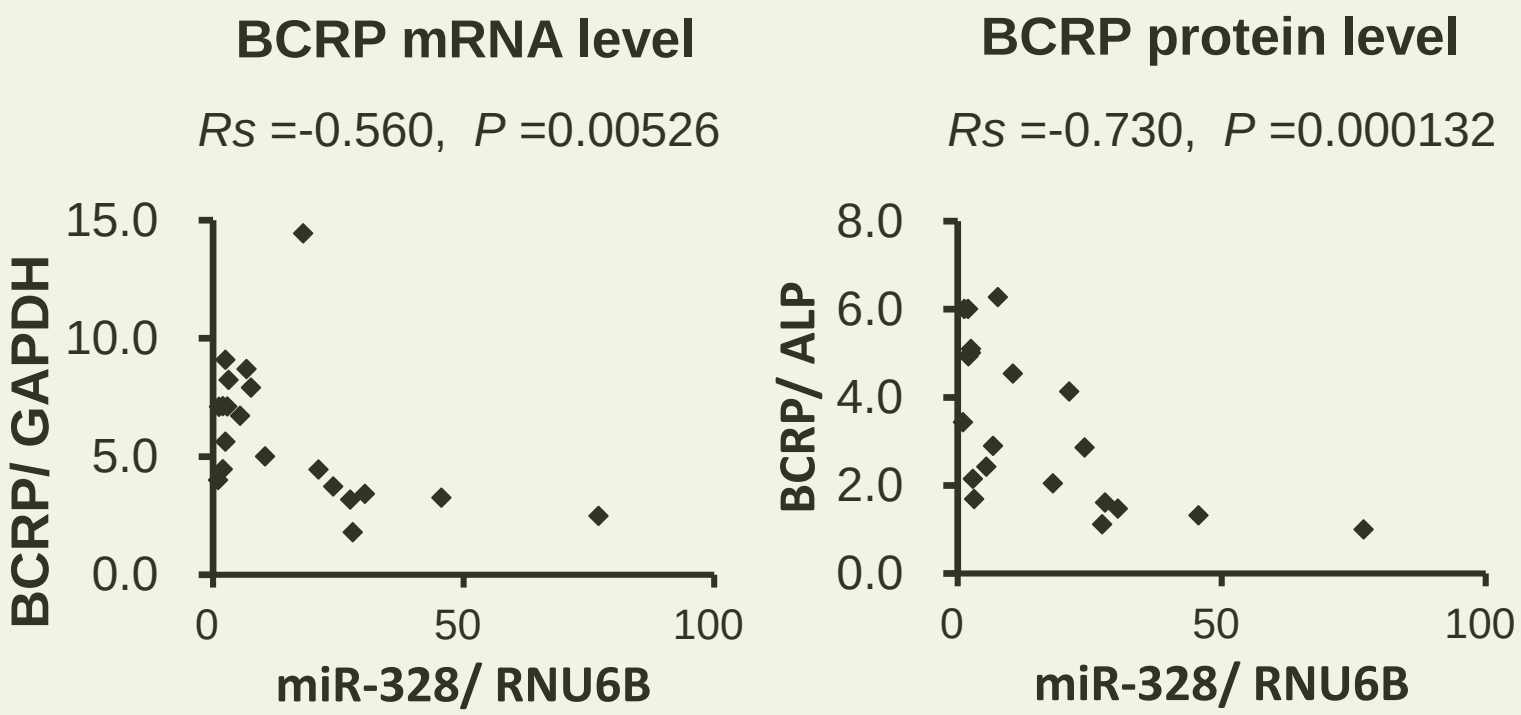


miR-328 and ABCG2 gene, BCRP protein expression, and efflux functions

Previous evidences;

1. *miR-328 negatively regulated the expression of (BCRP/ABCG2) in human cancer cells.*
   Mol Pharmacol 75:137 (2009)
2. *Downregulation of ABCG2 expression in glioblastoma cancer stem cells with miR-328 may decrease their chemo-resistance.*
   Med Sci Monit 16:27 (2010)
3. *MiR-328 expression is decreased in high-grade glioma and is associated with worse survival in primary glioblastoma.*
   PLOS ONE 7:e47270 (2012)
4. *MicroRNA expression profiling identifies miR-328 regulates cancer stem cell-like SP cells in colorectal cancer.*
   Bt J Cancer 106:1320 (2012)
5. *microRNA-328 is a favorable prognostic marker in human glioma via suppressing invasive and proliferative phenotypes of malignant cells.*
   Int J Neirosci 126:145 (2016)

The relationship between miR-328 and BCRP expression in human placentas



Correlation analysis between miR-328 and BCRP genes mRNA or protein expression levels in human placentas samples

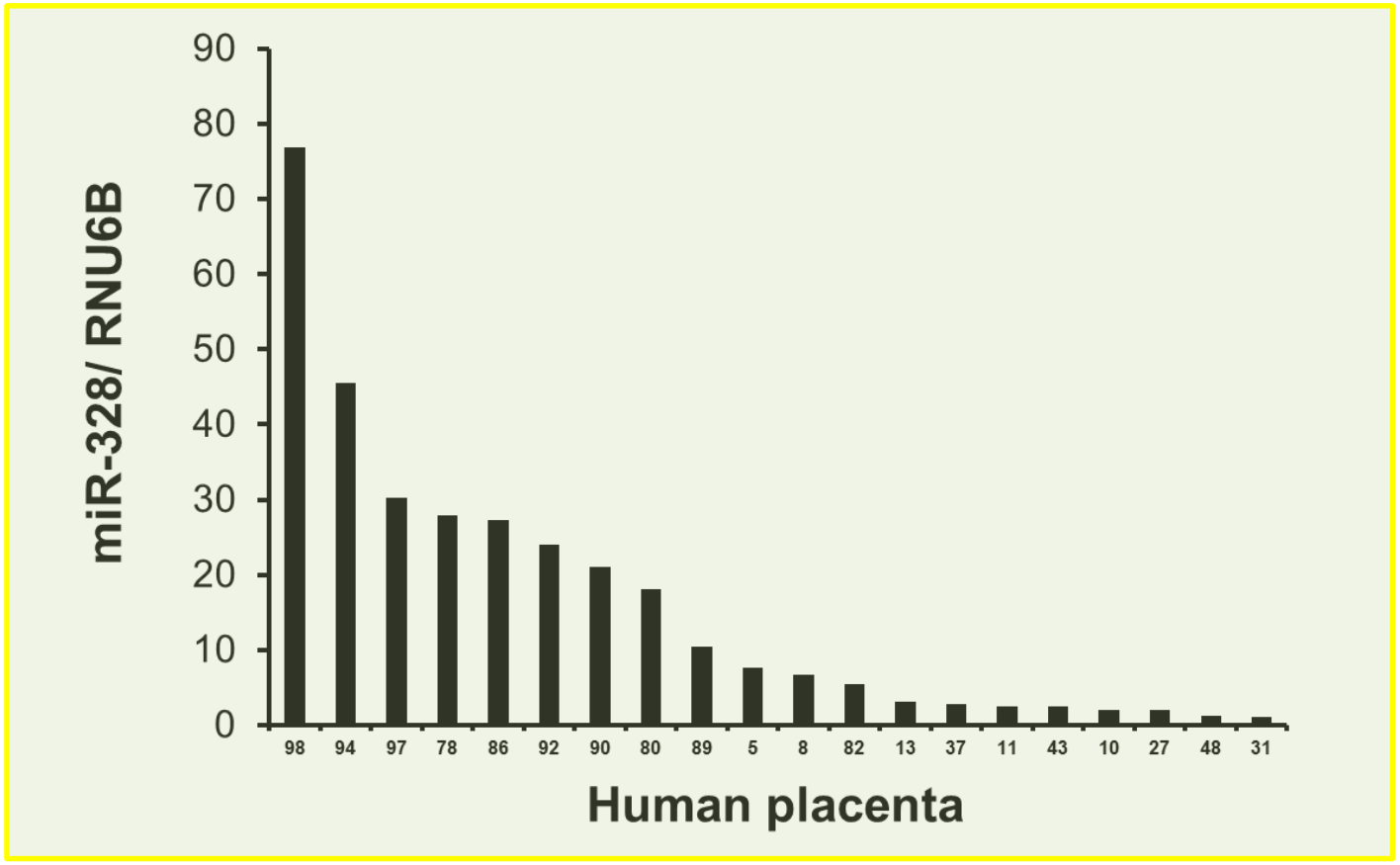
Wild type (421 C/C) homozygote samples were selected to eliminate the influence of BCRP genetic variants (n = 20)

These observations are consistent with previous reports showing BCRP expression is regulated by miR-328

Inter-individual difference in miR-328 expression in human placentas

MiRNA expression levels in normal tissues showed a large inter-individual variability

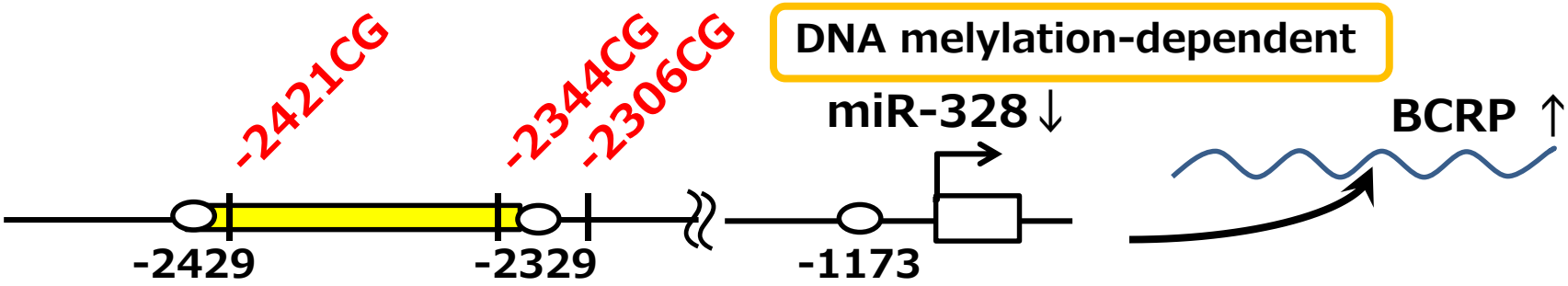
(Hirota T, et al., 2012, Yokoi T, et al., 2011)



Relative miR-328 expression levels in human placenta (n=20)

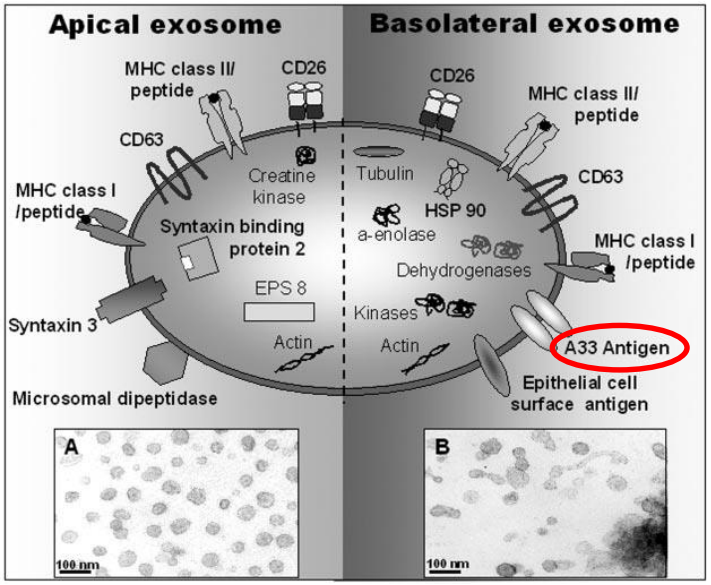
An over 80-fold inter-individual difference is observed in miR-328 levels

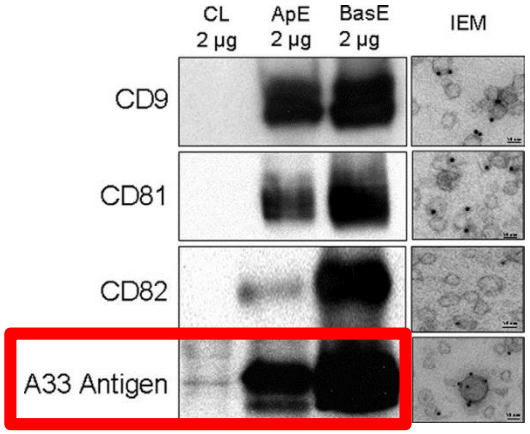
Methylation in the three CG dinucleotides in the 5'-flanking region of precursor of the miR-328, that is the promoter region, suppressed miR-328 expression, leading to BCRP up-regulation in the human placenta

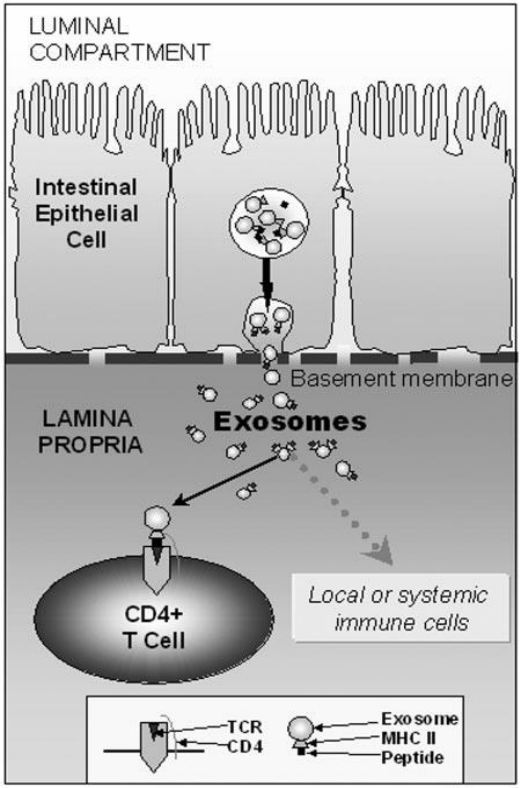


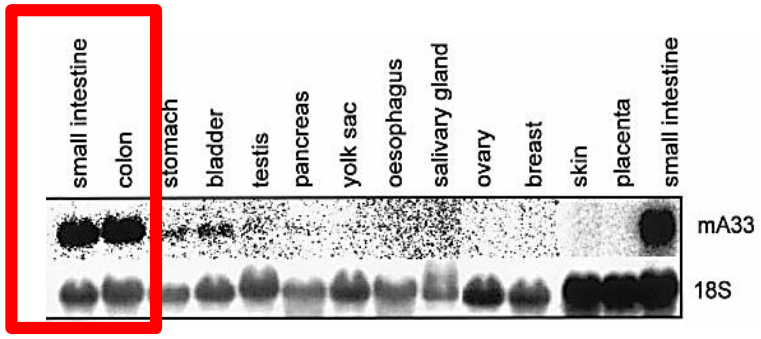
Glycoprotein A33 (GPA33) UniProtKB/Swiss-Prot:Q99795

- Cell surface antigen (319-aa)
- Expression in greater than 95% of human colon cancers





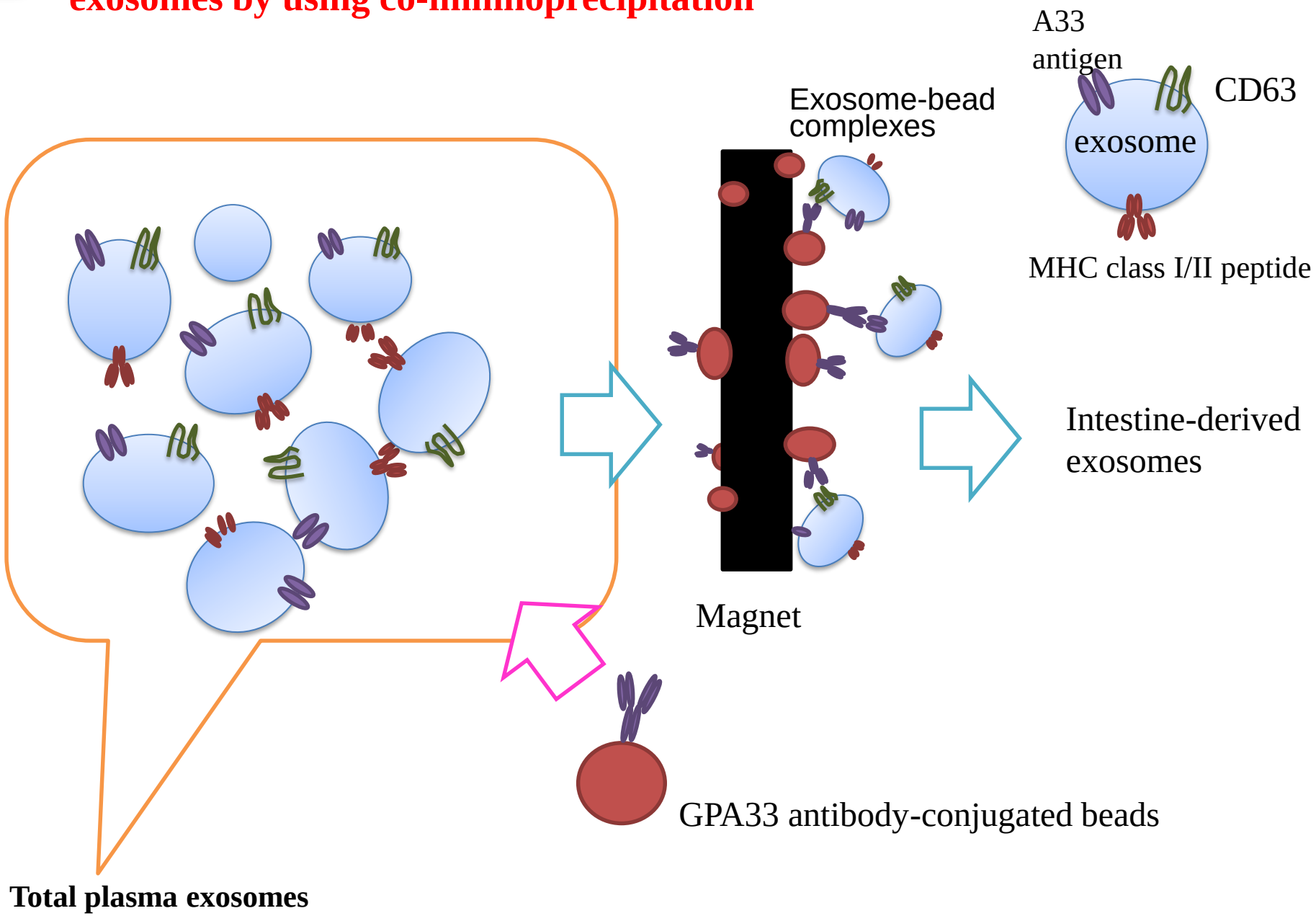




Western blots indicate that CD9, CD81, CD82, and **A33 antigen are expressed in apical (ApE) and basolateral (BasE) exosomes**

(Mallegol J et al., 2010)

Isolation of the intestine-derived exosomes from the total plasma exosomes by using co-immnoprecipitation



Relationship between exosomal miR-328 expression and the pharmacokinetics of sulfasalazine

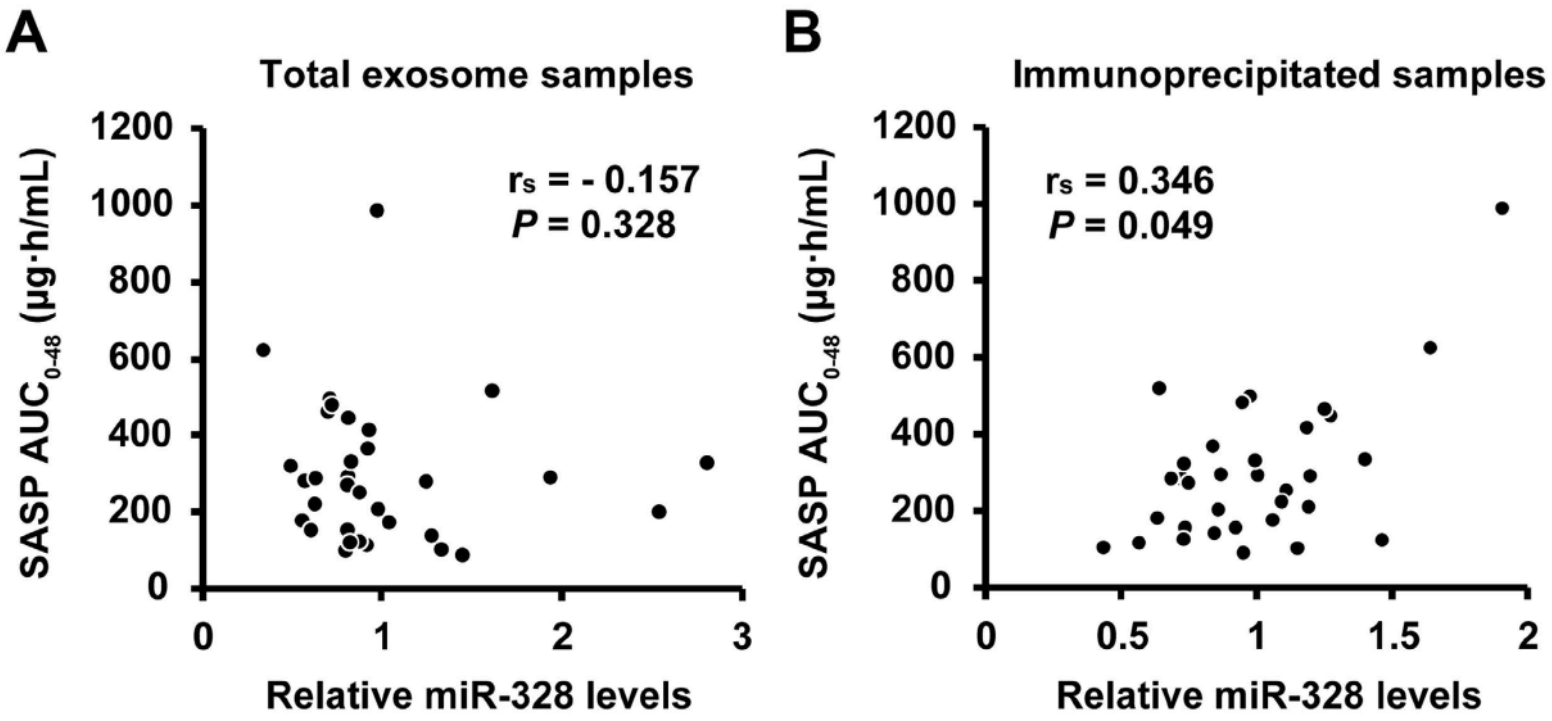


Figure 4. Relationship between miR-328 levels in total exosomes or intestine-derived exosomes in plasma and SASP AUC_{0-48}. MiR-328 levels were normalized with the most stable reference genes selected by geNorm for all samples. Significance was determined by Spearman's correlation test.

Subjects with high miR-328 levels in plasma show high SASP AUC, which suggest that subjects with high miR-328 levels in plasma show low intestinal BCRP function.

Plasma MiR-328 level in the intestine-derived exosomes can serve as a potential biomarker to predict the pharmacokinetics of the BCRP substrate.

British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2019) **85** 216–226 216

ORIGINAL ARTICLE

Plasma extracellular nanovesicle (exosome)-derived biomarkers for drug metabolism pathways: a novel approach to characterize variability in drug exposure

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Andrew Rowland[1,*], Warit Ruanglertboon[1], Madelé van Dyk[1], Dhilushi Wijayakumara[1], Linda S. Wood[2], Robyn Meech[1], Peter I. Mackenzie[1], A. David Rodrigues[2], Jean-Claude Marshall[2] and Michael J. Sorich[1]

[1]*College of Medicine and Public Health, Flinders University, Adelaide, Australia and* [2]*Pfizer Worldwide Research and Development, Groton, USA*

*Principle Investigator: Dr Andrew Rowland was the Principle Investigator for this paper; he had direct clinical responsibility for study participants.

Keywords ADME, biomarkers, cytochrome P450, exosomes

AIMS

Demonstrate the presence of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) proteins and mRNAs in isolated human plasma exosomes and evaluate the capacity for exosome-derived biomarkers to characterize variability in CYP3A4 activity.

METHODS

The presence of CYP and UGT protein and mRNA in exosomes isolated from human plasma and HepaRG cell culture medium was determined by mass spectrometry and reverse transcription–polymerase chain reaction, respectively. The concordance between exosome-derived CYP3A4 biomarkers and midazolam apparent oral clearance (CL/F) was evaluated in a small proof-of-concept study involving six genotyped (CYP3A4 *1/*1 and CYP3A5 *3/*3) Caucasian males.

RESULTS

Exosomes isolated from human plasma contained peptides and mRNA originating from CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2 J2, 3A4 and 3A5, UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10 and 2B15, and NADPH-cytochrome P450 reductase. Mean (95% confidence interval) exosome-derived CYP3A4 protein expression pre- and post-rifampicin dosing was 0.24 (0.2–0.28) and 0.42 (0.21–0.65) ng ml^{-1} exosome concentrate. Mean (95% confidence interval) exosome CYP3A4 mRNA expression pre- and post-rifampicin dosing was 6.0 (1.1–32.7) and 48.3 (11.3–104) $\times 10^{-11}$ $2^{-\Delta\Delta Ct}$, respectively. R^2 values for correlations of exosome-derived CYP3A4 protein expression, CYP3A4 mRNA expression, and *ex vivo* CYP3A4 activity with midazolam CL/F were 0.905, 0.787 and 0.832, respectively.

CONCLUSIONS

Consistent strong concordance was observed between exosome-derived CYP3A4 biomarkers and midazolam CL/F. The significance of these results is that CYP3A4 is the drug-metabolizing enzyme of greatest clinical importance and variability in CYP3A4 activity is poorly described by existing precision dosing strategies.

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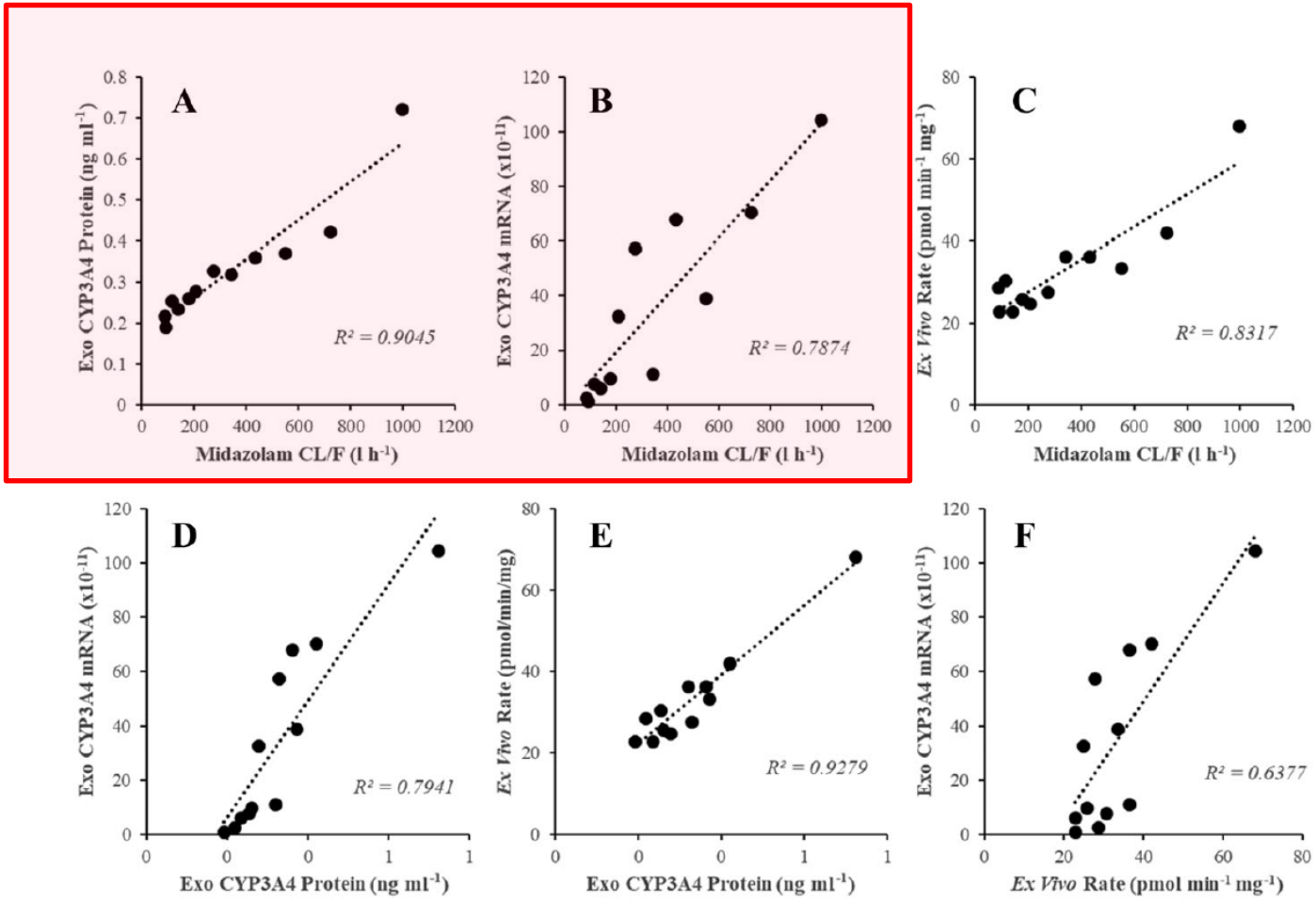

Figure 4

Concordance of exosome-derived CYP3A4 biomarkers and midazolam CL/F in a cohort of healthy males ($n = 6$). (A) Exosome-derived CYP3A4 protein expression *vs.* midazolam CL/F. (B) Exosome-derived CYP3A4 mRNA expression *vs.* midazolam CL/F. (C) *Ex vivo* CYP3A4 activity (rate of 1-hydroxymidazolam formation) *vs.* midazolam CL/F. (D) Exosome-derived CYP3A4 mRNA expression *vs.* exosome-derived CYP3A4 protein expression. (E) *Ex vivo* CYP3A4 activity *vs.* exosome-derived CYP3A4 protein expression. (F) *Ex vivo* CYP3A4 activity *vs.* exosome-derived CYP3A4 protein expression

From Endogenous Compounds as Biomarkers to Plasma-Derived Nanovesicles as Liquid Biopsy; Has the Golden Age of Translational Pharmacokinetics-Absorption, Distribution, Metabolism, Excretion-Drug–Drug Interaction Science Finally Arrived?

David Rodrigues[1,*] and Andrew Rowland[2]

It is now established that a drug's pharmacokinetics (PK) absorption, distribution, metabolism, excretion (ADME) and drug–drug interaction (DDI) profile can be modulated by age, disease, and genotype. In order to facilitate subject phenotyping and clinical DDI assessment, therefore, various endogenous compounds (in plasma and urine) have been pursued as drug-metabolizing enzyme and transporter biomarkers. Compared with biomarkers, however, the topic of circulating extracellular vesicles as "liquid biopsy" has received little attention within the ADME community; most organs secrete nanovesicles (e.g., exosomes) into the blood that contain luminal "cargo" derived from the originating organ (proteins, messenger RNA, and microRNA). As such, ADME profiling of plasma exosomes could be leveraged to better define genotype-phenotype relationships and the study of ontogeny, disease, and complex DDIs. If methods to support the isolation of tissue-derived plasma exosomes are successfully developed and validated, it is envisioned that they will be used jointly with genotyping, biomarkers, and modeling tools to greatly progress translational PK-ADME-DDI science.

Table 1 Summary examples of ADME biomarkers and studies utilizing plasma-derived, urine-derived, or cell culture medium-derived exosomes

Protein	Biomarker	References
OATP1B1 (*SLCO1B1*)	CP-I, GCDCA-S (plasma)	35–39
MRP2 (*ABCC2*)	CP-I (plasma, urine)	
OAT1 (*SLC22A6*)	Taurine (plasma, urine)	
OAT3 (*SLC22A8*)	6βHC, GCDCA-S (plasma, urine)	
MATE1 (*SLC47A1*)	NMN (plasma, urine)	
OCT2 (*SLC22A2*)	NMN (plasma, urine)	
CYP3A4/5	4βHC (plasma), 6βHC/cortisol (urine)	
Protein(s)	**Exosome preparation**	**References**
CYP3A4	Measurement of CYP3A activity and expression in plasma-derived exosomes following inducer (rifampicin) Assessment of CYP3A4 mRNA induction in HepaRG cell medium exosomes following rifampicin	63
CYPs, UGTs, AO, SULT	Immunoblotting and proteomic analysis of multiple DME in medium-derived exosomes of collagen-plated rat hepatocytes	55
Multiple CYPs	mRNA and immunoblot analysis of human plasma-derived exosomes CYP2E1 and CYP3A4 activity measurement	49
BCRP (*ABCG2*)	Correlation of intestine-derived exosome miR-328 levels vs. sulfasalazine plasma AUC	56
OCTN2 (*SLC22A5*)	Sodium-dependent carnitine uptake in HEK293 cell–derived and human urine–derived exosomes	57
Multiple UGTs	Measurement of UGT activity (4MU) and expression using human plasma-derived exosomes	63
P-gp (*ABCB1*)	Measurement of P-gp expression in exosomes in media of drug-resistant MCF-7 cells in culture	58

4βHC, 4β-hydroxycholesterol; 4MU, 4-methylumbelliferone; 6βHC, 6β-hydroxycortisol; ADME, absorption, distribution, metabolism, and excretion; AO, aldehyde oxidase; AUC, area under the plasma concentration vs. time curve; BCRP, breast cancer resistance protein; CP-I, coproporphyrin I; CYP, cytochrome P450; DME, drug-metabolizing enzymes; GCDCA-S, glycochenodeoxycholic acid 3-sulfate; HEK, human embryonic kidney; MATE, multidrug and toxin extrusion protein; MCF-7, Michigan Cancer Foundation-7; miR, micro RNA; MRP, multidrug resistance-associated protein; NMN, N^1-methylnicotinamide; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OCTN, carnitine/organic cation transporter; Pgp, P-glycoprotein; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

血液1滴でがん検査　東芝、21年にもキット実用化

エレクトロニクス ヘルスケア

2019/11/25 0:00

東芝は血液1滴から13種類のがんを発見できる検査キットを開発した。がんにかかっているかどうかを2時間以内に99%の精度で判定できるという。2020年にがん患者を対象に実証試験を始め、21～22年に人間ドックの血液検査などで実用化することを目指す。2万円以下で検査できるようにする考えだ。

画像の拡大

東芝が開発したがん検査キット

東芝が感染症検査用などで販売している遺伝子検査チップをもとに開発した。がんができると血液中に増える「マイクロRNA」という物質を検出する。東京医科大学や国立がん研究センターが開発に協力した。

過去に採取されたがん患者の血液で精度を検証した。大腸がんや肺がん、膵臓（すいぞう）がんなど13種類のがんについて、何らかのがんにかかっているかどうかを99%の精度で判定できたという。大きさが1センチメートルに満たない早期のがんも発見できた。医療現場ではこの検査を受けて、どの臓器にがんがあるかを画像診断などで確認する。

同様の技術は東レなども開発しているが、東レの検査は特定のがんを調べる手法で、多数のがんを調べるには数万円以上かかる見通し。東芝は13種類のいずれかのがんにかかっていることが1度で分かり、採血から2時間以内と、東レなど他社の数分の一程度の時間で結果が出るのも特長。半導体などの技術を活用し、電気的な方法でマイクロRNAを検出する。

20年に始める実証試験では、新たにがんと診断された患者などを対象により大規模に判定精度を検証する。この結果を受けて、まずは人間ドックなどで自費で受ける検査として実用化する考え。

アクセスランキング 一覧 ›

1. 日立製作所、日立化成を昭和電工に売却へ　デジタル注力
2. 働く高齢者の年金減額基準、65歳以上は据え置き
3. 香港の圧倒的な民意、中国に圧力

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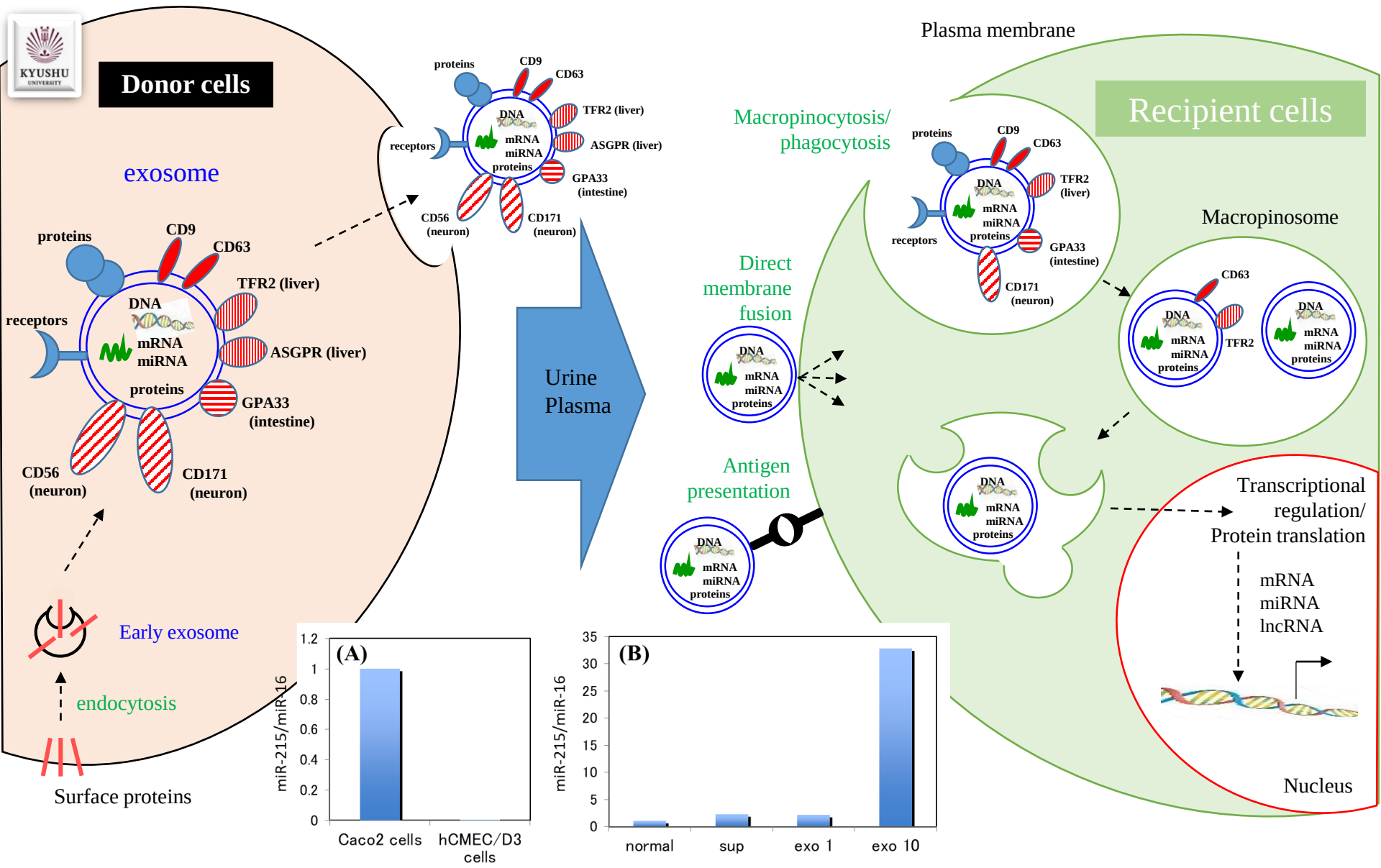

図4 Caco-2細胞由来exosomeをヒト脳血管内皮由来hCMEC/D3細胞に曝露した際のmiRNAの取り込み検討。(A) miR-215はCaco-2細胞にのみ発現し、hCMEC/D3細胞には見られない。しかし、Caco-2細胞由来exosomeをhCMEC/D3細胞に曝露すると、添加exosome濃度依存的にhCMEC/D3細胞内のmiR-215発現が観察される(B)。("normal", exosome非含有培地;"sup", exosomeを分取した後の上清のみを添加)

Acknowledgments

Laboratory stuffs

Kyushu Pharmacology Research Clinic