図 2

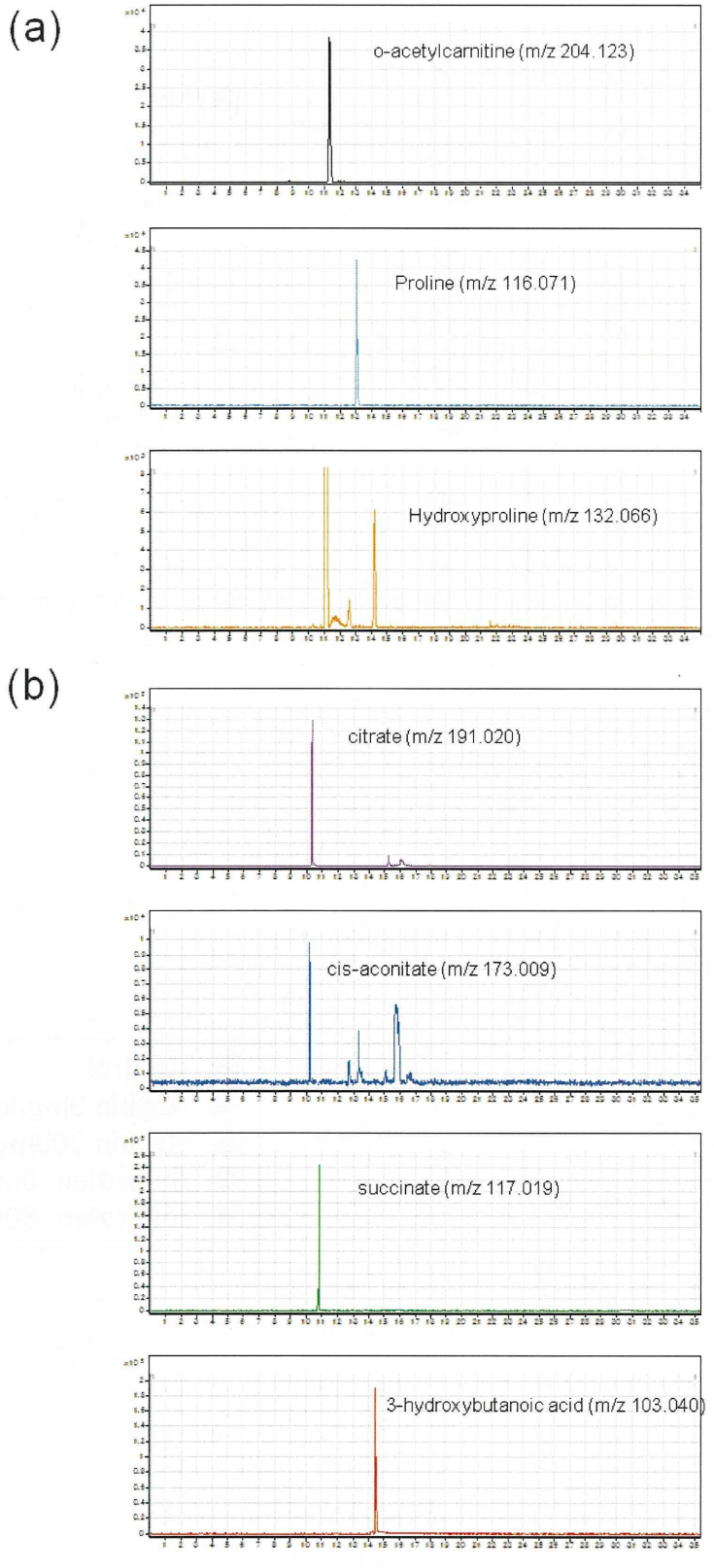

(a)
o-acetylcarnitine (m/z 204.123)
Proline (m/z 116.071)
Hydroxyproline (m/z 132.066)
(b)
citrate (m/z 191.020)
cis-aconitate (m/z 173.009)
succinate (m/z 117.019)
3-hydroxybutanoic acid (m/z 103.040)
Time (min)

図 3

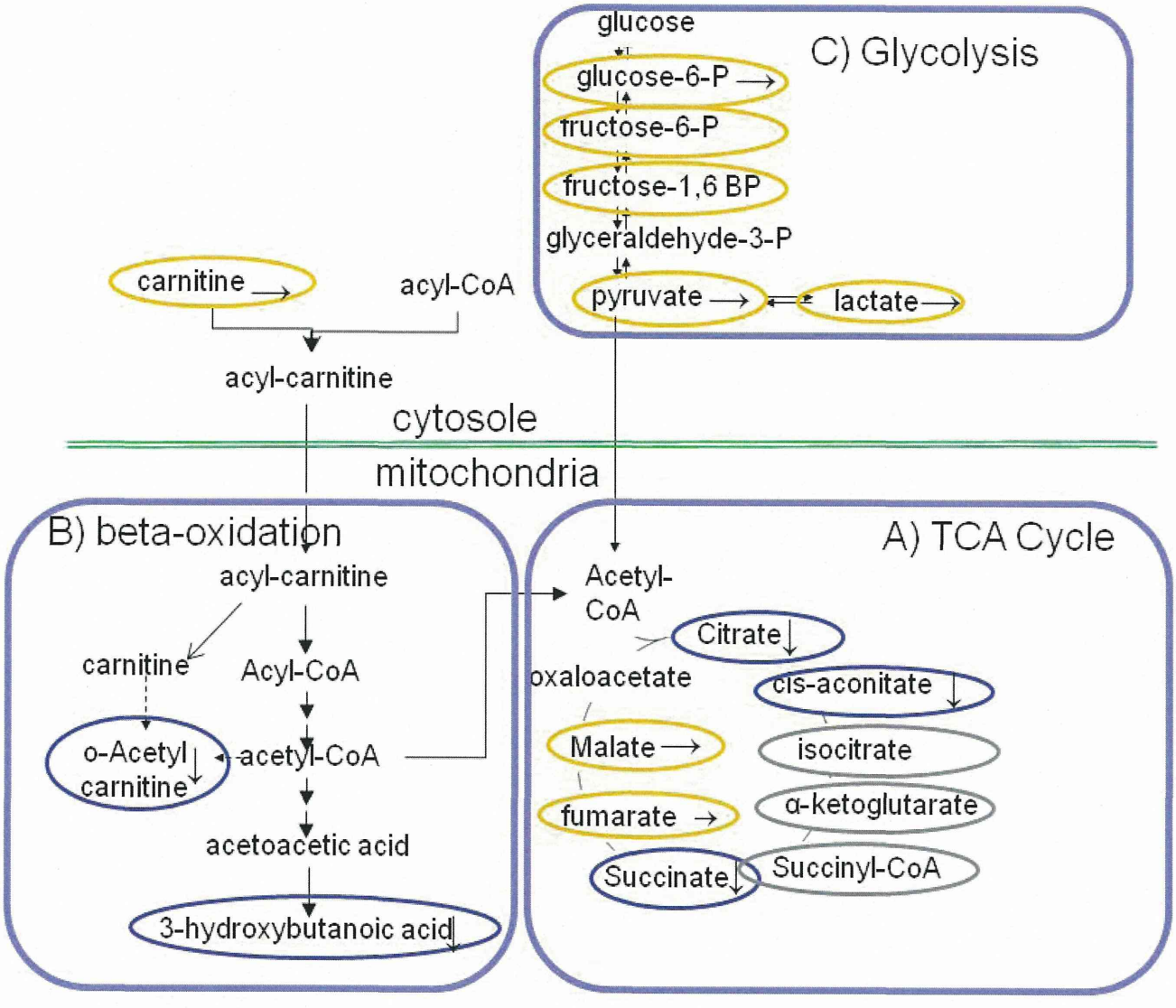

glucose
C) Glycolysis
glucose-6-P
fructose-6-P
fructose-1,6 BP
glyceraldehyde-3-P
pyruvate
lactate
carnitine
acyl-CoA
acyl-carnitine
cytosole
mitochondria
B) beta-oxidation
acyl-carnitine
carnitine
Acyl-CoA
o-Acetyl carnitine
acetyl-CoA
acetoacetic acid
3-hydroxybutanoic acid
A) TCA Cycle
Acetyl-CoA
Citrate
oxaloacetate
cis-aconitate
Malate
isocitrate
fumarate
α-ketoglutarate
Succinate
Succinyl-CoA

図 4

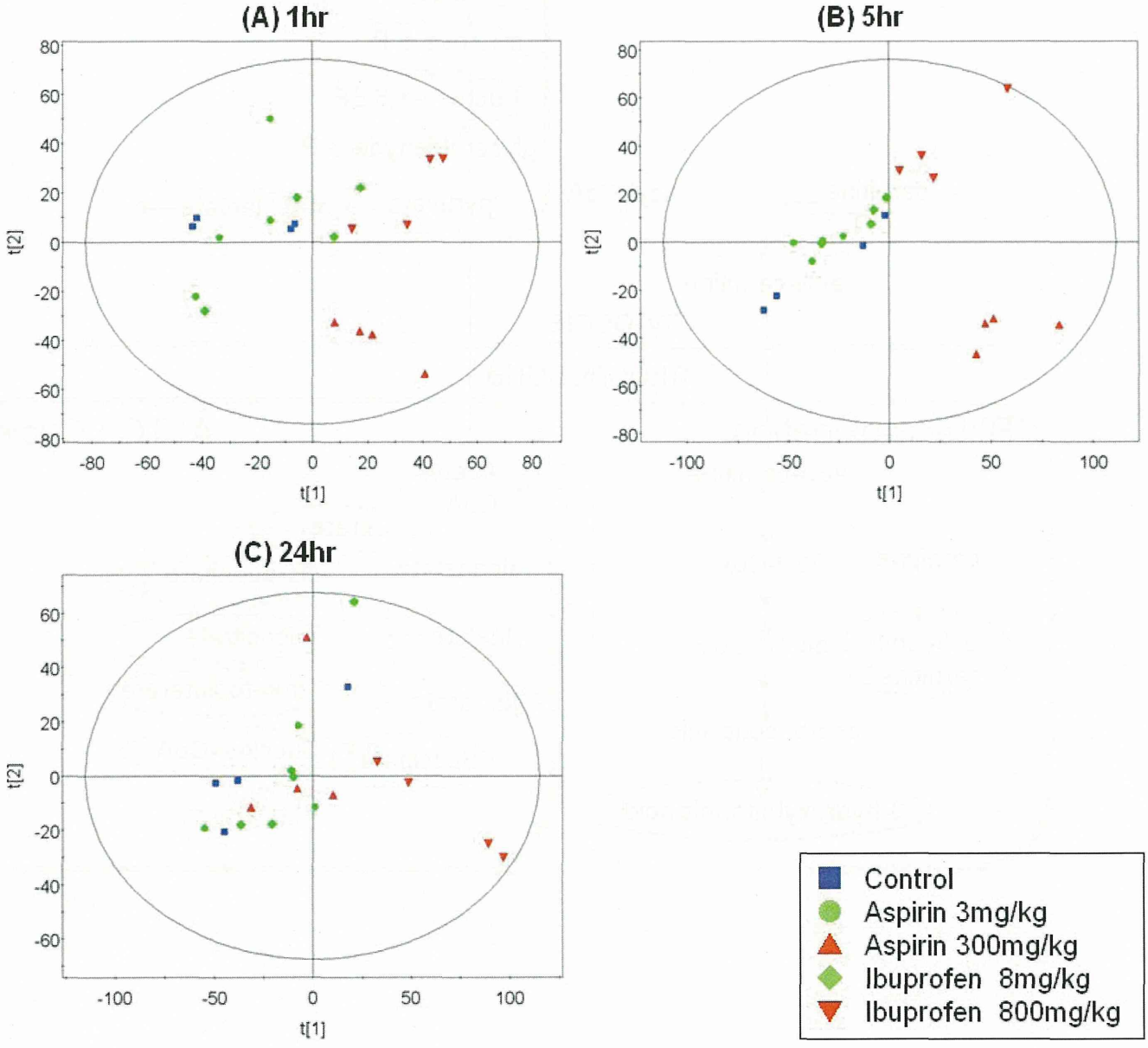

(A) 1hr
(B) 5hr
(C) 24hr
t[1]
t[2]
Control
Aspirin 3mg/kg
Aspirin 300mg/kg
Ibuprofen 8mg/kg
Ibuprofen 800mg/kg

図 5

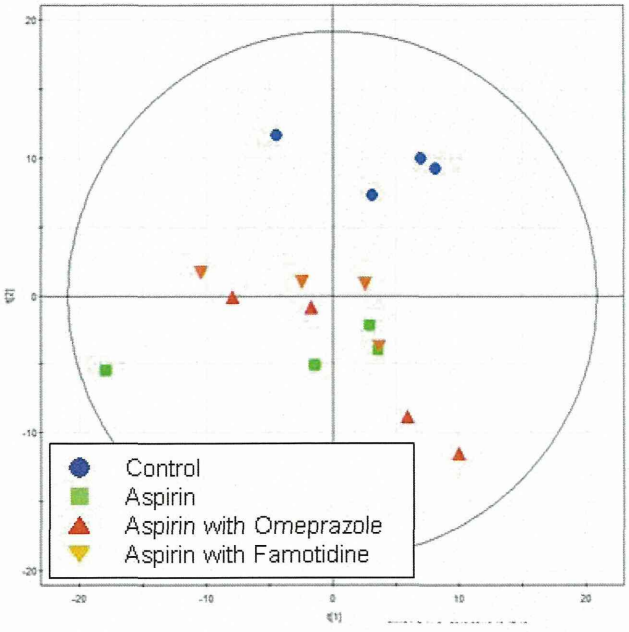
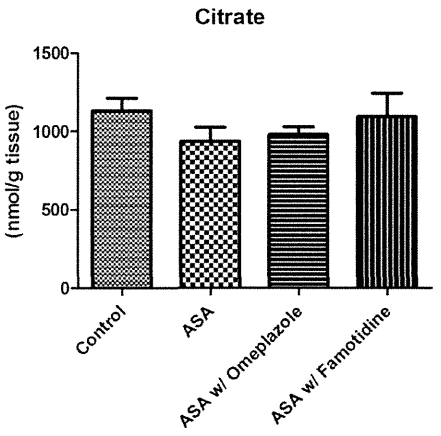
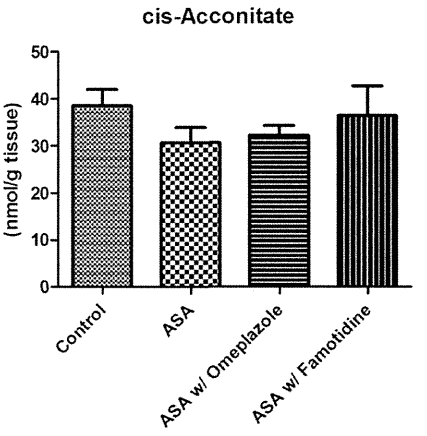

Control
Aspirin
Aspirin with Omeprazole
Aspirin with Famotidine

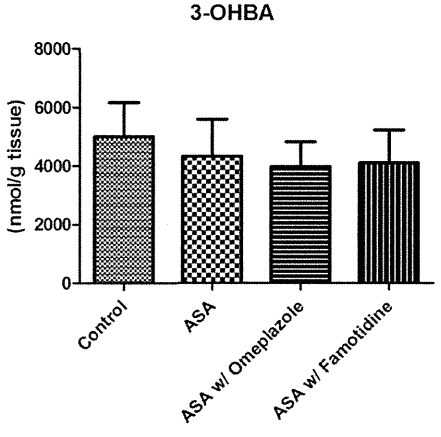
図 6

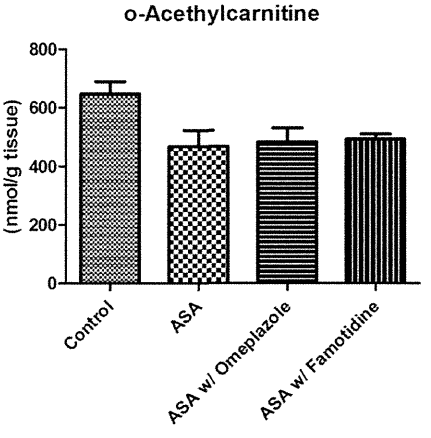
TCA Cycle



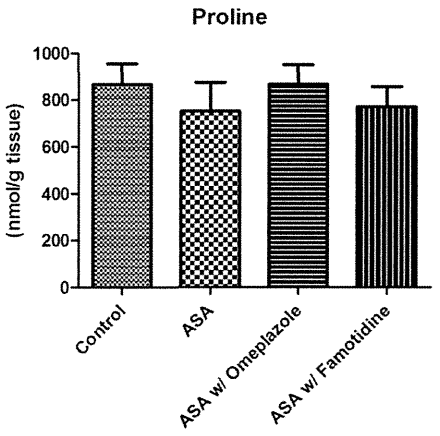


beta-Oxidization





Collagen metabolism



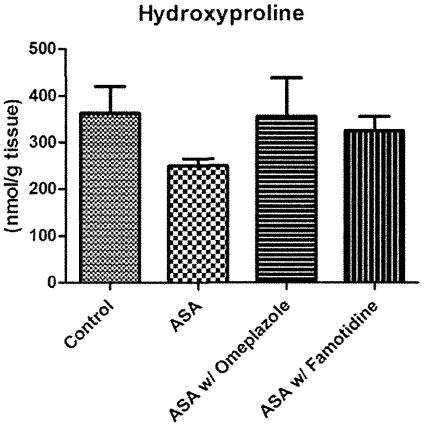


図 7

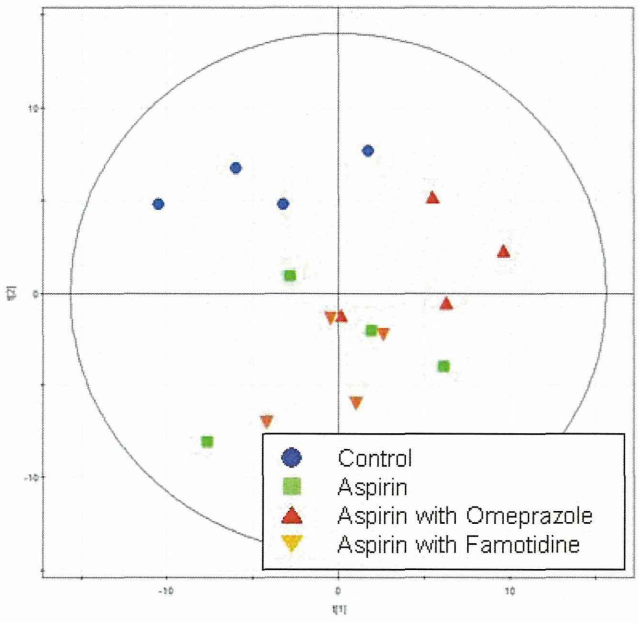
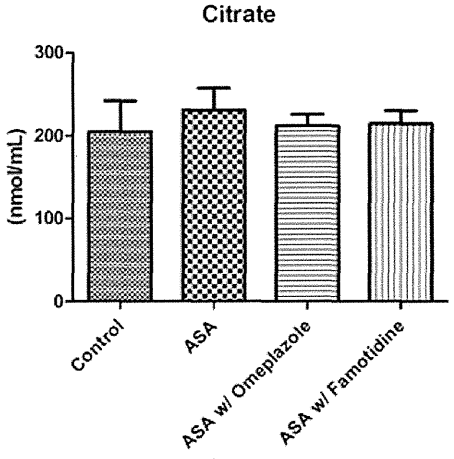
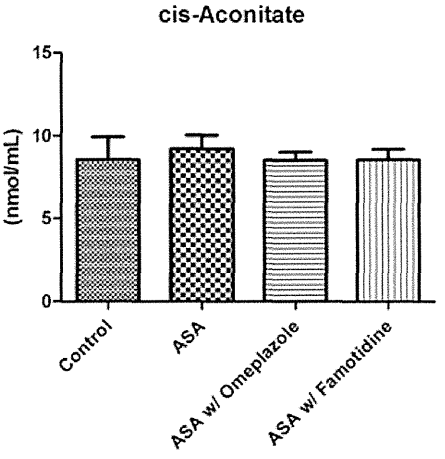

Control
Aspirin
Aspirin with Omeprazole
Aspirin with Famotidine

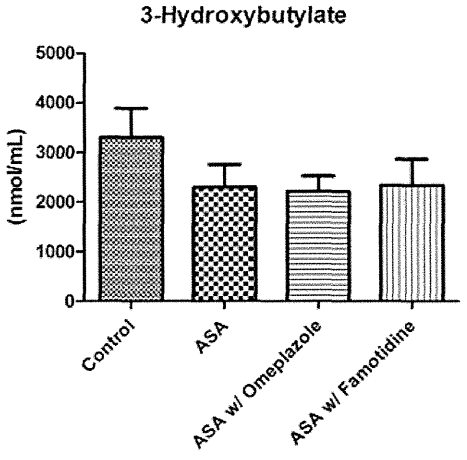
図 8

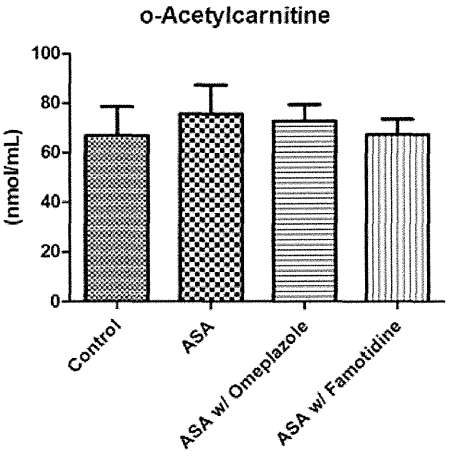
TCA Cycle



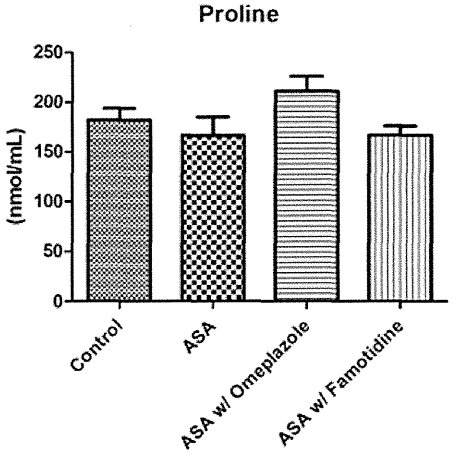


beta-Oxidization





Collagen metabolism



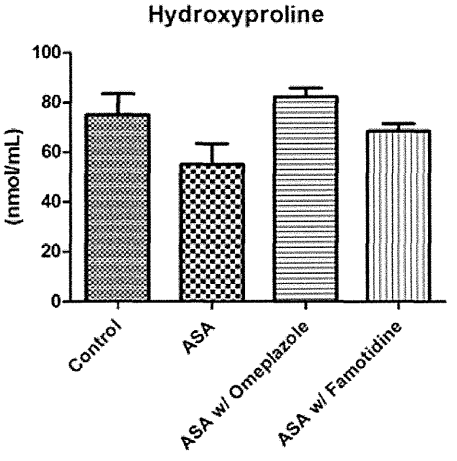


図 9

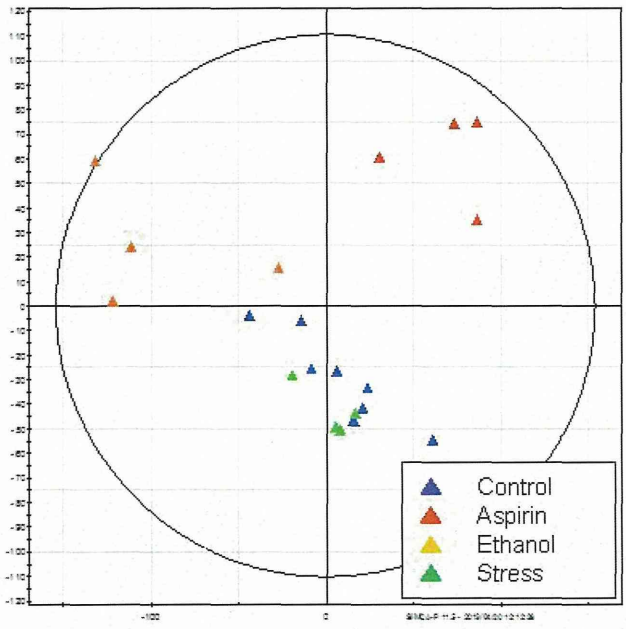


図 10

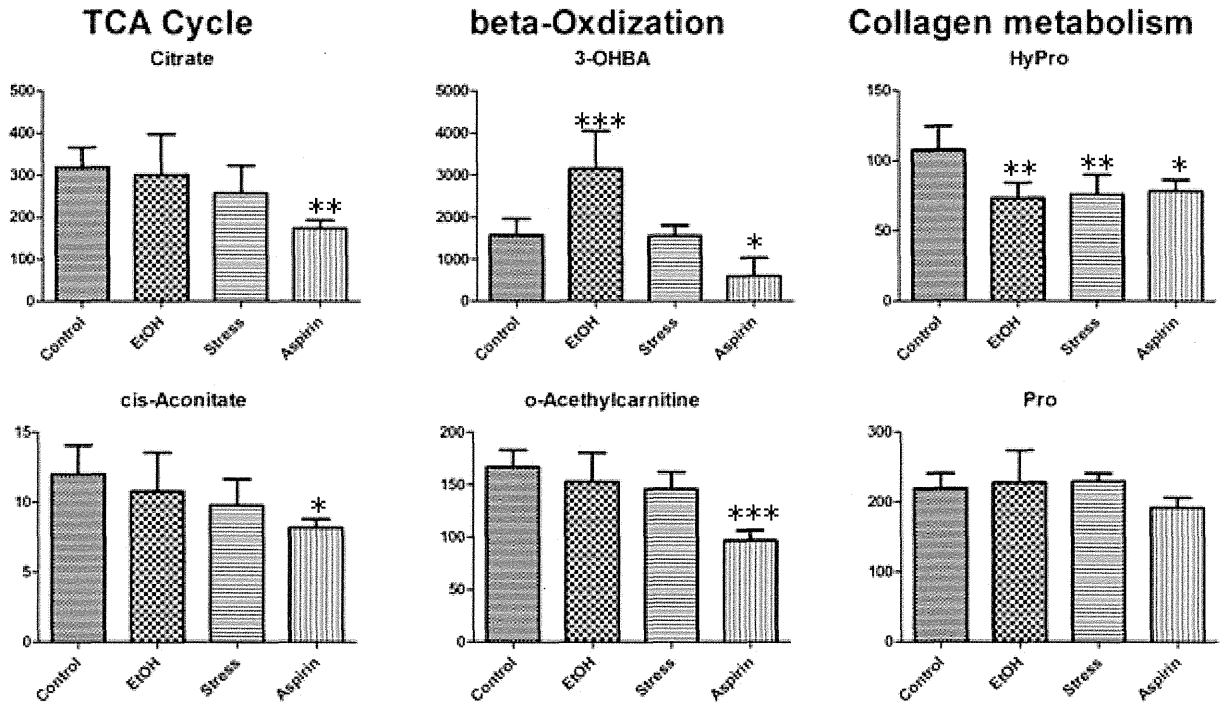
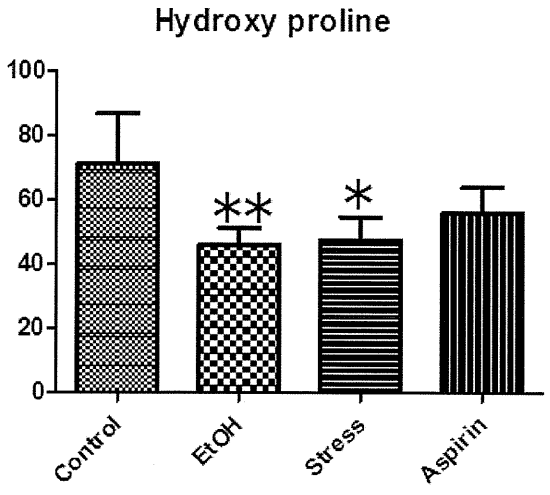

TCA Cycle
Citrate
500
400
300
200
100
0
**
Control
EtOH
Stress
Aspirin
beta-Oxdization
3-OHBA
5000
4000
3000
2000
1000
0
***
*
Collagen metabolism
HyPro
150
100
50
0
**
**
*
cis-Aconitate
15
10
5
0
*
o-Acethylcarnitine
200
150
100
50
0
***
Pro
300
200
100
0

図 11


Hydroxy proline
100
80
60
40
20
0
**
*
Control
EtOH
Stress
Aspirin

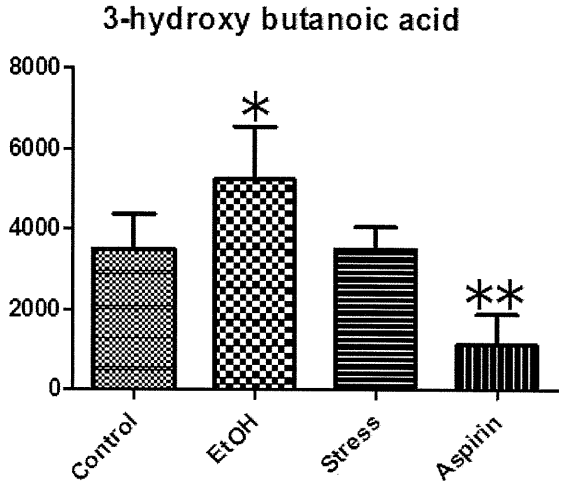

3-hydroxy butanoic acid
8000
6000
4000
2000
0
*
**
Control
EtOH
Stress
Aspirin

表 1　代謝物質標準液濃度

	各代謝物質濃度（μM）	内部標準物質(IS)濃度（μM）
陽イオン ALLSTD	20	200
陽イオン 112STD	20	200
陰イオン ALLSTD	20	200
陰イオン 112STD	50	200
陰イオン 追加依頼 3 物質 (アスピリン、サリチル酸、イブプロフェン)	50	200

表 2 胃潰瘍の面積（実験-1）

	Control	Aspirin		Ibuprofen	
		3 mg/kg	300 mg/kg	8 mg/kg	800 mg/kg
1 hr	ND (0/4)	ND (0/4)	ND (0/4)	ND (0/4)	ND (0/4)
5 hr	ND (0/4)	ND (0/4)	2.48 ± 1.14 (4/4)	ND (0/4)	18.08 ± 18.72 (4/4)
24 hr	ND (0/4)	ND (0/4)	0.08 ± 0.15 (1/4)	ND (0/4)	3.76 ± 4.33 (4/4)

Data are expressed as mean + standard deviation of the area of ulceration (mm2)

Values in parentheses are the incidence rate of animals with gastric ulceration.

ND: not detected

表 3　抽出された低分子代謝物の臓器内濃度の群平均及び有意差検定の結果

			Aspirin		Ibuprofen	
Metabolite	Time point	Control	3 mg/kg	300 mg/kg	8 mg/kg	800 mg/kg
Citrate	1 hr	194±21	202±23	120±25 (**)	202±26	135±39 (*)
	5 hr	190±18	187±33	120±8 (**)	172±23	124±19 (**)
	24 hr	269±75	203±42	213±50	175±60	169±52
Cis-aconitate	1 hr	4.7±0.3	4.4±0.5	2.8±0.7	4.6±0.6	3.6±1.0
	5 hr	5.4±0.6	4.6±1.0	3.1±0.4 (**)	4.1±1.0 (*)	3.1±0.9 (**)
	24 hr	7.7±2.0	5.6±0.7	5.7±1.0	4.9±1.6 (*)	3.7±1.1 (**)
Succinate	1 hr	235±34	252±30	227±28	229±29	139±27 (**)
	5 hr	220±7	231±14	195±8 (*)	205±11	155±11 (**)
	24 hr	349±60	323±44	295±80	270±110	278±87
o-Acetyl carnitine	1 hr	201±14	196±9	150±20 (*)	206±36	149±29 (*)
	5 hr	205±9	198±39	128±13 (**)	164±12	131±25 (**)
	24 hr	237±41	237±33	193±41	195±75	145±39
3-Hydroxybutanoic acid	1 hr	565±141	575±110	285±67 (*)	445±234	188±60 (**)
	5 hr	690±326	610±211	206±40 (*)	394±130	404±134
	24 hr	596±298	397±217	317±227	326±206	175±70 (*)
Proline	1 hr	339±23	325±28	274±24 (**)	329±25	317±18
	5 hr	274±23	303±24	247±10	292±22	233±17 (*)
	24 hr	434±78	433±35	428±131	367±134	385±98
Hydroxyproline	1 hr	158±14	130±10 (*)	113±8 (**)	128±19 (*)	130±10 (*)
	5 hr	118±23	128±13	65±11 (**)	118±24	89±21 (*)
	24 hr	171±46	154±28	145±36	122±37	100±26

Mean concentration (nmol/g tissue) and standard deviation.
Asterisks indicate statistically significant differences. **, $p<0.01$ *, $p<0.05$.

表 4　抽出された低分子代謝物の血中濃度の群平均及び有意差検定の結果

Metabolite	Time point	Control	Aspirin		Ibuprofen		Correlation
			3 mg/kg	300 mg/kg	8 mg/kg	800 mg/kg	
Citrate	1 hr	112±5	99±10	99±14	112±13	113±8	p<0.01
	5 hr	116±9	106±10	97±13	103±6	100±10	
	24 hr	106±7	96±6	94±18	96±5	84±3	
Cis-aconitate	1 hr	5.3±0.2	5.0±0.8	4.7±0.5	5.4±0.5	5.7±0.6	p<0.01
	5 hr	5.8±0.6	5.6±0.6	4.2±0.3 (**)	5.3±0.5	5.1±0.4	
	24 hr	5.8±0.9	5.4±0.1	5.0±0.8	5.5±0.1	5.2±0.3	
Succinate	1 hr	20±2	19±3	18±1	18±1	18±2	NS
	5 hr	22±2	20±1 (*)	24±1	20±1	19±0 (**)	
	24 hr	19±1	19±2	17±1	18±2	21±3	
o-Acetyl carnitine	1 hr	11.4±1.5	11.6±1.6	11.8±4.4	12.4±2.1	8.8±1.4	p<0.01
	5 hr	15.6±3.9	14.3±1.6	8.7±1.0 (**)	9.8±1.3 (*)	7.3±2.6 (**)	
	24 hr	15.1±3.6	17.0±2.6	15.1±0.7	20.6±8.9	8.0±2.4	
3-Hydroxy butanoic acid	1 hr	1299±241	1253±130	579±101 (**)	983±343	466±152 (*)	p<0.01
	5 hr	1515±731	1260±261	320±102 (**)	880±203 (*)	660±213 (**)	
	24 hr	971±477	725±235	501±299	699±293	311±128 (*)	
Proline	1 hr	160±18	157±19	116±18 (**)	137±13	135±14	p<0.01
	5 hr	150±14	141±10	118±14 (*)	149±16	104±16 (**)	
	24 hr	138±5	153±13	139±26	140±10	127±20	
Hydroxyproline	1 hr	57±5	51±5	40±8 (**)	50±3	51±3	p<0.01
	5 hr	52±6	53±4	28±4 (**)	52±6	41±5 (*)	
	24 hr	47±5	46±10	38±10	43±5	30±8 (*)	

Mean concentration (nmol/mL) and standard deviation.

Asterisks indicate statistically significant differences. **, p<0.01 *, p<0.05.

NS: not significant

表 5　胃潰瘍の面積（実験-2）

Group	Control	Aspirin	Aspirin with Omeprazole	Aspirin with Famotidine
Test article	Vehicle	Aspirin 100mg/kg	Aspirin 100mg/kg	Aspirin 100mg/kg
Co-administration (administrated 30 min. before test article administration)	Vehicle	Vehicle	Omeprazole 60 mg/kg	Famotidine 5mg/kg
Number of animals	4	4	4	4
Severity of gastric ulceration	ND (0/4)	0.863 ± 0.426 (4/4)	ND (0/4)	ND (0/4)

Data are expressed as mean + standard deviation of the area of ulceration (mm2)
Values in parentheses are the incidence rate of animals with gastric ulceration.
ND: not detected

表 5　胃潰瘍の発現例数(実験-2)

Group / Model	Control	Aspirin	Ethanol	Stress
Test article	Vehicle	Aspirin 100mg/kg	Ethanol 5mL/kg	Vehicle
Experimental room temperature	Room temperature	Room temperature	Room temperature	Cold room (4℃)
Number of animals	8	4	4	4
Gastric ulceration	ND	4/4	4/4	4/4

Values are the incidence rate of animals with gastric ulceration.
ND: not detected

参考文献

CHOI, E. Y., HWANG, H. J., KIM, I. H. & NAM, T. J. 2009. Protective effects of a polysaccharide from Hizikia fusiformis against ethanol toxicity in rats. *Food Chem Toxicol,* 47, 134-9.

GABRIEL, S. E. & FEHRING, R. A. 1992. Trends in the utilization of non-steroidal anti-inflammatory drugs in the United States, 1986-1990. *J Clin Epidemiol,* 45, 1041-4.

GABRIEL, S. E., JAAKKIMAINEN, L. & BOMBARDIER, C. 1991. Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis. *Ann Intern Med,* 115, 787-96.

HASEBE, T. 1987. Collagen and collagenase in ulcer tissue--2. Restraint and water immersion induced gastric lesions and effects of cimetidine and misoprostol. *Tokai J Exp Clin Med,* 12, 181-90.

HASEBE, T., HARASAWA, S., MIWA, T., SHIBATA, T. & INAYAMA, S. 1987. Collagen and collagenase in ulcer tissue--1. The healing process of acetic acid ulcers in rats. *Tokai J Exp Clin Med,* 12, 147-58.

HAWKEY, C. J. 2000. Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology,* 119, 521-35.

HONDA, K., FUKUDA, S., ISHIKAWA, S. E., KUZUYA, T. & SAITO, T. 1994. Role of endogenous vasopressin in development of gastric ulcer induced by restraint and water immersion. *Am J Physiol,* 266, R1448-53.

SHAKER, E., MAHMOUD, H. & MNAA, S. 2010. Anti-inflammatory and anti-ulcer activity of the extract from Alhagi maurorum (camelthorn). *Food Chem Toxicol,* 48, 2785-90.

SOMASUNDARAM, S., RAFI, S., HAYLLAR, J., SIGTHORSSON, G., JACOB, M., PRICE, A. B., MACPHERSON, A., MAHMOD, T., SCOTT, D., WRIGGLESWORTH, J. M. & BJARNASON, I. 1997. Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine. *Gut,* 41, 344-53.

STEINMEYER, J. 2000. Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. *Arthritis Res,* 2, 379-85.

SZEWCZYK, A. & WOJTCZAK, L. 2002. Mitochondria as a pharmacological target. *Pharmacol Rev,* 54, 101-27.

TAKEDA, M., TAKAGI, T., YASHIMA, Y. & MAENO, H. 1982. Effect of a new potent H2-blocker, 3-[[[2-(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-N2-sulfamoyl propionamidine (YM-11170), on gastric secretion, ulcer formation and weight of male accessory sex organs in rats. *Arzneimittelforschung,* 32, 734-7.

UM, S. Y., PARK, J. H., CHUNG, M. W., KIM, K. B., KIM, S. H., CHOI, K. H. & LEE, H. J. 2012. Nuclear magnetic resonance-based metabolomics for prediction of gastric damage induced by indomethacin in rats. *Anal Chim Acta,* 722, 87-94.

WALLACE, J. L. & GRANGER, D. N. 1996. The cellular and molecular basis of gastric mucosal defense. *FASEB J,* 10, 731-40.

研究成果の刊行に関する一覧表

雑誌　　2012年度

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takeuchi, K., Ohishi, M., Ota, S., Suzumura, K., Naraoka, H., Ohata, T., Seki, J., Miyamae, Y., Honma, M., **Soga, T.**	"Metabolic Profiling to Identify Potential Serum Biomarkers for gastric ulceration induced by nonsteroid anti-inflammatory drugs"	J. Proteome Res.	12(3)	1399-1407	2013
Tanaka, S., Taga, H., Maehara, K., Kaneshima, A., Machino, M., Onuma, H., Kaneko, M., Sakagami, H., Sugimoto, M., **Soga, T.** and Tomita, M.	"Pilot Study of Changes in Salivary Metabolic Profiles Induced by Template Therapy"	In Vivo.	26(6)	1015-1020	2012
Hirayama, A., Tomita, M., and **Soga, T.**	"Sheathless capillary electrophoresis-mass spectrometry with a high-sensitivity porous sprayer for cationic metabolome analysis"	Analyst	137(21)	5026–5033	2012
Kato, T., Niizuma, S., Inuzuka, Y., Kawashima, T., Okuda, J., Kawamoto, A., Tamai, Y., Iwanaga, Y., **Soga, T.,** Kita, T., Kimura, T., Shioi, T.	"Analysis of Liver Metabolism in a Rat Model of Heart Failure"	Int. J. Cardiol.	161(3)	130-136	2012
Sugimoto, M., Sakagami, H., Yokote, Y., Onuma, H., Kaneko, M., Mori, M., Sakaguchi, Y., **Soga, T.**, Tomita, M.	"Non-targeted metabolite profiling in activated macrophage secretion "	Metabolomics	8(4)	624-633	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
曽我朋義	メタボロミクスによるがんの診断マーカー探索	現代化学	3(504)	pp52-56	2013

Journal of proteome research

Article

pubs.acs.org/jpr

Metabolic Profiling to Identify Potential Serum Biomarkers for Gastric Ulceration Induced by Nonsteroid Anti-Inflammatory Drugs

Kenichiro Takeuchi,*[,†] Maki Ohishi,[‡] Sana Ota,[‡] Kenichi Suzumura,[§] Hitoshi Naraoka,[†] Takeji Ohata,[†] Jiro Seki,[†] Youichi Miyamae,[†] Masashi Honma,[∥] and Tomoyoshi Soga[‡]

[†]Drug Safety Research Laboratories, Astellas Pharma Inc., 1-6 Kashima 2-chome, Yodogawa-ku, Osaka 532-8514, Japan

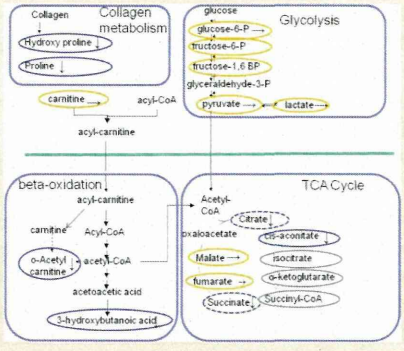
[‡]Institute for Advanced Bioscience, Keio University, 246-2 Mizukami, Kakuganji, Tsuruoka, Yamagata 997-0052, Japan

[§]Analysis and Pharmacokinetics Research Laboratories, Astellas Pharma Inc., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 532-8514, Japan

[∥]Department of Pharmacy, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Supporting Information

ABSTRACT: Nonsteroid anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs currently available. The most frequently reported serious side effects associated with NSAIDs are gastric mucosal ulceration and gastric hemorrhage. Presently, these side effects are only detectable by endoscopy, however, and no biomarkers have yet been identified. The ability to identify serum biomarkers would likely improve the safety of NSAID use. In this study we performed capillary electrophoresis-mass spectrometry (CE−MS)-based metabolomic profiling in stomach extract and serum from rats administered NSAIDs. Results showed drug-induced decreases in levels of citrate, *cis*-aconitate, succinate, 3-hydroxy butanoic acid, *o*-acetyl carnitine, proline, and hydroxyproline. We consider that these changes are due to NSAID-induced depression of mitochondrial function and activation of collagenase by lesions in the stomach. In addition, four of these changes in metabolite levels in the stomach were significantly correlated with changes in the serum. While further study is needed to clarify the mechanism of change in the level of these biomarkers, limitation of indications, and extrapolation to humans, these new serum biomarker candidates of gastric injury may be useful in the monitoring of NSAID-induced tissue damage.



KEYWORDS: *metabolomics, capillary electrophoresis−mass spectrometry, gastric injury, nonsteroid anti-inflammatory drugs, diagnostic marker candidate*

INTRODUCTION

Nonsteroid anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs available[1] and are commonly used to treat rheumatoid arthritis, osteoarthritis, acute pain, and fever.[2] The most frequently reported serious side effects associated with NSAIDs are gastric mucosal ulceration and gastric hemorrhage, with NSAID users having an approximately three times greater relative risk of serious adverse gastrointestinal events than nonusers.[3] Some 15−30% of chronic NSAID users suffer from gastrointestinal ulceration and bleeding,[4] and given that NSAIDs may mask the pain associated with gastric ulceration, patients may delay seeking medical attention, which can subsequently delay ulcer healing.[5] Presently, gastric ulceration and hemorrhage are only detectable by endoscopy,[1] and there is an unmet medical need to identify a noninvasive means of assessing gastric injury associated with the use of NSAIDs.

Several mechanisms to explain the gastric ulceration associated with NSAID use have been suggested, including increased permeability of the stomach,[6] inhibition of prostaglandin synthesis, mitochondria dysfunction,[7] or any combination of these. While details of the mechanism of gastric ulceration remain to be elucidated, a number of treatment strategies to minimize the gastrointestinal side effects of NSAID have been employed, including coadministration with proton pump inhibitors[8] or H_2 antagonists.[9]

"Metabolomics" is a rapidly evolving technology based on highly sensitive analytical methods which are used to comprehensively analyze endogenous metabolites in samples collected from both affected patients and control subjects. Comparing concentrations of endogenous metabolites between patients and controls can help identify metabolites or metabolic pathways that may function in the pathogenesis of the disease or injury in question. Several analytical methods have been used to analyze metabolites, including mass spectrometry (MS) with gas chromatography (GC−MS),[10] liquid chromatography (LC−MS),[11] or capillary electrophoresis (CE−MS)[12] and nuclear magnetic resonance (NMR) spectrometry.[13] CE−MS has emerged as a powerful tool for the simultaneous analysis of endogenous metabolites in serum, urine, and organ extracts, thanks to its rapid analysis and efficient resolution (CE) with high selectivity and sensitivity (MS).[14] Quantitative data for a number of metabolites at various time points and in different organs or matrices can be used to identify new biomarkers for

Received: November 6, 2012
Published: January 21, 2013

dx.doi.org/10.1021/pr3010452 | *J. Proteome Res.* 2013, 12, 1399−1407