5. 中山敬一: ワールブルグ効果とは何か？：全てを計ることによって明らかとなったがん代謝の真実. 第 72 回日本癌学会学術総会, 横浜, 2013 年 10 月 4 日
6. 中山敬一: 次世代プロテオミクスが拓く医学生物学の新地平：90 年来の謎を解く. 第 40 回日本神経内分泌学会学術集会・第 38 回日本比較内分泌学会大会・合同大会, 宮崎, 2013 年 10 月 25 日
7. 中山敬一: 次世代プロテオミクスが拓く生命科学の新地平：90 年来のがんの謎に挑む. 金沢大学がん進展制御研究所・共同利用共同研究拠点シンポジウム 2013, 金沢, 11/19, 2013.
8. 中山敬一: 次世代プロテオミクスを用いたがん代謝の統合的理解. 第 36 回日本分子生物学会年会, 神戸, 2013 年 12 月 3 日
9. 中山敬一: 次世代プロテオミクスが拓く新たな創薬アプローチ：「ネットワーク標的創薬」の実現に向けて 次世代医薬分子解析学講座 3 周年記念シンポジウム：日本版 NIH 元年に次世代創薬を考える, 東京, 2014 年 1 月 16 日

国際学会

（招待講演）

1. Nakayama, K.I.: Fbw7 is essential for maintenance of quiescence and function of cancer stem cells. 35th Naito Conference, Sapporo, 12 July, 2013.
2. Nakayama, K.I.: Cell cycle and cancer stem cells: Abrogation of quiescence by Fbw7 ablation eliminates leukemia stem cells. ISEH 42nd Annual Scientific Meeting, Vienna, 23 August, 2013.
3. Nakayama, K.I.: Absolute quantification oh human proteome by large-scale targeted proteomics. HUPO 2013 12th Annual World Congress, Yokohama, 17 September, 2013.
4. Nakayama, K.I.: Fbw7 is essential for maintenance of quiescence and function of cancer stem cells. The 23rd Hot Spring Harbor Symposium: Recent Advances in Stem Cell Biology 2013, Fukuoka, 5 November, 2013.
5. Nakayama, K.I.: What is the Warburg effect? Next-generation proteomics uncovers the secret of cancer. 3rd GDRI French Japanese Cancer Meeting, Toulouse, 21 November, 2013.
6. Nakayama, K.I.: Comprehensive profiling of cancer metabolism by the next generation proteomics. Kyushu University/Academia Sinica Biliateral Mini-Symposium on Cancer and Stem Cell, Taipei, 21 January, 2014.
7. Nakayama, K.I.: Comprehensive profiling of cancer metabolism by the next generation proteomics. International Symposium between Kyushu University Post-Global Centers of Excellence Program and School of Biomedical Sciences, Monash University: Cell-fate Decision: Function and Dysfunction in Homeostasis, Melbourne, 7 February, 2014.

H. 知的財産権の出願・登録状況

1. 特許取得

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出願人：国立大学法人九州大学

2. 実用新案登録

なし。

3. その他

なし。

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III. 研究成果の刊行に関する一覧表

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	ページ
平野 久他		日本プロテオーム学会	プロテオミクス辞典	講談社サイエンティフィーク	東京	2013	293-304

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kume, H., Muraoka, S., Kuga, T., Adachi, J., Narumi, R., Watanabe, S., Kuwano, M., Kodera, Y., Matsushita, K., Fukuoka, J., Masuda, T., Ishihama, Y., Matsubara, H., Nomura, F., Tomonaga, T.	Discovery of colorectal cancer biomarker candidates by membrane proteomic analysisand subsequent verification using selected reaction monitoring and tissue microarrayanalysis.	Mol. Cell Proteomics	In press		2014
Kuga, T., Nie, H., Kazami, T., Satoh, M., Matsushita, K., Nomura, F., Maeshima, K., Nakayama Y., Tomonaga, T.	Lamin B2 prevents chromosome instability by ensuring proper mitotic chromosome segregation.	Oncogenesis	In press		2014
Sano, S., Tagami, S., Hashimoto, Y., Yoshizawa-Kumagay, K., Tsunemi, M., Okochi, M., Tomonaga, T.	Absolute quantitation of low abundance plasma APL1β peptides at sub fmol/mL level by SRM/MRM without immunoaffinity enrichment.	J. Proteome Res.	In press.		2014

Yamaguchi S., Zhang B., Tomonaga T., Seino U., Kanagawa A., Segawa M., Nagasaka H., Suzuki A., Miida T., Yamada S., Sasaguri Y., Doi T., Saku K., Okazaki M., Tochino Y., Hirano K.	Selective evaluation of high density lipoprotein from mouse small intestines by an in situ perfusion technique.	J Lipid Res	In press		2014
Oji Y., Tatsumi N., Fukuda M., Nakatsuka S., Aoyagi S., Hirata E., Nanchi I., Fujiki F., Nakajima H., Yamamoto Y., Shibata S., Nakamura M., Hasegawa K., Takagi S., Fukuda I., Hoshikawa T., Murakami Y, Mori M, Inoue M., Naka T., Tomonaga T., Shimizu Y., Nakagawa M., Hasegawa J., Nezu R., Inohara H., Izumoto S., Nonomura N, Yoshimine T., Okumura M., Morii E., Maeda H., Nishida S., Hosen N, Tsuboi A, Oka Y., Sugiyama H.	The translation elongation factor eEF2 is a novel tumor-associated antigen overexpressed in various types of cancers.	Int J Oncol	In press		2014

Matsushita, K., Shimada, H., Ueda, Y., Inoue, M., Hasegawa, M., Tomonaga, T., Matsubara, H., Nomura, F.	Non-transmissible Sendai virus vector encoding c-myc suppressor FBP-interacting repressor for cancer therapy.	World J. Gastroenterol.	In press		2014
Liu, Y., Sogawa, K., Sunaga, M., Umemura, H., Satoh, M., Kazami, T., Yoshikawa, M., Tomonaga, T., Yokosuka, O., Nomura, F.	Increased concentrations of Apo A-I and Apo A-II fragments in the serum of patients with hepatocellular carcinoma by magnetic beads-assisted MALDI-TOF mass spectrometry.	Am. J. Clin. Pathol.	141	52-61	2014
Hara,Y., Kawasaki, N., Hirano, K., Hashimoto,Y., Adachi, J., Watanabe, S., Tomonaga, T.	Quantitative proteomic analysis of cultured skin fibroblast cells derived from patients with triglyceride deposit cardiomyovasculopathy.	Orphanet J. Rare Dis.	8	197	2013
Kuga, T., Kume, H., Kawasaki, N., Sato, M., Adachi, J., Shiromizu, T., Hoshino, I., Nishimori, T., Matsubara, H., Tomonaga, T.	A novel mechanism of keratin cytoskeleton organization through casein kinase I alpha and FAM83H in colorectal cancer.	J. Cell Sci.	126	4721-31	2013
Aoyama, K., Yuki, R., Horiike, Y., Kubota, S., Yamaguchi, N., Morii, M., Ishibashi, K., Nakayama, Y., Kuga, T., Hashimoto, Y., Tomonaga, T. Yamaguchi N.	Formation of long and winding nuclear F-actin bundles by nuclear c-Abl tyrosine kinase.	Exp. Cell Res.	319	3251-68	2013

Kubota, S., Fukumoto, Y., Aoyama, K., Ishibashi, K., Yuki, R., Morinaga, T., Honda, T., Yamaguchi, N., Hashimoto, Y., Kuga, T., Tomonaga, T., Yamaguchi, N.	Phosphorylation of KRAB-associated protein 1 (KAP1) at Tyr-449, Tyr-458, and Tyr-517 by nuclear tyrosine kinases inhibits the association of KAP1 and heterochromatin protein 1alpha (HP1alpha) with heterochromatin.	J. Biol. Chem.	288	17871-83	2013
Sogawa, K., Noda, K., Umemura, H., Seimiya, M., Kuga, T., Tomonaga, T., Nishimura, M., Kanai, F., Imazeki, F., Takizawa, H., Yoneda, M., Nakajima, A., Tsutsumi, M., Yokosuka, O., Nomura, F.	Serum fibrinogen alpha C-chain 5.9 kDa fragment as a biomarker for early detection of hepatic fibrosis related to hepatitis C virus.	Proteomics Clin. Appl.	7	424-31	2013
Guo, F., Hiroshima, K., Wu, D., Satoh, M., Abulazi, M., Nomura, F., Yoshino, I., Tomonaga, T., Nakatani, Y.	Prohibitin and its rapidly emerging role as a biomarker of systemic malignancies-reply.	Hum. Pathol.	44	679-80	2013
Matsushita, K., Tamura, M., Tanaka, N., Tomonaga, T., Matsubara, H., Shimada, H., Levens, D., He, L., Liu, J., Yoshida, M., Nomura, F.	Interactions between SAP155 and FUSE-binding protein-interacting repressor bridges c-Myc and P27Kip1 expression.	Mol. Cancer Res.	11	689-98	2013

Matsuzaki, S., Enomoto, T., Serada, S., Yoshino, K., Nagamori, S., Morimoto, A., Yokoyama, T., Kim, A., Kimura, T., Ueda, Y., Fujita, M., Fujimoto, M., Kanai, Y., Kimura, T., Naka, T.	Annexin A4- conferred platinum resistance is mediated by the copper transporter ATP7A.	Int. J. Cancer	134	1796-809	2014
Shimada, K., Serada, S., Fujimoto, M., Nomura,S., Nakatsuka, R., Harada, E., Iwahori,K., Tachibana ,I., Takahashi, T., Kumanogoh, K., Kishimoto, T., Naka, T.	The molecular mechanism underlying anti-proliferative effect of SOCS-1 in non-small cell lung cancer cells.	Cancer Sci..	104	1483-91	2013
Sadaoka, T., Serada, S., Kato, J., Hayashi, M., Gomi, Y., Naka, T., Yamanishi, K., Mori, Y.	Varicella-zoster virus ORF49 functions in the efficient production of progeny virus through its interaction with essential tegument protein ORF44.	J. Virol.	88	188-201	2014
Umegaki-Arao, N., Tamai, K., Nimura, K., Serada, S., Naka, T., Nakano, H., Katayama, I.	Karyopherin alpha2 is essential for rRNA transcription and protein synthesis in proliferative keratinocytes.	PLoS One	8(10)	E76416	2013

Takahashi, T., Serada, S., Ako, M., Fujimoto,M., Miyazaki,M., Nakatsuka, R., Ikezoe, I., Yokoyama,A., Taguchi, T., Shimada,K., Kurokawa,Y., Yamasaki, M., Miyata, H., Nakajima,K., Takiguchi, S., Mori, M., Doki, Y., Naka, T., Nishida, T.	New findings of kinase switching in gastrointestinal stromal tumor under imatinib using phosphoproteomic analysis.	Int. J. Cancer	133	2737-43	2013
Nishioka, C., Ikezoe, T., Furihata, M., Yang, J., Serada, S., Naka, T., Nobumoto, A., Kataoka, S., Tsuda, M., Udaka, K., Yokoyama, A.	CD34(+) /CD38(-) acute myelogenous leukemia cells aberrantly express CD82 which regulates adhesion and survival of leukemia stem cells.	Int. J. Cancer	132	2006-19	2013
Nagano, K., Kanasaki, S., Yamashita, T., Maeda, Y., Inoue, M., Higashisaka, K., Yoshioka, Y., Abe, Y., Mukai, Y., Kamada, H., Tsutsumi, Y., Tsunoda, S.	Expression of Eph receptor A10 is correlated with lymph node metastasis and stage progression in breast cancer patients.	Cancer Med.	2	972-7	2013

Yamashita,T., Kamada, H., Kanasaki, S., Maeda, Y., Nagano, K., Abe, Y., Inoue, M., Yoshioka, Y., Tsutsumi, Y., Katayama, S., Inoue, M., Tsunoda S.	Epidermal growth factor receptor localized to exosome membranes as a possible biomarker for lung cancer diagnosis.	Pharmazie	68	969–73	2013
平野 久	プロテオーム解析ータンパク質の網羅的解析で何がわかるか	化学と工業	67	128-31	2014
Hong, F., Liu, B., Mohammad, S., Debbie, R., Han, L. D., Huang X., Zhao, H., Kimura, Y., Hirano, H., Ohara, O., Udono, H.	Mapping the client network of a major mammalian endoplasmic reticulum heat shock protein 90 reveals its roles in innate immunity, Wnt signaling and organ development. Mol. Cell. Proteomics, in press	Mol. Cell. Proteomics	In press		2014
Kudoh, A., Takahama, S., Sawasaki, T., Ode, H., Yokoyama, M., Okayama, A., Ishikawa, A., Miyakawa, K., Matsunaga, S., Kimura, H., Sugiura, W., Sato, H., Hirano, H., Ohno, S., Yamamoto, N., Ryo, A.	The phosphorylation of HIV-1 Gag by atypical protein kinase C facilitates viral infectivity by promoting Vpr incorporation into virions.	Retrovirology	11	9	2014

Akama, K., Horikoshi, T., Nakayama, T., Otsu, M., Imaizumi, N., Nakamura, M., Toda, T., Inuma, M., Hirano, H., Kondo, Y., Suzuki, Y., Inoue, N.	Proteomic identification of differentially expressed genes during differentiation of cynomolgus monkey (Macaca fascicularis) embryonic stem cells to astrocyte progenitor cells in vitro.	Biochim. Biophys. Acta	1834	601-10	2013
Arakawa, N., Miyagi, E., Nomura, A., Morita, E., Ino, Y., Ohtake, N., Miyagi, Y., Hirahara, F., Hirano, H.	Secretome-based identification of TFPI2, a novel serum biomarker for detection of ovarian clear cell adenocarcinoma.	J. Proteome Res.	12	14340-50	2013
Kimura, Y., Kurata, Y., Ishikawa, A., Okayama, A., Kamita, M., Hirano. H.	N-Terminal methylation of proteasome subunit Rpt1 in yeast.	Proteomics	13	3167-74	2013
Masuishi, Y., Nomura, A., Okayama, A., Kimura, Y., Arakawa, N., Hirano H.	Mass spectrometric identification of glycosylphosphatidyl-inositol-anchored peptides.	J. Proteome Res.	12	4617-26	2013

Sato, Y., Akitsu, M., Amano, Y., Yamashita, K., Ide, M., Shimada, K., Yamashita, A., Hirano, H., Arakawa, N., Maki, T., Hayashi, I., Ohno, S., Suzuki , A.	A novel PAR-1-binding protein, MTCL1, plays critical roles in organizing microtubules in polarizing epithelial cells.	J. Cell Sci.	126	4671-83	2013
Masuda, M., Chen, WY., Miyanaga, A., Nakamura, Y., Kawasaki, K., Sakuma, T., Ono, M., Chen, CL., Honda, K., Yamada, T.	Alternative mTOR signal activation in sorafenib-resistant hepatocellular carcinoma cells revealed by array-based pathway profiling.	Mol. Cell Proteomics,	In press	2365-77	2014
Kobayashi, E., Satow, R., Ono, M., Masuda, M., Honda, K., Sakuma, T., Kawai, A., Morioka, H., Toyama, Y., Yamada, T.	MicroRNA expression and functional profiles of osteosarcoma.	Oncology	86	94-103	2014
Noro, R,, Honda, K., Tsuta, K., Ishii, G., Maeshima, AM., Miura, N., Furuta, K., Shibata, T., Tsuda, H., Ochiai, A., Sakuma, T., Nishijima, N., Gemma, A., Asamura, H., Nagai, K., Yamada, T.	Distinct outcome of stage I lung adenocarcinoma with ACTN4 cell motility gene amplification.	Ann. Oncol.	24	2594-600	2013

Makuuchi, Y., Honda, K., Osaka, Y., Kato, K., Kojima, T., Daiko, H., Igaki, H., Ito, Y., Hoshino,S., Tachibana,S., Watanabe, T., Furuta, K., Sekine, S., Umaki, T., Watabe, Y., Miura, N., Ono, M., Tsuchida, A., Yamada, T.	Soluble interleukin-6 receptor is a serum biomarker for the response of esophageal carcinoma to neoadjuvant chemoradio-therapy.	Cancer Sci.	104	1045-51	2013
Ishikawa, Y., Hosogane, M., Okuyama, R., Aoyama, S., Onoyama, I., Nakayama, K.I., Nakayama, K.	Opposing functions of Fbxw7 in keratinocyte growth, differentiation and skin tumorigenesis mediated through negative regulation of c-Myc and Notch.	Oncogene	32	1921-32	2013
Furutachi, S., Matsumoto, A., Nakayama, K.I., Gotoh, Y.	p57 controls adult neural stem cell quiescence and modulates the pace of lifelong neurogenesis.	EMBO J.	32	970-81	2013
Yumimoto, K., Matsumoto, M., Onoyama, I., Imaizumi, K., Nakayama, K.I.	F-box and WD repeat domain-containing-7 (Fbxw7) protein targets endoplasmic reticulum-anchored osteogenic and chondrogenic transcriptional factors for degradation.	J. Biol. Chem..	288	28488-502	2013

Yumimoto, K., Muneoka, T., Tsuboi, T., Nakayama, K.I.	Substrate binding promotes formation of the Skp1-Cul1-Fbxl3 (SCF^{Fbxl3}) protein complex.	J. Biol. Chem.	288	32766-76	2013
Zhao, H., Bauzon, F., Fu, H., Lu, Z., Cui, J., Nakayama, K. Nakayama,K.I., Locker, J., Zhu, L.	Skp2 deletion unmasks a p27 safeguard that blocks tumorigenesis in the absence of pRb and p53 tumor suppressors.	Cancer Cell	24	645-59	2013
Ohnishi, T., Shirane, M., Hashimoto, Y., Saita, S., Nakayama, K.I.	Identification and characterization of a neuron- pecific isoform of protrudin.	Genes Cells	19	97-111	2014
Lu, Z., Bauzon, F., Fu, H., Cui, J., Zhao, H., Nakayama, K., Nakayama, K.I., Zhu, L.	Skp2 suppresses apoptosis in Rb1-deficient tumours by limiting E2F1 activity.	Nat. Commun.	5	3463	2014
Hashimoto,Y., Shirane, M., Matsuzaki, F., Saita, S., Ohnishi, T., Nakayama, K.I.	Protrudin regulates endoplasmic reticulum morphology and function associated with the pathogenesis of hereditary spastic paraplegia.	J. Biol. Chem.	In press		2014
Matsumoto, A., Takeishi, S., Nakayama, K.I.	p57 regulates T cell development and prevents lymphomagenesis by balancing p53 activity and pre-TCR signaling.	Blood	In press		2014

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Discovery of colorectal cancer biomarker candidates by membrane proteomic analysis and subsequent verification using selected reaction monitoring and tissue microarray analysis

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Running title: Discovery and validation of membrane protein tumor markers

Abbreviations

iTRAQ: isobaric tag for relative and absolute quantitation

SILAC: stable isotope labeling by amino acids in cell culture

SRM: selected reaction monitoring

MRM: multiple reaction monitoring

PTS: phase-transfer surfactant

SI-peptide: stable isotope-labeled peptide

CID: collision-induced dissociation

HCD: higher energy collision-induced dissociation

IHC: immunohistochemistry

LC-MS/MS: liquid chromatography tandem mass spectrometry

CE: collision energy

LTQ: linear ion trap

FDR: false discovery rate

TMA: tissue microarray

Summary

Recent advances in quantitative proteomic technology have enabled the large-scale validation of biomarkers. We here performed a quantitative proteomic analysis of membrane fractions from colorectal cancer tissue to discover biomarker candidates, and then extensively validated the candidate proteins identified. A total of 5566 proteins were identified in six tissue samples, each of which was obtained from polyps and cancer with and without metastasis. GO cellular component analysis predicted that 3087 of these proteins were membrane proteins, while TMHMM algorithm predicted that 1567 proteins had a transmembrane domain. Differences were observed in the expression of 159 membrane proteins and 55 extracellular proteins between polyps and cancer without metastasis, while the expression of 32 membrane proteins and 17 extracellular proteins differed between cancer with and without metastasis. A total of 105 of these biomarker candidates were quantitated using selected (or multiple) reaction monitoring (SRM/MRM) with stable synthetic isotope-labeled peptides as an internal control. The results obtained revealed differences in the expression of 69 of these proteins, and this was subsequently verified in an independent set of patient samples (polyps (n=10), cancer without metastasis (n=10), cancer with metastasis (n=10)). Significant differences were observed in the expression of 44 of these proteins,

including ITGA5, GPRC5A, PDGFRB, and TFRC, which have already been shown to be overexpressed in colorectal cancer, as well as proteins with unknown function, such as C8orf55. The expression of C8orf55 was also shown to be high not only in colorectal cancer, but also in several cancer tissues using a multi-cancer tissue microarray, which included 1150 cores from 14 cancer tissues. This is the largest verification study of biomarker candidate membrane proteins to date; our methods for biomarker discovery and subsequent validation using SRM/MRM will contribute to the identification of useful biomarker candidates for various cancers. Data are available via ProteomeXchange with identifier PXD000851.

Introduction

Recent advances in proteomic technology have contributed to the identification of biomarkers for various diseases. Improvements in LC-MS technology have led to an increase in the number of proteins that have been identified. In addition, a stable isotopic labeling method using iTRAQ and SILAC has enabled the quantitative analysis of multiple samples (1, 2). Therefore, a large number of proteins have already been identified as biomarker candidates; however, only a few of these have been used in practical applications because most have not yet progressed to the validation stage, in

which potential biomarker candidates are quantified on a large scale. The validation of biomarker candidates is generally accomplished using Western blotting and enzyme-linked immunosorbent assays (ELISA) if specific and well-characterized antibodies for these candidates are available. However, highly specific antibodies are not currently available for most novel biomarker candidate proteins, and it takes a significant amount of time and money to obtain these antibodies and optimize ELISA assay systems for many candidates; therefore, another validation assay system needs to be developed. Selected (or multiple) reaction monitoring (SRM or MRM) was previously shown to be a potentially effective method for the validation of biomarker candidates (3-5). The SRM/MRM assay can measure multiple targets at high sensitivity and throughput without antibodies; hence, it is useful for initial quantitative evaluations and the large-scale validation of biomarker candidates, which defines validation of hundreds of biomarker candidate proteins simultaneously.

In addition to these technical improvements, the fractionation process also plays an important in proteome analysis for biomarker discovery. This procedure very effectively analyzes the proteomes of specific cellular compartments or organelles in detail, which reduces sample complexity. The preparation of a membrane fraction was previously shown to be useful for identifying membrane proteins that are generally

expressed at relatively low levels. Membrane proteins play critical roles in many biological functions, such as signal transduction, cell-cell interactions, and ion transport, account for ~38% of all proteins encoded by the mammalian genome and more than one-third of biomarker candidates, and are also potential targets for drug therapy (6, 7). Therefore, membrane proteome analysis is important for biomarker discovery. However, difficulties have been associated with extracting and solubilizing membrane proteins and subsequent protease digestion. Many procedures have consequently been developed to improve the solubilization and digestion of membrane proteins (8-11), and a protocol using phase transfer surfactant (PTS) was shown to be suitable for membrane proteomics using LC-MS/MS (12, 13).

The selection of a control group for comparisons is also important for identifying potential biomarkers. Tissue samples from cancer patients have been used in many studies to discover biomarker candidates by proteomic analysis. Previous studies, including our own, attempted to compare cancer tissues with matched normal tissue (14-17). However, marked differences have been reported in the histology, genetics, and proteomics of normal and cancer tissues, and many biomarker candidates have been identified, by making it difficult to narrow down more reliable candidates for further validation. Lazebnik recently emphasized that the features of malignant, but

not benign tumors could be used as a hallmark of cancer (18), and also that premalignant lesions were more appropriate controls for cancer tissue than normal tissue for the identification of biomarker candidates involved in cancer progression. Moreover, comparisons of cancer with and without metastasis may also assist in the discovery of biomarker candidates involved in cancer metastasis. Therefore, the identification of biomarker candidates that can be used to diagnose and determine the prognosis of cancer should become more effective by comparing cancer tissues at different stages, including benign tumors.

We performed a shotgun proteomic analysis of membrane fractions prepared from colorectal cancer tissue and benign polyps in the present study to identify biomarker candidates for the diagnosis and treatment of cancer. We identified a large number of biomarker candidate proteins associated with the progression of colon cancer by using membrane protein extraction with PTS followed by iTRAQ labeling. SRM/MRM confirmed the altered expression of these biomarker candidates, and these results were further verified using an independent set of tissue samples. A protein with uncharacterized function, C8orf55, was also validated with a tissue microarray that included various types of cancers.