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Editorial

Insight into Protein Variants/Isoforms and Post-Translational Modifications in a Proteome

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Editorial

With the rapid development of human genomics, human structural genome has been completely sequenced accounting for 25,000-30,000 genes [1]. Transcriptomics and proteomics are two important approaches to annotate functions of human genome, or called as functional genomics. However, Transcriptomics and proteomics analyses of the same human tissues reveal that coefficient of relationship of proteome and transcriptome is very low, and the number of proteins is much more than the number of genes [2-4]. It is estimated that the number of human proteins reaches up to over 100,000 or even 1,000,000 if variants or isoforms [5]. It clearly demonstrates that one gene corresponds to multiple proteins are considered which is present as "one gene-multiple proteins" model, but not "one gene-one protein" model [6,7]. Those protein variants or isoforms coded by the same gene are mainly derived from splicing [8-10] and Post-Translational Modifications (PTMs) [6,11]. Moreover, PTMs are not controlled by genes, and dynamically alter with different conditions such as different physiological conditions, different pathological conditions and different disease stages, etc. Therefore, a gene-coded protein is not only a protein expression event but also involves many post-transcriptional/translational regulations such as splicing, modifications, translocation, and spatial conformation. Those protein post-transcriptional/translational regulations play very important roles in different physiological and pathological processes. Thus, it emphasizes the scientific importance of investigating post-transcriptional/translational regulations such as splicing, PTMs and spatial conformation in the human proteome.

Since concepts "proteome" and "proteomics" were proposed in 1994 [12], a huge number of publications have focused on protein expression alterations related to different given conditions and proteomic reference maps. Whereas, protein variations/isoforms and PTMs have not been extensively and in-depth studied al all in the field of proteomics, although some PTMs such as phosphorylation [13-15], glycosylation [16,17], acetylation [18,19], methylation [20,21], nitration [22-24], ubiquitylation [25,26], sumoylation [27,28], succinylation [29,30], sulfation [31,32], myristoylation [33,34], palmitoylation [35,36], deamidation [37], prenylation [38], and hydroxylation [39] have been explored. These mentioned PTMs are only a "window" in the field of PTM database that contains hundreds of PTMs (http://web.expasy.org/findmod/findmod_masses.html; http://www.uniprot.org/docs/ptmlist). Even though phosphorylation and glycosylation are the most extensively studied PTMs; however, only partial phosphorylation- and glycosylation-sites have identified in the human proteome by now. Also, each kind of PTM been dynamically alters with different conditions. Therefore, PTMs that lead to different protein variants/isoforms are very complicated.

Detection and identification of protein variants/isoforms and PTMs in a proteome are essential to clarify biological significance of a given protein variants/isoforms and PTMs in a biological system. For detection techniques, gel and gel-free methods are used [6,7]. Gel-based methods mainly include One-Dimensional Gel Electrophoresis (1DGE), Two-Dimensional Gel Electrophoresis (2DGE), and Two- Dimensional Difference In-Gel Electrophoresis (2D DIGE) [6,11]. Those gel-methods are commonly coupled with corresponding antibody to detect variants/isoforms of a given protein [11], or a kind of PTM [24,40]. For example, 2DGE-based Western blot coupled with growth hormone antibody [11], nitrotyrosine antibody [24,40], and phosphotyrosine antibody [41] is used to detect the corresponding growth hormone variants/isoforms in a human pituitary proteome, tyrosine nitration in an astrocytoma and pituitary proteome, and tyrosine phosphorylation in a glioma proteome, respectively. Gel-free methods mainly include C4 or C5 Reverse Phase Liquid Chromatography (RPLC) with pore size particles of 300 Å [42,43], Hydrophobic Interaction Chromatography (HIC) that is used to separate large bimolecular such as proteins [44, 45], Weak-Cation Exchange Chromatography (WCX) coupled with HIC in a single column with a single phase (2D-LC; from WCX to HIC mode) [46], Capillary Electrophoresis (CE)- ElectroSpray Ionization- Mass Spectrometry (CE-ESI-MS) [47], and multiplexed gel-eluted liquid fraction entrapment electrophoresis (mGELFrEE; size-based separation) with 8 parallel glass gel column [48].

MS is the key technique to identify protein variants/isoforms and PTMs with determination of amino acid sequence of intact proteins, splicing sites [6], and PTM-sites [24,40,41]. Tandem mass spectrometry (MS/MS) can determine amino acid sequence of a protein to directly identify amino acid sequence errors, variations, and modifications, which leads to characterization of protein variants/isoforms and PTMs on different types of mass spectrometers including Fourier Transform Ion Cyclotron Resonance (FTICR) [49,50], Matrix-Assisted Laser Desorption Ionization-Time Of Flight-

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Time Of Flight (MALDI-TOF-TOF) [51,52], Triple TOF 5600 or 6600 systems [53], and LTQ Orbitrap system [54-56] with different ion fragmentation models such as Collision Induced Dissociation (CID) [57,58], Electron Transfer Dissociation (ETD) [59], and Electron Capture Dissociation (ECD) [60,61]. High-resolution FTICR MS is especially suitable for high-mass accuracy measurement of intact protein ion mass [62]. For analysis of PTMs, a preferential enrichment of PTM-proteins or PTM-enzymatic peptides is necessary prior to MS analysis because of low-abundance of PTM in a proteome and the limited detection sensitivity of MS [22,23,63]. Furthermore, the MS parameter should be adjusted with the features of each variants/isoforms and PTMs.

Moreover, quantification of protein variants/isoforms and PTMs plays very important roles in understanding their biological significance. Currently, three main categories of quantitative strategies are used: 2DGE-based quantitative methods [64,65], stable isotope-labeled quantitative methods such as isobaric tags for relative and absolute quantification iTRAQ [66,67], and label-free quantitative methods [68,69] including Selected or Multiple Reaction Monitoring (SRM/MRM) [70, 71] and Sequential Window Acquisition Of All Theoretical Mass Spectra (SWATH) [72,73]. Also, the use of structural proteomics to interpret the spatial conformation of protein variants/isoforms and PTM-proteins would in-depth understand the biological functions of protein variants/isororms and PTMs in a biological system [22,74].

In summary, protein variants/isoforms and PTMs play very important roles in many physiological and pathological processes, and are potential biomarkers and therapeutic targets, which are more promising aspects in the field of proteomics relative to traditional protein expressions. However, studies of protein variants/isoforms and PTMs are much insufficient in the width and depth relative to traditional expression proteomics. Much more efforts should be made to insight into protein variants/isoforms and PTMs in a proteome. Some methods that detect and identify protein variants/isoforms and PTMs have been developed. However, more effective, accuracy, sensitive, and high-throughout detection and identification techniques are needed to maximize the coverage of protein variants/isoforms and PTMs in a proteome.

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References

1. Liao P, Yong TF, Liang MC, Yue DT, Soong TW. Splicing for alternative structures of Cav1.2 Ca^{2+} channels in cardiac and smooth muscles. Cardiovasc Res. 2005; 68: 197-203.
2. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol. 1999; 19: 1720-1730.
3. Anderson L, Seilhamer J. A comparison of selected mRNA and protein abundances in human liver. Electrophoresis. 1997; 18: 533-537.
4. Zhan X, Desiderio DM. Comparative proteomics analysis of human pituitary adenomas: current status and future perspectives. Mass Spectrom Rev. 2005; 24: 783-813.
5. Hu R, Wang X, Zhan X. Multi-parameter systematic strategies for predictive, preventive and personalised medicine in cancer. EPMA J. 2013; 4: 2.
6. Zhan X, Giorgianni F, Desiderio DM. Proteomics analysis of growth hormone isoforms in the human pituitary. Proteomics. 2005; 5: 1228-1241.
7. Stastna M, Van Eyk JE. Analysis of protein isoforms: can we do it better? Proteomics. 2012; 12: 2937-2948.
8. Blencowe BJ. Alternative splicing: new insights from global analyses. Cell. 2006; 126: 37-47.
9. Perrin BJ, Ervasti JM. The actin gene family: function follows isoform. Cytoskeleton (Hoboken). 2010; 67: 630-634.
10. Black DL. Mechanisms of alternative pre-messenger RNA splicing. Annu Rev Biochem. 2003; 72: 291-336.
11. Kohler M, Thomas A, Püschel K, Schänzer W, Thevis M. Identification of human pituitary growth hormone variants by mass spectrometry. J Proteome Res. 2009; 8: 1071-1076.
12. Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery-Smith I. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol Genet Eng Rev. 1996; 13: 19-50.
13. von Stechow L, Francavilla C, Olsen JV. Recent findings and technological advances in phosphoproteomics for cells and tissues. Expert Rev Proteomics. 2015; 12: 469-487.
14. Iliuk AB, Arrington JV, Tao WA. Analytical challenges translating mass spectrometry-based phosphoproteomics from discovery to clinical applications. Electrophoresis. 2014; 35: 3430-3440.
15. Ruprecht B, Lemeer S. Proteomic analysis of phosphorylation in cancer. Expert Rev Proteomics. 2014; 11: 259-267.
16. Christiansen MN, Chik J, Lee L, Anugraham M, Abrahams JL, Packer NH. Cell surface protein glycosylation in cancer. Proteomics. 2014; 14: 525-546.
17. Pan S, Chen R, Aebersold R, Brentnall TA. Mass spectrometry based glycoproteomics--from a proteomics perspective. Mol Cell Proteomics. 2011; 10: 110.
18. Svinkina T, Gu H, Silva JC, Mertins P, Qiao J, Fereshetian S, et al. Deep, Quantitative coverage of the lysine acetylome using novel anti-acetyl-lysine antibodies and an optimized proteomic workflow. Mol Cell Proteomics. 2015; 14: 2429-2440.
19. Rardin MJ, Newman JC, Held JM, Cusack MP, Sorensen DJ, Li B, et al. Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways. Proc Natl Acad Sci U S A. 2013; 110: 6601-6606.
20. Plank M, Fischer R, Geoghegan V, Charles PD, Konietzny R, Acuto O. Expanding the yeast protein arginine methylome. Proteomics. 2015; 15: 3232-3243.
21. Carlson SM, Gozani O. Emerging technologies to map the protein methylome. J Mol Biol. 2014; 426: 3350-3362.
22. Zhan X, Wang X. Mass spectrometry analysis of nitrotyrosine-containing proteins. Mass Spectrom Rev. 2015; 34: 423-448.
23. Zhan X, Wang X, Desiderio DM. Pituitary adenoma nitroproteomics: current status and perspectives. Oxid Med Cell Longev. 2013; 2013: 580710.
24. Peng F, Li J, Guo T, Yang H, Li M, Sang S, et al. Nitroproteins in human astrocytomas discovered by gel electrophoresis and tandem mass spectrometry. J Am Soc Mass Spectrom. 2015.
25. Porras-Yakushi TR, Hess S. Recent advances in defining the ubiquitylome. Expert Rev Proteomics. 2014; 11: 477-490.
26. Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP, et al. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. Nature. 2013; 496: 372-376.
27. Tu J, Chen Y, Cai L, Xu C, Zhang Y. Functional proteomics study reveals

sumoylation of tfii-i is involved in liver cancer cell proliferation. J Proteome Res. 2015; 14: 2385-2397.

28. Galisson F, Mahrouche L, Courcelles M, Bonneil E, Meloche S, Chelbi-Alix MK, et al. A novel proteomics approach to identify SUMOylated proteins and their modification sites in human cells. Mol Cell Proteomics. 2011; 10: M110.

29. Yang M, Wang Y, Chen Y, Cheng Z, Gu J, Deng J, et al. Succinylome analysis reveals the involvement of lysine succinylation in metabolism in pathogenic Mycobacterium tuberculosis. Mol Cell Proteomics. 2015; 14: 796-811.

30. Li X, Hu X, Wan Y, Xie G, Li X, Chen D, et al. Systematic identification of the lysine succinylation in the protozoan parasite Toxoplasma gondii. J Proteome Res. 2014; 13: 6087-6095.

31. Zaia J, Li XQ, Chan SY, Costello CE. Tandem mass spectrometric strategies for determination of sulfation positions and uronic acid epimerization in chondroitin sulfate oligosaccharides. J Am Soc Mass Spectrom. 2003; 14: 1270-1281.

32. Paciotti R, Coletti C, Re N, Scuderi D, Chiavarino B, Fornarini S, et al. Serine O-sulfation probed by IRMPD spectroscopy. Phys Chem Chem Phys. 2015; 17: 25891-25904.

33. Kimura A, Kurata Y, Nakabayashi J, Kagawa H, Hirano H. N-myristoylation of the Rpt2 subunit of the yeast 26S proteasome is implicated in the subcellular compartment-specific protein quality control system. J Proteomics. 2015; pii: S1874-3919: 30112-303113.

34. Traverso JA, Giglione C, Meinnel T. High-throughput profiling of N-myristoylation substrate specificity across species including pathogens. Proteomics. 2013; 13: 25-36.

35. Ivaldi C, Martin BR, Kieffer-Jaquinod S, Chapel A, Levade T, Garin J,et al. Proteomic analysis of S-acylated proteins in human B cells reveals palmitoylation of the immune regulators CD20 and CD23. PLoS One. 2012; 7: e37187.

36. Martin BR. Chemical approaches for profiling dynamic palmitoylation. Biochem Soc Trans. 2013; 41: 43-49.

37. Adav SS, Qian J, Ang YL, Kalaria RN, Lai MK, Chen CP, et al. iTRAQ quantitative clinical proteomics revealed role of Na(+) K(+) -ATPase and its correlation with deamidation in vascular dementia. J Proteome Res. 2014; 13: 4635-4646.

38. Suzuki T, Ito M, Ezure T, Shikata M, Ando E, Utsumi T, et al. Protein prenylation in an insect cell-free protein synthesis system and identification of products by mass spectrometry. Proteomics. 2007; 7: 1942-1950.

39. Ono M, Matsubara J, Honda K, Sakuma T, Hashiguchi T, Nose H, et al. Prolyl 4-hydroxylation of alpha-fibrinogen: a novel protein modification revealed by plasma proteomics. J Biol Chem. 2009; 284: 29041-29049.

40. Zhan X, Desiderio DM. The human pituitary nitroproteome: detection of nitrotyrosyl-proteins with two-dimensional Western blotting, and amino acid sequence determination with mass spectrometry. Biochem Biophys Res Commun. 2004; 325: 1180-1186.

41. Guo T, Wang X, Li M, Yang H, Li L, Peng F, et al. Identification of glioblastoma phosphotyrosine-containing proteins with two-dimensional western blotting and tandem mass spectrometry. Biomed Res Int. 2015; 2015: 134050.

42. Staub A, Zurlino D, Rudaz S, Veuthey JL, Guillarme D. Analysis of peptides and proteins using sub-2 Î¼m fully porous and sub 3-Î¼m shell particles. J Chromatogr A. 2011; 1218: 8903-8914.

43. Hong G, Gao M, Yan G, Guan X, Tao Q, Zhang X. [Optimization of two-dimensional high performance liquid chromatographic columns for highly efficient separation of intact proteins]. Se Pu. 2010; 28: 158-162.

44. Cummins PM, O'Connor BF. Hydrophobic interaction chromatography. Methods Mol Biol. 2011; 681: 431-437.

45. Goheen SC, Engelhorn SC. Hydrophobic interaction high-performance liquid chromatography of proteins. J Chromatogr. 1984; 317: 55-65.

46. Geng X, Ke C, Chen G, Liu P, Wang F, Zhang H, et al. On-line separation of native proteins by two-dimensional liquid chromatography using a single column. J Chromatogr A. 2009; 1216: 3553-3562.

47. Sikanen T, Aura S, Franssila S, Kotiaho T, Kostiainen R. Microchip capillary electrophoresis-electrospray ionization-mass spectrometry of intact proteins using uncoated Ormocomp microchips. Anal Chim Acta. 2012; 711: 69-76.

48. Tran JC, Doucette AA. Multiplexed size separation of intact proteins in solution phase for mass spectrometry. Anal Chem. 2009; 81: 6201-6209.

49. Mao Y, Valeja SG, Rouse JC, Hendrickson CL, Marshall AG. Top-down structural analyses of an intact monoclonal antibody by electron capture dissociation-Fourier transform ion cyclotron resonance-mass spectrometry. Anal Chem. 2013; 85: 4239-4246.

50. Tipton JD, Tran JC, Catherman AD, Ahlf DR, Durbin KR, Lee JE, et al. Nano-LC FTICR tandem mass spectrometry for top-down proteomics: routine baseline unit mass resolution of whole cell lysate proteins up to 72 kDa. Anal Chem. 2012; 84: 2111-2117.

51. Fagerquist CK, Zaragoza WJ. Shiga toxin 2 subtypes of enterohemorrhagic E. coli O157: H-E32511 analyzed by RT-qPCR and top-down proteomics using MALDI-TOF-TOF-MS. J Am Soc Mass Spectrom. 2015; 26: 788-799.

52. Fagerquist CK, Sultan O. Induction and identification of disulfide-intact and disulfide-reduced ß-subunit of Shiga toxin 2 from Escherichia coli O157:H7 using MALDI-TOF-TOF-MS/MS and top-down proteomics. Analyst. 2011; 136: 1739-1746.

53. Tveen-Jensen K, Reis A, Spickett CM, Pitt AR. Targeted mass spectrometry methods for detecting oxidative post-translational modifications. Free Radic Biol Med. 2014; 75: 52-53.

54. Forstenlehner IC, Holzmann J, Scheffler K, Wieder W, Toll H, Huber CG. A direct-infusion- and HPLC-ESI-Orbitrap-MS approach for the characterization of intact PEGylated proteins. Anal Chem. 2014; 86: 826-834.

55. Scheffler K. Top-down proteomics by means of Orbitrap mass spectrometry. Methods Mol Biol. 2014; 1156: 465-487.

56. Brunner AM, Lössl P, Liu F, Huguet R, Mullen C, Yamashita M, et al. Benchmarking multiple fragmentation methods on an orbitrap fusion for top-down phospho-proteoform characterization. Anal Chem. 2015; 87: 4152-4158.

57. Takayama M, Sekiya S, Iimuro R, Iwamoto S, Tanaka K. Selective and nonselective cleavages in positive and negative CID of the fragments generated from in-source decay of intact proteins in MALDI-MS. J Am Soc Mass Spectrom. 2014; 25: 120-131.

58. Wang Z, Chen X, Deng L, Li W, Wong YL, Chan TW. Letter: Evaluation and comparison of collision-induced dissociation and electron-capture dissociation for top-down analysis of intact ribonuclease B. Eur J Mass Spectrom (Chichester, Eng). 2015; 21: 707-711.

59. Riley NM, Westphall MS, Coon JJ. Activated Ion electron transfer dissociation for improved fragmentation of intact proteins. Anal Chem. 2015; 87: 7109-7116.

60. Mao Y, Valeja SG, Rouse JC, Hendrickson CL, Marshall AG. Top-down structural analysis of an intact monoclonal antibody by electron capture dissociation-Fourier transform ion cyclotron resonance-mass spectrometry. Anal Chem. 2013; 85: 4239-4246.

61. Zhang H, Cui W, Wen J, Blankenship RE, Gross ML. Native electrospray and electron-capture dissociation in FTICR mass spectrometry provide top-down sequencing of a protein component in an intact protein assembly. J Am Soc Mass Spectrom. 2010; 21: 1966-1968.

62. Carrera M, Cañas B, Vázquez J, Gallardo JM. Extensive de novo sequencing of new parvalbumin isoforms using a novel combination of bottom-up proteomics, accurate molecular mass measurement by FTICR-MS, and selected MS/MS ion monitoring. J Proteome Res. 2010; 9: 4393-4406.

63. Doll S, Burlingame AL. Mass spectrometry-based detection and assignment of protein posttranslational modifications. ACS Chem Biol. 2015; 10: 63-71.

64. Arentz G, Weiland F, Oehler MK, Hoffmann P. State of the art of 2D DIGE. Proteomics Clin Appl. 2015; 9: 277-288.

65. Collier TS, Muddiman DC. Analytical strategies for the global quantification of intact proteins. Amino Acids. 2012; 43: 1109-1117.

66. Nie S, Lo A, Zhu J, Wu J, Ruffin MT, Lubman DM. Isobaric protein-level labeling strategy for serum glycoprotein quantification analysis by liquid chromatography-tandem mass spectrometry. Anal Chem. 2013; 85: 5353-5357.

67. Karabudak AA, Hafner J, Shetty V, Chen S, Secord AA, Morse MA, et al. Autoantibody biomarkers identified by proteomics methods distinguish ovarian cancer from non-ovarian cancer with various CA-125 levels. J Cancer Res Clin Oncol. 2013; 139: 1757-1770.

68. Merl J, Deeg CA, Swadzba ME, Ueffing M, Hauck SM. Identification of autoantigens in body fluids by combining pull-downs and organic precipitations of intact immune complexes with quantitative label-free mass spectrometry. J Proteome Res. 2013; 12: 5656-5665.

69. Russell JD, Scalf M, Book AJ, Ladror DT, Vierstra RD, Smith LM, et al. Characterization and quantification of intact 26S proteasome proteins by real-time measurement of intrinsic fluorescence prior to top-down mass spectrometry. PLoS One. 2013; 8: e58157.

70. Oeckl P, Steinacker P, von Arnim CA, Straub S, Nagl M, Feneberg E, et al. Intact protein analysis of ubiquitin in cerebrospinal fluid by multiple reaction monitoring reveals differences in Alzheimer's disease and frontotemporal lobar degeneration. J Proteome Res. 2014; 13: 4518-4525.

71. Janecki DJ, Bemis KG, Tegeler TJ, Sanghani PC, Zhai L, Hurley TD, et al. A multiple reaction monitoring method for absolute quantification of the human liver alcohol dehydrogenase ADH1C1 isoenzyme. Anal Biochem. 2007; 369: 18-26.

72. Sidoli S, Lin S, Xiong L, Bhanu NV, Karch KR, Johansen E, et al. Sequential window acquisition of all theoretical mass spectra (swath) analysis for characterization and quantification of histone post-translational modifications. Mol Cell Proteomics. 2015; 14: 2420-2428.

73. Collins BC, Gillet LC, Rosenberger G, Rost HL, Vichalkovski A, Gstaiger M, et al. Quantifying protein interaction dynamics by SWATH mass spectrometry: application to the 14-3-3 system. Nat Methods. 2013; 10: 1246-1253.

74. Hyung SJ, Ruotolo BT. Integrating mass spectrometry of intact protein complexes into structural proteomics. Proteomics. 2012; 12: 1547-1564.

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