Trends in Medicine

Commentary

ISSN: 1594-2848

Commentary regarding; Quantification of the *Lamin* A/C transcript variants in cancer cell lines by targeted absolute quantitative proteomics and correlation with mRNA expression

Wedad Saeed Al-Qahtani*

Biology Department, Faculty of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia

Abstract

LMNA proteins are important in nuclear chromosomal stability and structural integrity. LMNA cumulative proteins expression of variants is done through Western blotting (semi-quantitative). To present day, there are not particular antibodies for individual *laminA/C* (LMNA) transcript variants. The mass spectrometric technique of quantifying *laminA/C* (LMNA) transcript variants has been developed and applied in the quantification. In the quantification, signature peptides for every particular *laminA/C* (LMNA) splice variant can be used. The LC–MS/MS assay technique based on targeted signature peptides as well as their internal standards (labeled) was identified in order to measure representation of *LaminA/C* (LMNA) transcript varied concentrations. Validation of the approach was conducted in accordance with the guidelines of Food and Drug Administration (FDA). The levels of expression for Lamin A/C (LMNA) transcript variations were examined in samples obtained from U937 and MCF7 cell lines. In addition, RT-qPCR assay approach was applied to compare and quantitate splice variants' mRNA expression for *Lamin A/C* (LMNA). The validated and established approach portrayed great precision, sensitivity, and linearity. The various expressed Lamin A/C (LMNA) variants in the various lines of cell were determined while their levels matched with the qRT-PCR results. The technique is reliable, sensitive, and reproducible in measuring Lamin A/C (LMNA) transcript variations in various cell lines.

Background

The nucleus enclosure that encircles the nucleus has two coverings of membrane, divided by the intermembranous area. The nuclear enclosure is highlighted by the nucleus lamina, made up of polypeptides known as lamins that interlink heterochromatin and membrane proteins [1,2]. Lamins are intermediary filament proteins (type V) grouped as type A and type B in respect of their structural and sequence configurations. Whereas type A is explicit in many modified somatic cells, type B is almost in all cells. Type B lamins interact with type A lamins in a highly uncharacterized process of assembly in order to form the nucleus lamina. Type A lamins, constituted by lamins C and A, are laminA/C (LMNA) gene products formed through alternative splicing [3-5].

The recent resolve of full human laminA/C (LMNA) gene has offered a good understanding into the procedure through which lamins C and A are produced from one gene [6,7]. The LMNA gene consist of 12 exons, substitute splicing that creates various lamin isoforms. LMNA (laminA/C) gene has five transcript variants: laminC, progerin(laminAΔ50), lamina, laminC2, and laminAΔ10. Lamins help in physical matching of cytoskeleton with the nucleus [8]. Lamins being a crucial constituent of the nucleus matrix, facilitate organization of chromatin, moderate attachment of different transmembrane proteins. In addition, they are part of transcriptional activities, replication of DNA as well as repair processes [9-11]. LaminA/C gene's mutations have been associated to different disorders referred to as laminopathies. LaminA/C (LMNA) have been associated with a number of heritable disorders that include EmeryDreifuss muscular dystrophy, cardiomyopathy, lipodystrophy, neuropathy, premature aging syndrome, and cerebellar disorders [3,7,10,12]. Particular proteins' absolute quantification by use of MS-techniques has been created to examine concentrations of proteins in several researches using mass spectrometry (triple quadrupole) with multiple reactions monitoring (MRM) – the most used technique for analyte's quantitation by LCMSMS [13,14].

The signature peptides for targeted proteins are normally selected on the basis of different factors such as peptide length, targeted protease, intra- and inter-species uniqueness, probable places for post-translational modification (PTM) as well as history of detection by mass spectrometry [15,16]. Bioinformatics tools that include Peptide Atlas, BLAST, and Skyline can be applied in this selection. Targeted proteins are obtained from biological specimens and cleaved by use of particular enzymes in order to release fragments of signature peptides with distinct primary sequences. The signature peptides are used as surrogates of matching parent proteins such that little molecular peptides may be quantified in order to approximate concentration of proteins. This technique has been applied in study areas e.g. occupational asthma [11,17,18], or in diagnosis involving inborn metabolism errors, oral cancer, and pancreatic cancer [3,19-21].

Correspondence to: Wedad Saeed Al-Qahtani, Biology Department, Faculty of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia, E-mail: wed-mw@hotmail.com

Received: April 07, 2020; **Accepted:** April 21, 2020; **Published:** April 24, 2020

The principle of developed method

The LC–MS/MS technique facilitates assessment of diagnostic and biological potential of LMNA (Lamin A/C) transcript varied proteins. The LC–MS/MS bioanalytical technique is validated in accordance with the international guidelines and showed great selectivity with reduced matrix impact, good linearity, precision, accuracy, stability, and recovery [1,6,22,23]. The developed method of separation indicated acceptable recovery values. The chromatogram developed has appropriately resolved Lamin A/C (LMNA) transcript variations proteins with no interference and can easily be utilized in researches regarding human laminopathies. Every Lamin A/C (LMNA) transcript variation protein's sensitivity can vary over a broad range because they depend on the molecular proton's affinity under the common parameters of mass spectrometry such as post-source stability of chemicals, ion source, temperature and gas pressure, and collision energy. Under conditions of optimum chromatographic, targeted Lamin A/C (LMNA)'s transcript variations proteins peaks were separated adequately with retention periods of between nine and ten minutes. Lamin A level of expression was obtained by subtracting the proteins' expression level of lamin A/C (LMNA) from the proteins' expression level for different lamin splice variations (lamin Δ50, lamin Δ10, and lamin C as follows:

lamin A = *lamin* A/C − (*lamin* Δ10 + *lamin* C + *lamin* Δ50)

Discussion

Signature peptides lack potential sites for PTM, for instance cystine. Additionally, signature peptides have good history and size for detection by mass spectrometry. Uncovering the particular biological function of every lamin A/C (LMNA) transcript variant can offer a better understanding into laminopathies' pathophysiology. Most published researches have identified lamin A/C (LMNA) gene transcript varieties as a single protein and not separate proteins having separate biological roles. Nonetheless, the LC–MS/MS technique presents a specific and sensitive approach of quantifying proteins compared to the traditional semi-quantitative Western blotting approach or relative RT-qPCR quantitation.

Currently, lamin AΔ10 has no particular antibody and trials to identify its particular antibody have failed [24]. Due to lack of sensitivity in lamin Δ50 antibody, lamin C and lamin A can only be quantified by IHC or Western blotting (semi-quantitative). RNA's stability would present an obstacle in routine utilization of lamina m RNA/lamina C ratio as the diagnostic test [25,26]. Furthermore, the novel technique may be utilized to examine different transduction pathways entailed in laminopathies linked with the four transcript variations. The results from LC–MS/MS can be compared with RT-qPCR's result that is a quantitative technique for mRNA expression of mRNA expression (LMNA) transcript variants.

Lamin C and lamin A have great abundance, while progerin and lamin AΔ10 have little abundance in U937 and MCF7 cells. The four lamin A/C transcript variants in U937 that is monocytic line of cell have smaller values in comparison to MCF7 cells. Lamin A/C (LMNA) expression is present in monocytes [7,20,22,24,27,28]. Furthermore, lamin A/C (LMNA) gene inactivation by the CpG island hypermethylation promoter has been linked to leukemic monocytes. In addition, the developed technique utilizing particular signature peptides for LC–MS/MS detects expression of four transcripts variations of LMNA (lamin A/C) in leukemia monocytes of U937 cell lines. RT-qPCR is unable to detect lamin AΔ50 or lamin AΔ10 in mononucleus cells of leukemic and normal patients. Therefore, this technique could be used as leukemia diagnostic tool through lamin A/C (LMNA) transcript variations ratios quantitation in monocytes. There is an inverse relation between progerin and lamin AΔ10 expression, where induction of progerin inhibits lamin AΔ10. Lamin A/C (LMNA) transcript varied proteins are greatly expressed in properly differentiated tissues and cells although they are poorly represented in the stem cells [14,18]. From the findings, lamin A/C (LMNA) transcript varied proteins are represented rather than being fully absent in monocytic or breast cancer cell lines. Differences in findings depend on the techniques utilised. This shows the importance of established sensitivity of LC–MS/MS.

Progerin expression quantification by specific and sensitive technique may permit exploration of smaller progerin levels in the aged as well as other diseases [2,28,29]. Furthermore, progerin and lamin C have exhibited opposite and distinct functions in respect of lifespan and energy expenditure [30-33]. Therefore, measurement of the transcript variants could likely lead to comprehending their function in expenditure of energy. Splice variants of Lamin A have been recognized as probable cellular aging biomarkers. Additionally, Splice variants of Lamin A have been associated to aging disorders (premature), such as Hutchinson-Gilford progeria syndrome (HGPS) as well as adults' Werner syndrome [13,34-36], and in lipodystrophies, cardiomyopathies, and muscular dystrophy [3,5,12,17,21]. The levels of expression for Lamin A/C transcript variations were examined in samples obtained from U937 and MCF7 cell lines. RT-qPCR assay procedure was utilised to compare and quantitate splice variants' mRNA expression for Lamin A/C. The various expressed Lamin A/C variants in the various lines of cell were determined while their levels matched with the qRT-PCR results.

Conclusion

Lamins being a crucial constituent of the nucleus matrix, facilitate organization of chromatin, moderate attachment of different transmembrane proteins. They also consist of transcriptional activities, replication of DNA and repair processes. Most published studies have identified lamin gene transcript varieties as a single protein and not separate proteins having separate biological roles. Nonetheless, the LC–MS/MS technique presents a specific and sensitive approach of quantifying proteins compared to the traditional semi-quantitative Western blotting approach or relative RT-qPCR quantitation. This technique will be applicable to groups willing to perform laminopathic profiling on tissues or cells. The study has indicated that agents of ion pairing, for example tributylamine are normally avoided for usage in the positive status LC/MS because of their ability to suppress ions. In this process involving quadrupole triple instrument, there were excellent sensitivity as well as designed transactions to shun tributylamine ion's detection. The levels of expression for *Lamin A/C* transcript variations were examined in samples obtained from U937 and MCF7 cell lines. In addition, RT-qPCR assay approach was applied to compare and quantitate splice variants' mRNA expression for *Lamin A/C*. The validated and established approach portrayed great precision, sensitivity, and linearity. Measurement of the transcript variants could likely lead to comprehending their function in expenditure of energy. Splice variants of Lamin A have been recognized as probable cellular aging biomarkers. Splice variants of Lamin A have been associated to pre-mature aging disorders. The various expressed *Lamin A/C* variants in the various lines of cell were determined while their levels matched with the qRT-PCR results. The results from LC–MS/MS can be compared with RT-qPCR's result that is a quantitative technique for mRNA expression of mRNA expression (LMNA) transcript variants. The technique is reliable, sensitive, and reproducible in measuring

Lamin A/C transcript variations in various cell lines. The LC–MS/MS technique facilitates assessment of diagnostic and biological potential of Lamin A/C transcript varied proteins. The recent resolve of full human laminA/C gene has offered a good understanding into the procedure through which lamins C and A are produced from one gene. The approach is validated in accordance with the international guidelines and showed great selectivity with reduced matrix impact, good linearity, precision, accuracy, stability, and recovery.

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