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Studying Protein Arginine Methylation: Approaches and Methods

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Editorial

Protein arginine methylation is a posttranslational modification that is involved in the regulation of a wide spectrum of biological processes[1,2,3]. The nine members of the protein arginine methyltransferases (PRMTs) family can methylate arginines in a monomethyl and di-methyl symmetric or asymmetric manner. Understanding the functional consequences and cataloging the methylated substrates of PRMTs have dramatically advanced the understanding of the PRMT enzymes and facilitated their linkage to disease[2]. Additionally, the development of potent and selective inhibitors has enabled chemical biology-based approaches to study PRMTs and has accelerated the clinical development of the PRMT targeting agents[3]. As the field of PRMTs progresses, novel methods and their applications will further enable elucidation of the regulation and biology of these enzymes.

In this methods collection, an article on the recombinant production of the PRMT proteins provides a detailed account of a baculovirus-based expression system[4]. Several PRMTs can be generated using the bacterial expression; however, larger or complex dependent PRMTs such as PRMT7 or PRMT5 require an insect cell expression system. This method has already benefited numerous *in vitro* efforts to determine the substrates of PRMTs.

Studying PRMT substrates in the cellular environment requires sophisticated detection approaches. Two articles in this collection present distinct methodologies that rely on antibody enrichment of methylated arginines followed by mass spectrometry quantitation or antibody-independent quantification using nuclear magnetic resonance (NMR). Several antibodies recognizing distinct methylation states were developed and have been used in studies identifying the PRMT substrates[5]. The mass spectrometry method enables the quantitative evaluation of cellular protein arginine methylation by employing metabolic labeling, subsequent sophisticated separation techniques, and antibody-based enrichment[6]. Several members of the PRMT family prefer methylating arginines that are found in repetitive RGG motifs residing in the intrinsically disordered regions of proteins[7]. The NMR spectroscopy-based method enables determining the methylation state and position of all the arginine derivatives (mono and di, symmetric and asymmetric) where

they can be quantified reliably within complex biological samples[8].

Finally, to identify methylated arginine residues in specific proteins, two articles provide approaches based on western blotting and the proximity ligation assay (PLA). Both of these methods utilize antibody-based detection but differ in the detection method and the information provided. The western blot method is quantitative and has been valuable in characterizing chemical probes[9]. The PLA provides information on the spatial location of the methylated arginine since it uses the target protein antibody and the generic antibody for mono or di-methyl arginine, thus circumventing the need to generate specific antibodies and can remarkably be used on fixed tissues[10].

Together, these diverse methodologies will enable robust discovery of methylated arginines in various cell systems and disease states and further advance the fundamental knowledge of the biological function of the PRMT enzymes.

Disclosures

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