Novel antibody reagents for the characterization of protein ADP-ribosylation

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INTRODUCTION

Poly-ADP-ribosylases (PARPs) catalyze the transfer of ADP-ribose from NAD⁺ and release nicotinamide in the process. ADP-ribosylation is predominantly involved in processes, occurring on protein amino acid side chains of proteins, such as arginine, glutamate, aspartate, cysteine, and lysine. PARPs, which include DNA repair, RNA processing, and transcription. The PARylation process is known to be involved in various cellular processes, including cell stress responses, mitotic spindle formation, chromosome condensation, and transcription. Even though PARylation is a central point of cellular function, there are no commercially available antibodies that recognize both PARylated and PARyalted proteins. Therefore, novel rabbit monoclonal antibodies have been produced and characterized against this modification on proteins, and their utility for the detection of ADP-ribosylated proteins by IFA, western blot, dot blot, and immunofluorescence assays has been validated.

METHODS

Polyclonal antibodies were produced by modifying lysine residues on KLH using periodate chemistry. Rabbits were selected for monoclonal antibody development based on reactivity in ELISA and Western blot assays. Rabbit monoclonal antibodies were then produced, tested, and selected for scale-up and additional testing. ELISA, Western blot, and immunofluorescence assays were performed as described. Hydrogen peroxide treatment was performed at 0.5 mM for 5 min. PDE1 treatment was performed at 0.5 mM for 4 hr at 37°C. The immunofluorescence assay was performed as described by Jones et al. and cells were imaged on an ImagingXpress Micro XLS and quantified using MetaXpress.

CONCLUSIONS

Four antibody clones were isolated and characterized which showed distinct binding patterns and levels of induction of cellular ADP-ribosylation on Western blots of cells stimulated with PARylation-activating treatments such as hydrogen peroxide. Clone E6F6A which was selected for commercial release was validated for use in Western blot, dot blot, ELISA, and immunofluorescence assays. Initial studies also show that the antibody is a useful tool for the enrichment of PARylated proteins or peptides prior to LC-MS/MS analysis. Further work is aimed at the understanding of the specificity of the four antibody clones. Development of new tools to study this critical post-translational modification should facilitate new discoveries of PARylated PARylated proteins and their function during growth, development, and in disease settings.

REFERENCES