MMSL
Military Medical
Science Letters
Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2018, 87(supplementum 1)
ISSN 2571-113X (Online)

MEETING ABSTRACTS

MASS SPECTRAL DETECTION OF DIETHOXYPHOSPHO-TYROSINE ADDUCTS ON PROTEINS FROM HEK293 CELLS USING MONOCLONAL ANTIBODY DEPY FOR ENRICHMENT

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Chronic illness from exposure to organophosphorus toxicants is hypothesized to involve modification of unknown proteins. Tyrosine readily reacts with organophosphorus toxicants in proteins that have no active site serine. We developed a monoclonal antibody, depY, that specifically recognizes diethoxyphospho-tyrosine in proteins and peptides, independent of the surrounding amino acid sequence [1]. Our goal was to identify diethoxyphosphorylated proteins in human HEK293 cell lysate treated with chlorpyrifos oxon. Cell lysates treated with chlorpyrifos oxon were examined by ELISA and capillary electrophoresis Western blot. Tryptic peptides were analyzed by liquid chromatography-tandem mass spectrometry. The depY antibody recognized diethoxyphospho-tyrosine containing proteins by ELISA and Western blotting. Mass spectrometry identified 40 diethoxyphospho-tyrosine peptides from 24 proteins in immunopurified samples, but found only 9 diethoxyphospho-tyrosine peptides from 6 proteins when the same sample was not immunopurified on depY. The most abundant proteins in the cell lysate, Histone H4, Heat shock 70 kDa protein 1A/1B, Heat shock protein HSP 90 beta, and Alpha-enolase, were represented by several diethoxyphospho-tyrosine peptides. It was concluded that use of immobilized depY improved the number of diethoxyphospho-tyrosine peptides identified in a complex mixture. The mass spectrometry results confirmed the specificity of depY for diethoxyphospho-tyrosine peptides independent of the context of the modified tyrosine, which means depY could be used to analyze modified proteins in any species.

Keywords: chlorpyrifos oxon; diethoxyphospho-tyrosine antibody; mass spectrometry; ELISA; Western blot

References

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