ANALYSIS OF MANTLE CELL LYMPHOMA PHOSPHOPROTEOME

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The phosphoproteomic study of lymphoid cells may contribute to an understanding of their origin by identifying pathways responsible for their escape from cell control. The proteomic approach was used for an initial study of GRANTA-519, an established cell line for mantle cell lymphoma, by making an initial inventory and identifying proteins with post translational modifications, especially phosphoproteins. Initially, cells were cultured in an appropriat medium. Proteins were extracted with 7.7M urea, 2.2M thiourea, 4.4% chaps and a mixture of protease inhibitors. After extraction of proteins, aliquots of proteins were treated with ?PPase and submitted to isoelectric focusing pH 4,0-7,0. SDS PAGE was performed in acrylamide gels prepared in our laboratory. Proteins were detected by colloidal Coomassie G-250 staining, specific staining for phosphorylated proteins and specific antibodies against phosphoserine and phosphotyrosine residues in proteins. Two dimensional maps were analyzed using the software ImageMaster. More than twenty eight spots of two hundred, about ± 14 %, disappeared after treatment with ?PPase. Proteins, equivalent to those which have disappeared after the treatment, were digested *in situ* with trypsin and the tryptic peptides were submitted to MS or MS/MS in a MALDI-TOF or in an ESI triple quadrupole spectrometers. Twelve of the digested proteins whose spots disappeared after ?PPase treatment were identified. The following proteins were identified in more than one spot: Nucleophosmin, a phosphorylated protein involved with nucleolar acetylation, Annexin A6, a highly conserved Ca^{2+} regulated protein Other proteins chosen after disaperance with the treatment were identified as: Heat shock 70 kDa protein 1, Stress-70 protein, mitochondrial, Heterogeneous nuclear ribonucleoprotein H, Tumor protein D52, Actin-related protein 2/3 complex subunit 5, Eukaryotic translation initiation factor 5A-1 and Rho GDP-dissociation inhibitor 1.

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