Analysis of Rps19 protein and Rps19 missense mutant proteins interactome

ID - 248

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Motivation

Ribosomal protein S19 (Rps19) is a 16 kDa protein found mainly as a component of the ribosomal 40S subunit. Its mutations are responsible for Diamond Blackfan Anemia (DBA), a congenital disease characterized by defective erythroid progenitor maturation (1). Dysregulation of Rps19 has therefore been implicated in this defective erythropoiesis, though the link between them is still unclear. A loss of function not directly connected with Rps19 structural role in the ribosome have been proposed. We use proteomic strategies to look for proteins interacting with Rps19 in order to determine its functions (2). Finally we identify proteins interacting with Rps19 missense mutant proteins (R62W, R101H) (3).

Methods

Protein complexes are isolated by affinity purification using GST-Rps19, GST-Rps19R62W GST-Rps19_R101H recombinant proteins and identified using LCMS/MS analysis. To define the interactome of Rps19 and Rps19 missense mutant proteins we subtracted species common to the GST . Raw data from LCMS/MS analyses are converted into a Mascot format text in order to identify proteins by means of the MatrixScience software (www.matrixscience.com). Protein search is ruled by the following parameters: non-redundant protein sequence data base (NCBInr; 01242006 download - 3229765 sequences); specificity of the proteolytic enzyme used for the hydrolysis (trypsin); taxonomic category of the sample (Homo sapiens); no protein molecular weight was considered; up to 1 missed cleavage; cysteines as S-carbamidomethylcysteines; unmodified N- and C-terminal ends; methionines both unmodified and oxidized; putative pyroGlu formation by Gln; precursor peptide maximum mass tolerance of 150 ppm and a maximum fragment mass tolerance of 300 ppm. All the MS/MS spectra displaying a Mascot score higher than 38 show a good signal/noise ratio leading to an unambiguous interpretation of the data. Individual MS/MS spectra for peptides with a Mascot score equal to 38 are inspected manually and only included in the statistics if a series of at least four continuous y or b ions are observed. In silico analysis was performed to identify proteins known to directly or indirectly interact with RPS19 by using databases HPRD Human Protein Reference Database (www.hprd.org), PubMED www.ncbi.nlm.nih.gov, Nucleolar Proteome Database (http://www.lamondlab.com/NoPDB) and the Pre-Ribosomal Network yeast database (http://www.pre-ribosome.de/Home.html).

Results

We identify 159 proteins interacting with wild type Rps19 from the following Gene Ontology categories: NTPases (ATP- and GTPases; 5 proteins), hydrolases/helicases (19 proteins), isomerases (2 proteins), kinases (3 proteins), splicing factors (5 proteins), structural costituents of ribosome (29 proteins), transcription factors (11 proteins), transferases (5 proteins), transporters (9 proteins), DNA/RNA-binding protein species (53 proteins), other (1 dehydrogenase protein, 1 ligase protein, 1 peptidase protein, 1 receptor protein, 1 translation elongation factor) and 13 proteins of still unknown function (2). Proteomic results are validated by western blotting and by co-immunoprecipitation using a monoclonal Rps19 antibody. Many interactors are nucleolar proteins and thus expected to take part in the Rps19 interactome; however, some proteins suggest additional functional roles for Rps19. Interactomes of Rps19 mutant proteins are compared to the wild type protein one in order to elucidate the loss of function hypothesis suggested for DBA pathogenesis. We also carry out an in silico proteomic analysis of proteins known to directly or indirectly interact with Rps19 by using databases HPRD and PubMED. A list of primary (direct) and secondary (indirect) protein-protein interactions of RPS19 is created using the web available Human Protein Reference Database. Primary interactions of Rps19 are screened for protein interactors to define an in silico interaction map with the indirect protein partners. This map is then compared with the Rps19 protein partners identified by experimental proteomic strategies. In addition a list of primary interactions is created by HPRD for each identified protein. The list of Rps19 protein partners is compared with the Nucleolar Proteome Database and with the Pre-Ribosomal Network yeast database. The characterization of

Rps19 interacting proteins provides information for studies aimed at more cleary defining the role of the protein in the DBA. _ (1) Campagnoli, M.F et al. (2004) Haematologica. (2) Orru`, S. et al. (2007) MCP. (3) Da Costa L. et al. Blood (2003).
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