rilación en treonina o truncaciones “naturales”, así como determinar cambios relativos de estas modificaciones en situaciones patológicas (enfermedad autoinmune *vs* controles sanos).

Bibliografía

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Regulación de la Metiltioadenosina Fosforilasa por oxido-reducción en células hepáticas. Mecanismo e implicaciones funcionales

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La enzima 5´-metiltioadenosina fosforilasa (MTAP) cataliza de la formación de adenina y 5´-metiltioribosa 1 fosfato a partir de la 5´-metiltioadenosina (MTA). En humanos, una deficiencia en la actividad MTAPasa se ha correlacionado con diversas enfermedades, incluyendo cirrosis y hepatocarcinoma. En el presente trabajo se ha investigado la regulación de la actividad MTAPasa por especies reactivas del oxígeno. Los datos obtenidos muestran la inactivación de la enzima MTAP hepática tanto en un modelo de ratón tratado con lipopolisacárido bacteriano (LPS) como en células HepG2 incubadas con de tert-butil hidroperóxido. Por otra parte, la MTAP recombinante purificada se inactivó de forma reversible en presencia de peróxido de hidrógeno. La pérdida de actividad de la MTAP mediada por radicales libres resulta de la reducción de la Vmax y ocurre por la oxidación específica de los residuos de cisteína 136 y 223 a ácido sulfénico, que podrían ser estabilizados mediante la formación de intermediarios sulfenil amidas. Además, identificamos un puente disulfuro entre las cisteínas 145 y 211 tras la exposición de la enzima a peróxido de hidrógeno. Sin embargo, esta modificación no participa en la inactivación de la actividad MTAPasa, como se pudo comprobar mediante experimentos de mutagénesis dirigida.

From liver tissue to phosphorylation sites

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Non-alcoholic steatohepatitis (NASH) is a progressive disease that develops from hepatic steatosis to cirrhosis and liver failure. S-adenosylmethionine (SAMe) is considered a key metabolite that regulates hepatocyte growth, death and differentiation. A chronic hepatic SAMe deficiency facilitates the development of fatty liver and its progression to NASH and hepatocellular carcinoma (HCC). Methionine adenosyltransferase 1A (MAT1A) is expressed exclusively in the liver and in the pancreas in adults and synthesizes SAMe. Another key player in the metabolism of methionine is Glycine N-methyltransferase

(GNMT). GNMT is an abundant enzyme in liver that catalyzes the methylation of glycine by using S-adenosylmethionine (AdoMet). GNMT KO mice spontaneously develop steatohepatitis and fibrosis. It has been shown that eight months old MAT1A KO mice developed spontaneous NASH and the majority of them developed HCC at the age of eighteen months. Consequently, these KOs mice have been chosen as model for the study of the metabolic pathways implicated in the development of NASH.

Phosphorylation is a key regulation event in cell signalling and in consequence, in the function of biological systems. The use of phosphoprotein enrichment procedure is a method to simplify the proteome of KO and WT liver mice. Phosphoprotein enrichment was performed using Qiagen kit for phosphoprotein purification. The phosphoproteins were loaded into a 2D SDS-PAGE, visualized with Sypro Ruby and further analyzed by PDQuest software. In parallel, the phosphoproteins were digested by trypsin and titanium oxide and IMAC enrichment was performed on the tryptic peptides. Analysis of the phosphopeptides recovered for this second enrichment step at the peptide level was performed by LC-MS/MS using a nanoAcquity-UPLC system (Waters) coupled to a QToF Premier (Waters). The characterization of the proteins was carried out using Mascot Database Searching.

Differences in phosphorylation have been observed by Sypro Ruby staining of 2D gels for the phospho-proteomes of KOs and WTs mice. The biological analysis of these changes in phosphorylation levels of phosphoproteins between KO and WT liver homogenate mice it will provide valuable information about the role of phosphorylation in the development of the disease.

Major targets of iron-induced protein oxidative damage in frataxin-deficient yeasts are magnesium-binding proteins

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Introduction

Iron accumulation has been associated with several pathological conditions such as Friedreich ataxia (FRDA). This human disorder is caused by decreased expression of frataxin (Roy & Andrews, 2001). Oxidative stress due to iron-overload promoted selective damage to proteins. Such damage can be evaluated by analyzing protein- carbonyl content (Tamarit et al, 1998, Cabiscol et al 2002, Schacter et al 1994). In yeast cells lacking the frataxin ortholog *YFH1*, we have identified a set of 14 carbonylated proteins which include mitochondrial ATP synthase, phosphoglycerate kinase, pyruvate kinase and molecular chaperones. The fact that most of the target proteins are magnesium and/or nucleotide-binding proteins, leads us to postulate that when iron accumulates, it replaces magnesium at the corresponding metal-binding site, promoting selective damage to these proteins.

Materials and methods

- *Western blot Analysis and carbonyl content quantitation*.- Oxidative damage to proteins was evaluated by carbonyl-group derivatization with 2,4-dinitrophenyl hydrazine (DNPH). Antibodies against DNPH allow the immunodetection of this compound bound to proteins by classic western-blot techniques. Crude extracts were separated in one- or two-dimensional gels (Irazusta et al, 2006). In both cases, antibodies against DNPH (Dako) were used at 1:5,000 dilution. Images were acquired in a ChemiDoc XRS System (Bio-Rad) and analyzed with PDQuest or Quantity One software (Bio-Rad). Proteins were identified by peptide mass fingerprinting after tryptic digestion and MALDI-TOF analysis.