IDENTIFICATION OF THE PROTEIN TARGETS OF THIOREDOXIN AND GLUTATHIONE PATHWAYS IN HUMAN CELLS BY *ocSILAC* STRATEGY

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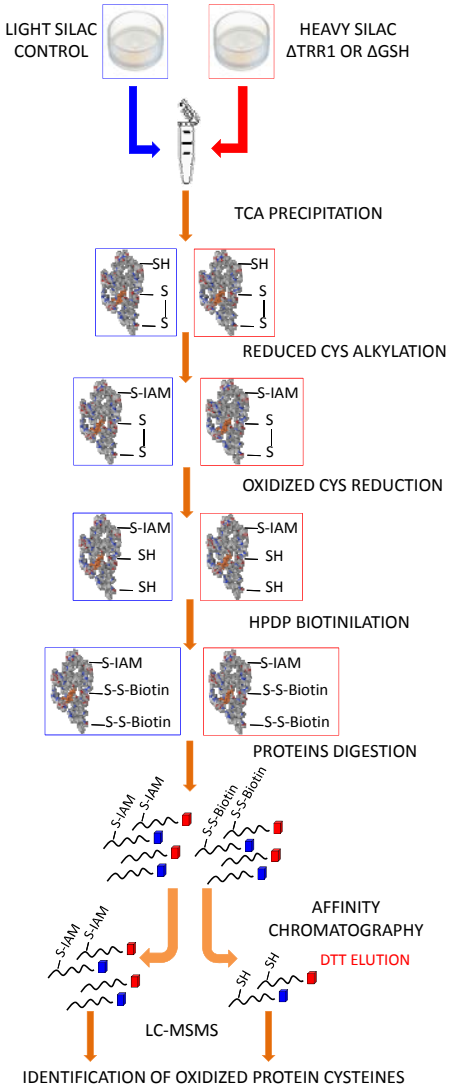
INTRODUCTION

Identification of proteins involved in glutathione (GSH) and Thioredoxin (Trx) pathways in mammals is a key step to decipher the molecular mechanisms regulated by the thiol redox biochemistry. *Isotope Coded Affinity Tag* (ICAT) based strategies were developed in order to characterize the thiol oxidation state of the proteome. However, this kind of approach it was shown to be lacking if applied to cell cultures without considering the protein expression profiles (1).

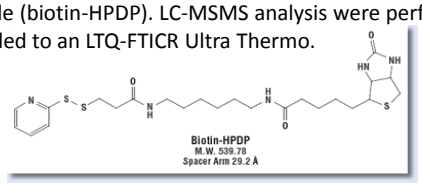
Here we show a strategy to localize oxidized cysteine residues and to simultaneously generate protein expression profiles. This strategy is based on the SILAC technology (ocSILAC). Our study was extent to the identification of the protein targets Trx and GSH pathways . This was performed by the redoxome analysis of GSH and Thioredoxin reductase (TRR1) silenced HELA cell lines.

ocSILAC STRATEGY



METHODS

GSH synthesis was inhibited in HELA cells by exposition for 24h to the L-buthionine-[S,R]-sulfoximine (BSO). TRR1 expression was silenced by short hairpin RNA technology. Cell lysis was performed following our thiol trapping procedure using trichloroacetic acid (TCA) (2). Reduced thiols were alkylated byiodoacetammide, oxidized thiols were selectively labeled by (N-(6-(Biotinamido)hexyl)-3'-(2'-pyridyldithio)-propionamide (biotin-HPDP). LC-MSMS analysis were performed with a Dionex coupled to an LTQ-FTICR Ultra Thermo.



RESULTS

IDENTIFICATION. Identified up to 491 oxidized cysteine residues in 254 different proteins. Un-specific peptides were detected under 15% of the assigned peptides. The enrichment of the peptides containing oxidized cysteines allowed to extend the analysis to the less aboundant proteins, 81% of the proteins detected in the enriched fraction were not detected in the unbound fraction.

MPRI_HUMAN	1	absent
GSLG1_HUMAN	2	absent
LAMB1_HUMAN	3	absent
GALT2_HUMAN	4	absent
GLU2B_HUMAN	5	403

KPYM_HUMAN	6	2
EF2_HUMAN	7	18
EGFR_HUMAN	8	absent
P3H1_HUMAN	9	absent
PGK1_HUMAN	10	25

PLOD3_HUMAN	11	absent
DAF_HUMAN	12	absent
ERO1A_HUMAN	13	absent
FBN2_HUMAN	14	absent
FKB10_HUMAN	15	absent

PROTEIN EXPRESSION PROFILES. Protein expression profiles of 370 proteins were generated using un-treated HELA cells as control. Protein over-expression was observed more important in ΔGSH cell lines. TRR1 that is roghly 70% silenced in ΔTRR1 cell line is more than two folds overxpressed in ΔGSH cell line.