Profiling the cysteine redox proteome by isobaric Tandem Mass Tag reagents (oxiTMT)

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Introduction

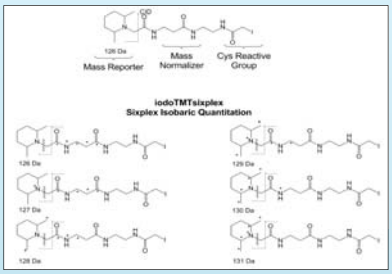
Reversible cysteine oxidation to form disulfide (-S-S-), sulfenic acid (-SOH-), S-nitroso (-SNO-) is a post-translational modification serving in many cellular functions. A systematic characterization of the cysteine redox state at proteomic scale (redoxome) is a key step to understand the thiol-redox based molecular mechanisms. Nowadays many strategies are available to reach this aim (1), meaning that the study of the redoxome is an important field of application of proteomics. The major issue of this kind of analysis is the high percentage of reduced cysteines in the cytoplasmic and nucleus fractions (2) that could constitute 64% of the proteome (3). Moreover, among the shotgun proteomics strategies available, little attention was paid to the changes in protein expression between control and treated samples.

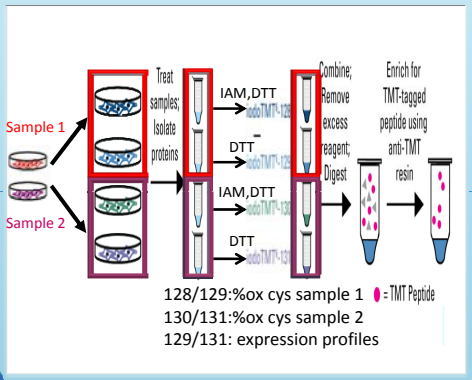
Aims

Our group has already developed a proteomic strategy, called OcSILAC (Chiappetta G. et al. SFEAP 2012 and Hupo 2012, see also poster P312), allowing to profile protein expression and cysteine oxidation. In order to extend the field of application achievable also to samples unsuitable with SILAC protocol (tissues, sera, etc…) here we show an adaptation of OcSILAC workflow using isobaric Iodoacetyl-Tandem Mass Tag (iTMT) reagents (oxiTMT).

Material and Methods

iTMT belongs to isobaric tandem Mass-Tag reagents. Its iodoacetamide function allows to label cysteine-thiol function. Resin activated with an anti-TMT antibody allows to enrich iTMT labeled peptides.

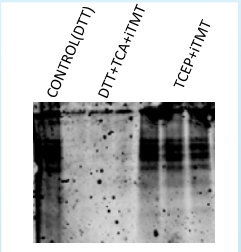




iTMT manufacturer protocol was tested and adapted in order to obtain an efficient saturation of reduced thiols and a selective labeling of oxidated thiols.

Differential cysteine labeling with iTMT, as presented in scheme, leads to obtain in the same MS/MS spectrum information about cysteine oxidation percentage and protein expression profiles.

Results

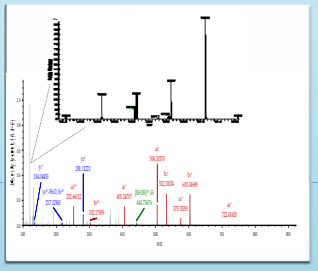


In order to test oxidized thiol saturation a fluorophore maleimmide was added after iTMT alkylation. The default protocol consisting in cysteine labeling in presence of the reductant TCEP, does not allow to saturate oxidized thiols. We modified the protocol by adding a TCA precipitation before each step and succeeded to fully saturate oxidized thiols. One iTMT tube can be used to saturate 100ug of protein extract.

Control

Carcinoma

Thyroid cancer redoxome: Galectin-I case



Galectin-I (GAL-I) gene expression increases up to 100-fold in oncogene-transformed rat thyroid cells (4). GAL-I is secreted as a non-covalently linked homodimer with two carbohydrate-recognizing domains that are capable to mediate tumor growth throw regulation of T-cell apoptosis (5). Gal-I reduced cysteines have essential function in lectin binding.

In our study we submitted to the analysis a normal and papillary carcinoma thyroid biopsies. By our approach it was possible to confirm the increased levels of Galectin-1 in tumor tissues. Moreover by our redoxome strategy it was underlined that the newly synthesized protein is in the reduced form.

Conclusions & Perspectives

Redoxome analysis could give an exceptional view of the molecular mechanisms based on redox signaling. Here we presented an alternative quantitative protocol to our OcSILAC strategy . Although the reagent cost could be higher respect to OcSILAC , oxiTMT led to extend the analysis to tissues or *non culture* samples. Moreover oxiTMT multiplex allows to calculate the percentage of oxidized cysteine residues *per* protein, a feature not possible with OcSILAC duplex.

Submitting to oxiTMT thyroid biopsies it was possible to underline the overexpression of Galectin-I in carcinoma tissues. For the first time we can show that the increase of Galectin-I level is mostly correlated to the increase of its reduced form. This reduced form is the active form that can generate an immuno-suppressive environment allowing to escape tumor cell from immunity system.

References

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