Abstract

Chemical proteomics has acquired a pivotal role in chemical biology because of its peculiar capability to deeply analyze the proteome from many points of view. Proteins identification, characterization and quantification provide a detailed portrait of a biological system. In particular, pharmaceutical research is looking with interest at chemical proteomics because the mechanism of action of bioactive molecules remains one of the main challenge[1]. Particularly, the identification of target proteins and investigation of ligand-receptor interactions are today considered essential steps in the drug discovery and development process. Affinity purification-based mass spectrometry approaches (AP-MS) have emerged as a valuable mean to link bioactive compounds to their cellular targets[2]. In recent years, the application of such techniques led to successful results in determining the macromolecular partners of many interesting bioactive molecules[3,4]. These techniques require the chemical modifications of the molecule of interest onto a solid matrix, in order to allow the bioactive compound to "fish out" its specific interactors from a cell lysate or a tissue extract. Once eluted, these cellular targets are identified by MS and bioinformatics analysis[5,6]. Later on, the biological profile of the selected compound toward its cellular interactors is investigated by *in vitro* and/or *in vivo* assays.

The application of this strategy to the cases of suvanine (SUV)[7], heteronemin (HET)[8,9] and scalaradial (SLD)[10,11] led to the identification of their main cellular targets. The identified interactions were then validated by means of surface plasmon resonance, whereas their biological relevance was established through *in vitro* and *in vivo* assays.

An in cell *fishing for partners* procedure was also developed and applied to the case of SLD and Oleochantal (OLC)[12].

Eventually, a competition variant of the standard AP-MS approach was also performed to analyze the interactome of the endogenous metabolite, 3'-5'-cyclic cytidine monophosphate (cCMP).

HSP60 has been identified as the main biological target of SUV in HeLa cells, and its ability in inhibiting the HSP60 activity was demonstrated *in vitro,* evaluating the reduction of HSP60 mediated refolding of citrate synthase.

HET was found to bind TDP43, a nucleic acid-binding protein involved in some neurodegenerative diseases. A marked effect of HET in lowering the binding affinity between TDP43 and the TAR32 oligonucleotide has been established by alpha screen technology. Moreover, a high tendency of TDP43 to aggregate upon HET treatment was demonstrated *in vitro*, by using recombinant TDP43, and in cell, trough western blot and immunofluorescence analyses.

Peroxiredoxin 1 and 14-3-3 ε were recognized as main cellular partners of SLD, by applying the described AP-MS approach.

Later on, SLD was chosen as a probe for the development of an in cell *fishing for partners* experiment based on bio-orthogonal chemistry[13,14]. SLD was first decorated with an azide-containing linker and then a living cell sample was treated with the tagged molecule. The SLD interactors, selected in the cellular environment, were then fished out by promoting an azide-alkyne cycloaddition between the tagged SLD and an acetylenic functionalized matrix. Peroxiredoxin 1 and 14-3-3 ε, along with proteasome, were recovered as specific and main SLD partners. The effectiveness of bio-orthogonal chemistry in affinity-based target discovery experiments was further confirmed assessing the ability of Oleocanthal to select HSP90, its already known target[15].

The cCMP interactome was deeply analyzed by means of AP-MS and competition experiments showing, along with the known partners PKA and

PKG[16], the heterogeneous nuclear ribonucleoproteins as a new class of potential cCMP effectors.

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