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 \textit{in vitro} and/or \textit{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 \textit{in vitro} and \textit{in vivo} assays.

An in cell \textit{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, through 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\textsuperscript{[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\textsuperscript{[15]}. The cCMP interactome was deeply analyzed by means of AP-MS and competition experiments showing, along with the known partners PKA and
Abstract

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

