Abstract

The emerging field of mass spectrometry-based chemical proteomics provides a powerful instrument in the target discovery of bioactive small-molecules, such as drugs or natural products[1]. The identification of their macromolecular targets is required for a comprehensive understanding of their bio-pharmacological role and for unraveling their mechanism of action[1, 2]. Indeed, the target discovery of bioactive molecules endowed with intriguing pharmacological profiles is one of the main issues in the field of pharmaceutical sciences, since this is necessary for a rational development of potential drugs. Nevertheless, several bioactive compounds have been mainly evaluated for their pharmacological effects, whereas the exact mechanism of action at molecular level still remains unknown[3, 4].

Moreover, a complementary point of view about the effect of a small bioactive molecule on a cellular system can be given by label-based quantitative proteomic analysis[5]. Indeed, the identification of biologically relevant changes in the expression of proteins in a cell, after a treatment with a bioactive compound, could help to understand the exact mechanism of action of such active compound.

Here, we report the application of chemical proteomics to the analysis of the cellular interactome of three marine bioactive metabolites, all showing an intriguing anti-inflammatory pharmacological profile, and the application of quantitative chemical proteomics to the platelets activation mechanism by collagen. In more detail, the chemical proteomic approach was applied to Petrosaspongiolide M (PM)[6-8], Bolinaquinone (BLQ)[9-11] and Perthamide C (PRT)[12] target discovery. Thus, these molecules were immobilized onto agarose beads through an α,ω-diamino polyethylene glycol spacer. The

modified beads were then used as baits for fishing the potential partners of the bioactive compounds in macrophages cell lysate. The application of such technique allowed us to identify 20S proteasome, clathrin and endoplasmin (GRP94) as main partners of PM, BLQ and PRT, respectively.

Then, *in vitro* and *in cell* fluorescence assays were developed to assess the effect of PM onto the 20S proteasome enzymatic system, allowing us to measure the inhibition potency of this sesterterpene on the different proteolytic sites of the proteasome machinery.

The BLQ ability to modulate clathrin mediated endocytosis has been assessed through cytofluorimetric and microscopy analysis, suggesting a new application of BLQ as biotechnological tool in the modulation of trafficking pathways.

SPR technology has been employed to prove the ability of PRT to interact with GRP94 and Hsp90, opening the way to further investigations on the role of PRT in the modulation of heat shock protein functions.

Finally, we report the application of quantitative chemical proteomics to discover the effect of collagen on platelet activation. Since cAMP and cGMP plays a key role in platelet activation[13], we combined quantitative chemical proteomics approach with the specific enrichment of cAMP/cGMP signaling nodes[14], to investigate how PKA but also cGMP-dependent protein kinases (PKG) spatially reorganizes in activated human platelets.

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