Abstract

The identification of natural products and synthetic compounds target proteins is pivotal to understand their mechanism of action for the development of molecular probes and/or potential drugs. Functional proteomics is a mass spectrometry-based discipline focused on the analysis of the interactome of *small molecules* and their targets discovery. Functional proteomics has become an invaluable tool in targets identification of *small molecules* since *Fishing for Partners* strategy, also named affinity purification mass spectrometry coupled approach (AP-MS), successfully disclosed a multitude of bioactive compounds interacting proteins in the past 15 years (Rix and Superti-Furga, 2009).

Unfortunately, this strategy is not universally applicable being limited by the need of a covalent modification of the molecular probe that should contain at least one reactive chemical group and, most importantly, the compound modification should not influence its original bioactivity. Thus, an alternative functional proteomics platform, based on a combination of untargeted Drug Affinity Responsive Target Stability (DARTS) with targeted Limited Proteolysis coupled to Multiple Reaction Monitoring (t-LiP-MRM), has been exploited during my PhD project to disclose and characterize the interacting proteins of bioactive compounds (Lomenick *et al.*, 2009; Feng *et al.*, 2014).

At first, DARTS has been exploited to identify *small molecules* most reliable cellular partners, then t-LiP-MRM has been carried out to investigate the molecules/target proteins interaction features. Moreover, the proteomics results were validated by Western Blotting to confirm *small molecules* interaction with their DARTS-identified targets and by *in silico* molecular docking to corroborate t-LiP-MRM information about the target region(s) involved in the binding. For an in-deep analysis of the binding between the investigated compounds and their protein counterparts and for moving through the activity of such compounds on their putative targets, proper *in vitro* and/or *in cell* biological assays were also employed.

In particular, during this PhD project, MS-based proteomics approaches have been exploited to profile the interactomes of two natural compounds (Artemetin and Tatridin A), abundant in the extracts from *Achillea millefolium* (De Souza *et al.*, 2011) and from *Anthemis melanolepis* (Saroglou *et al.*, 2010), respectively, and a synthetic benzodiazepine derivative, called 1g (Parenti *et.al*., 2016). The interactomes of Artemetin, 1g and Tatridin A in HeLa, U87MG and THP-1 cells proteome have been examined unveiling, respectively, the Filamin A and Filamin B (crucial role in the organization of the cytoskeleton interacting with F-actin) (Zhou *et al.*, 2021; Xu *et al.*, 2017), the Brain Glycogen Phosphorylase (crucial role in the degradation of the glycogen clusters in the brain and in the regulation of the cellular glucose concentrations) (Mathieu *et al*., 2017) and the Phosphoglycerate Kinase 1 (crucial role in glycolysis) (Zieker *et al.*, 2010) as their principal cellular interactors.

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