Targeting of the cysteines surface domain of the Transcription Enhancer Associate Domain (TEAD) for anticancer drug discovery.

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The Hippo signalling is a metabolic control pathway of cells proliferation and apoptosis, thus it represents an emerging topic in tumour suppression regulation and regenerative medicine. This pathway is activated by anti-proliferative signals which come from the extracellular environment received by membranes proteins. In the cytosol compartment, the pathway is regulated by a phosphorylation cascade of four main proteins with Serin-Threonine kinase activity [1]. At the end-point of this cascade, the phosphorylation of YAP/TAZ paralogues proteins, act as TEAD1-4 transcriptional coactivators. TEAD Ser127 phosphorylation activates YAP/TAZ proteasomal degradation, and this prevents its migration to the nucleus for YAP:TEAD interaction, thus preventing the transcription of genes activating cell proliferation. hTEAD protein shows three binding surfaces, of which the lipid site is usually bound to palmitic/myristic acid. The functional roles of the interfaces are still unclear. It was estimated that 50% of solid tumor masses present a functional alteration of this pathway, therefore YAP/TAZ:TEAD interaction represents a valuable target for anticancer drug discovery [2]. Despite a large number of medicinal chemistry programs have been developed in the recent five years, the potency and biological profile is still limited, and further work is necessary to better understand the protein features and druggability before reaching the expected optimal lead inhibitors.

We started a program with the aim of better characterizing the protein reactivity and functional properties by developing in parallel molecular probes that may become early hits suitable for further drug discovery advances. We focused our studies specifically on TEAD4 protein, that shows three surface cysteines (Cys310, Cys335, Cys367, Cys410) that are not hindered when YAP is bound, but closeby to its binding area. Therefore, compounds directed to the cysteine area may suggest novel allosteric binding sites for a new inhibitor class. The specific focus of this study is the characterization of the biophysical/chemical properties of TEAD4, followed by cysteines tethering studies through disulphides compounds with structural diversity. This approach allows the preliminary exploration of the targeted region with a small compound library, before a large library screening approach. With this purpose, we performed a visual inspection of the available x-ray crystallographic structures of these cysteine regions. Then, through a UPLC coupled mass spectrometry assay we were able to identify a few small molecules capable of binding through a disulphide bridge the three mentioned cysteines. In particular, thiophenols and thio-nitrobenzoic acids showed some affinity for the recombinant protein and covalent disulphide bond formation. The protein exposure through the cysteines residues was confirmed by protein fragmentation analysis after trypsin digestion, by fluorescent probes derivatization by conjugation procedures and fluorescence spectroscopy and by HPLC-UV/Vis studies. Furthermore, we assessed that a larger part of the recombinant protein fragment undergoes myristoylation on Cys367 when expressed *in-vivo*. The presence of myristic acid in the lipid pocket allows the protection for the active site protein and permits a regioselective derivatization of the surface proteins with the disulphides. This finding supports the concept that a selective targeting of the TEAD surface cysteines can be achieved without sinking ligands in the lipidic site.

The preparation of the first large library screening is ongoing in parallel with the assay development for affinity ligands binding detection.

[1] Santucci M, Vignudelli T, Ferrari S, Mor M, Scalvini L, Bolognesi ML, Uliassi E, Costi MP. The Hippo Pathway and YAP/TAZ-TEAD Protein-Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment. J Med Chem. 2015 Jun 25;58(12):4857-73.
[2] Yueh C, Rettenmaier J, Xia B, Hall DR, Alekseenko A, Porter KA, Barkovich K, Keseru G, Whitty A, Wells JA, Vajda S, Kozakov D. Kinase Atlas: Druggability Analysis of Potential Allosteric Sites in Kinases. J Med Chem. 2019 Jul 25;62(14):6512-6524.

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