Structural insight into YAP-TEAD4 protein-protein interactions as target for cancer treatment

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The Hippo pathway is a signalling network which plays a key role in tissue homeostasis and organ size control, by regulating cell growth, proliferation and apoptosis. Once activated, the signalling transduction involves a core kinase-cascade, resulting in the phosphorylation, cytoplasmic retention and subsequent degradation of the Yes-associated protein (YAP). YAP is a DNA transcription co-activator without an intrinsic DNA binding domain, which, in its hypo-phosphorylated status, translocates into the nucleus and directly interacts with several DNA-binding partners. In mammalian cells, YAP primarily binds all four transcriptional enhancer associate domain (TEAD1-4) family members [1]. Since TEADs transcription factors are, in turn, unable to induce gene transcription, the interaction between YAP and TEAD is essential for the expression of Hippo pathway-downstream genes, involved in cell proliferation and apoptosis [2]. All four human TEADs (hTEADs) allocate an acylation binding site that is occupied by palmitic/myristic acid in physiological condition, but the influence of TEAD acylation on YAP:TEAD interaction is yet not fully understood [3]. Dysregulations of the Hippo pathway are associated with tumorigenesis, thus targeting YAP:TEAD interaction is an emerging, attractive therapeutic strategy in the oncology field [4]. To date, very few YAP:TEAD4 inhibitors have been reported and the development of new molecules targeting this protein complex remains challenging [5]. Structural information is missing and more work is necessary to contribute to function and ligand design.

Here, we report the development of reliable protocols for co-expression, co-purification and crystallization of the TEAD-binding domain (TBD) of human YAP (hYAP) (fragment 50-171) in complex with the C-terminal YAP-Binding Domain (YBD) of hTEAD4 (residues 217-434). Crystals of the hYAP-hTEAD4 complex were obtained using the microseeding crystallization technique, leading us to obtain the first structural characterization of the de-acylated state of this complex. The overall structure of de-acylated TEAD4 closely resembles that of the acylated protein in agreement with the biochemical and cellular assays of Mesrouze et al [3], assessing that acylation is not required for the interaction with hYAP, but it contributes to TEAD4 stability. However, some differences have been detected in the conformation of hYAP1 and in the orientation of its flexible N-terminal region.

Our investigation aims to the unveil the mechanisms regulating YAP:TEAD4 protein-protein interactions to support the rational design of new TEAD4 binder, preventing the formation of the complex.

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