Expression, purification, and characterization of Transcriptional Enhancer Associated Domain (hTEAD4), a promising target for anticancer agents

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Protein preparation for a broad technological application represents one of the highest expertise requests in the biotechnology research and application. Our work is focused on the optimization process for the preparation of a novel non-catalytic protein from the Hippo Pathway, necessary for anticancer research studies.
The Hippo Signalling pathway represents an emerging topic in tumour suppression regulation and regenerative medicine. The pathway is activated by extracellular anti-proliferative signals and is finely regulated by a cytosolic phosphorylation cascade of four main proteins with serin-threonine kinase activity, LATS, MOB, MST1/2 and YAP (YES Associated Protein). The downstream phosphorylation of YAP/TAZ paralogues proteins act as TEAD1-4 (Transcriptional Enhancer Associated Domain 4) transcriptional coactivators, while YAP Ser127 phosphorylation activates YAP/TAZ proteasomal degradation, thus preventing its migration to the nucleus for YAP-TEAD interaction, and the transcription of genes activating cell proliferation [1].

Despite being a promising pharmaceutical target, the disruption of YAP-TEAD complex is still under preliminary screenings, and further investigations are necessary to better understand how the pathway can be inhibited. Moreover, the difficulty in obtaining enough recombinant protein is a limiting factor in Hippo Pathway drug discovery and there is a need to optimize its preparation process. This requires the exploration of different purification technologies. Herein, we have developed an efficient purification protocol transforming ArcticExpress (Agilent) competent cells, able to grow at low temperatures, with pET15b plasmid encoding for 6xHis-Tagged hTEAD4 YAP binding domain (aa 217-434). The transformed cells are grown at low temperature in autoinducing enriched medium for 60h. The target protein is purified by the harvested cells in a four-step protocol consisting of two His-trap nickel-affinity chromatography and two desalting steps. The overall yield is 40mg/L of bacterial culture.

Protein is characterized with LC-MS in its acylated and non-myristoilated form. PTM's (phosphorylation) are mapped through bottom-up sequencing after trypsin hydrolyzation. Having no catalytic activity, its correct folding has been validated proving its ability to bind a YAP-mimicking peptide [2] with a FRET assay (Förster resonance energy transfer). The assay requires the conjugation of both purified hTEAD4 and YAP-mimicking peptide with fluorescence probes, fluorescein and tetramethyrhodamine respectively, on two exposed and reactive cysteines. The complex formation is confirmed by the emission of tetramethylrhodamine due to non-radiative energy transfer, allowed by the proximity of the probes, once fluorescein is excited.

Furter steps will include the development of a YAP-TEAD displacement assay to test the ability of a library of molecular disrupters to dissociate the complex.

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