ADVANCES IN THE SET-UP OF A FLUORESCENCE-ANISOTROPY ASSAY FOR THE SEARCH OF NOVEL INHIBITORS OF THE TEAD-4 COMPLEXES

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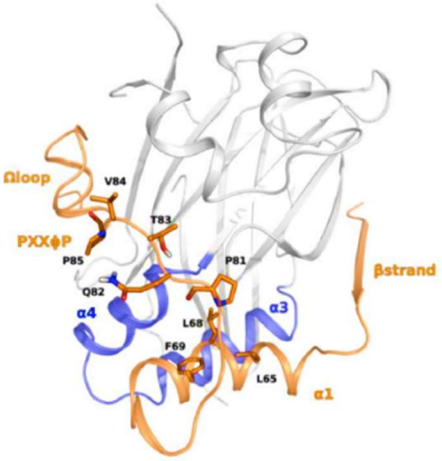
Background. TEAD-4 is a protein of the transcriptional enhancer factor family known as TEA (TEAD-4 = TEA domain family member 4). This is found at the end of the Hippo pathway; when activated, it shows antiproliferative and proapoptotic properties. Such a pathway is made of consecutive cytoplasmatic kinases, whose main feature is the phosphorylation, from Lats1/2, of a regulative protein known as YAP. This happens every time the Hippo pathway is activated. Should this be inactive, YAP would be able to migrate inside the nucleus, bind TEAD-4 and activate it, allowing transcription of genes regulative of cellular proliferation. This pathway is fundamental in regulating the growth of mammalian organs; a small change in this process (such as changes to the main proteins responsible for the kinasic core) leads to a failure in phosphorylation of YAP, thus allowing solid tumor formation to happen. The main objective of this study is to interfere with the interaction between YAP and TEAD to halt cancer progression[1].

YAP interacts with TEAD with three main mechanisms: a strand-strand interaction (antiparallel β-sheet), a triple α-helix with a highly conserved *LXXLX* motif and a twisted-coil region that comprises an Ω loop, which occupies a highly hydrophobic region[2].Therefore, TEAD-4 inhibitors represent a promising therapeutic strategy to address unmet medical needs in antiblastic medicine, above all colorectal adenocarcinoma, breast cancer and falloppian tube carcinoma. Very few inhibitors have been published and are available for drug discovery development.



Objectives of the present project is the discovery and development of new TEAD binders affecting YAP-TEAD interactions showing anticancer activity. Within the project a novel HIT series was identified. The communication topic is related to TEAD recombinant protein extraction and purification and to the target-inhibitor interaction assay set-up.

Results. For protein production, competent BL21 *E. coli* cells, were transformed with the *PGEX* plasmid and harvested in an adequate growth medium, then treated with isopropyl *β d-1-* thiogalactopyranoside to induce GST-TEAD4 transcription through lac operon activation. The protein suspension in the cell lysate obtained by sonication was submitted to an *FPLC* purification using *GTS-HiTrap™FF*, followed by cleavage by thrombin to separate the recombinant protein from the GST tag. The collected eluate was run on SDS page to evaluate the amount of tag free TEAD. Although a small amount of target protein was recovered, this was characterized and a fluorescence-ansotropy displacement assay was set-up and used on the GST-TEAD4 complex and then on the purified TEAD4, after thrombin linker hydrolysis.

Conclusions and future developments. The results obtained show a higher amount of TEAD4 protein obtained, after thrombin cleavage compared with previous purification experiments. The first step to set up the displacement assay was successful as it shows a concentration dependent increase of anisotropy when the protein was added to the fluorescent inhibitor S049 in the sample cell. This

REFERENCES

[1] Smith A.S., R. B Sessions et al. *Antiproliferative and antimigratory effects of a novel YAP-TEAD interaction inhibitor identified using in silico molecular docking,* Journal of Medicinal Chemistry, 2019; 62,3, 1291-1295

[2] Elisi G. M., Santucci M, D'Arca D, Lauriola A, Marverti G, Losi L, Scalvini L, Bolognesi M. L, Mor M, Costi M.P, *Repourposing of Drugs Targeting YAP-TEAD Functions*, Cancers 2018 Sept 14, 10(9)

indication may suggest an effective binding between the two molecules, which however will need further experiments for confirmation and will be presented in the poster presentation.