Molecular and functional characterization of TEAD family members in pleural mesothelioma

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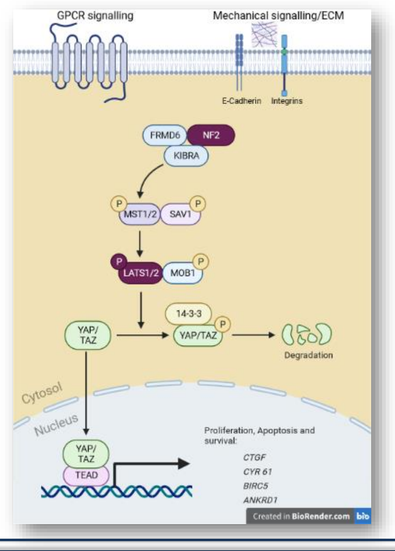
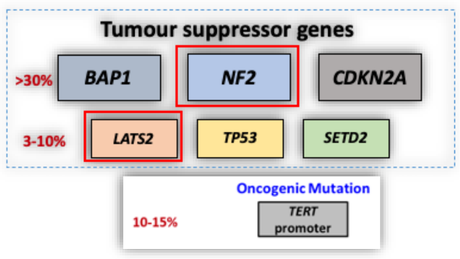
Background

Pleural mesothelioma (PM) is a heterogeneous cancer with a high medical need to identify novel therapeutic targets. The transcription factors of the TEAD family (TEAD1 to TEAD4), are the key transcriptional modulators of the Hippo signalling pathway. They show oncogenic properties by regulating cell growth, proliferation and invasiveness in several cancers. In PM, frequent inactivation of the Hippo pathway, notably by genetic alterations in genes like *NF2* or *LATS2*, is likely to be associated with increased oncogenic activity of TEAD.

Hippo signalling pathway



Main mutated genes in PM



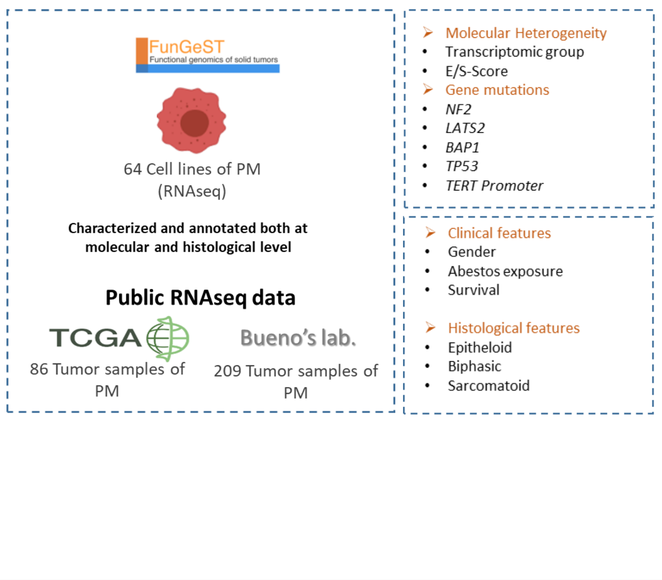
Aim

1. Determine the expression of the four different TEAD protein family members (TEAD1-TEAD4) in PM.
2. Identify which TEAD isoform is the main driver of PM cell growth.
3. Understand the overlap and the redundancy of TEAD family members.

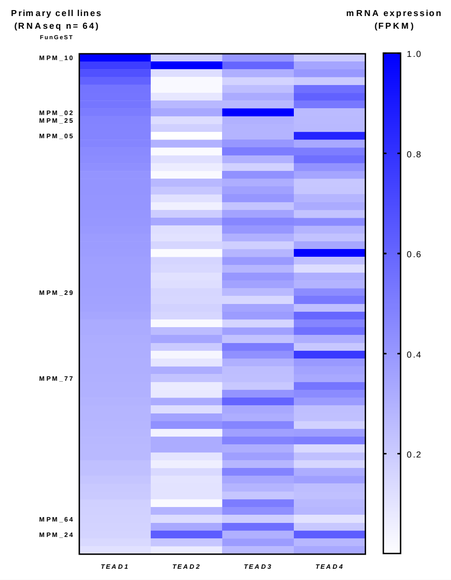
Methodology

RNA-seq data from PM primary cell lines (n=64), well annotated at the molecular and histological level, and public datasets (TCGA and Bueno's lab) were analysed to identify potential associations with clinical features, histological and molecular phenotypes of PM tumours (**A**). To study the effects of TEADs downregulation *in vitro*, we performed specific knock-down (KD), using RNA interference with two specific siRNA of each TEAD isoform, in eight primary PM cell lines, selected according to *TEAD1-4* expression profile (**B**). Gene expression of *TEAD1-4* and TEAD target genes (*CYR61, CTGF, ANKRD1*) was monitored by RT-qPCR, and PM cell proliferation was measured using a high content screening system (Operetta®, PerkinElmer) after nuclei Hoechst staining. We also performed comparative studies using the commercial TEAD inhibitors K-975 (panTEAD inhibitor) and VT103 (TEAD1 inhibitor).

A



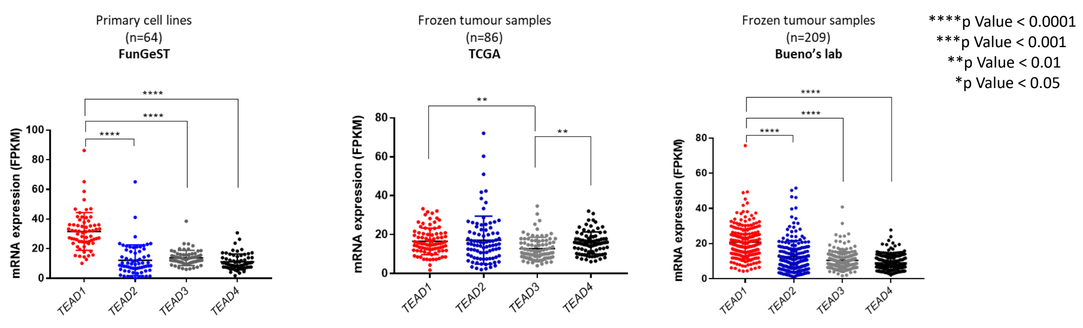
B



Results

TEAD expression

A



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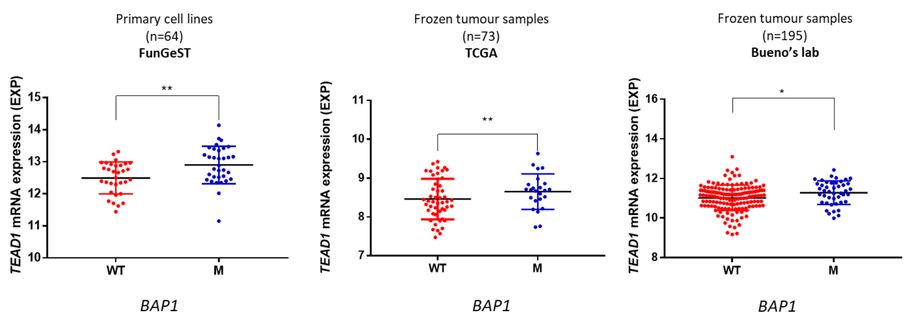
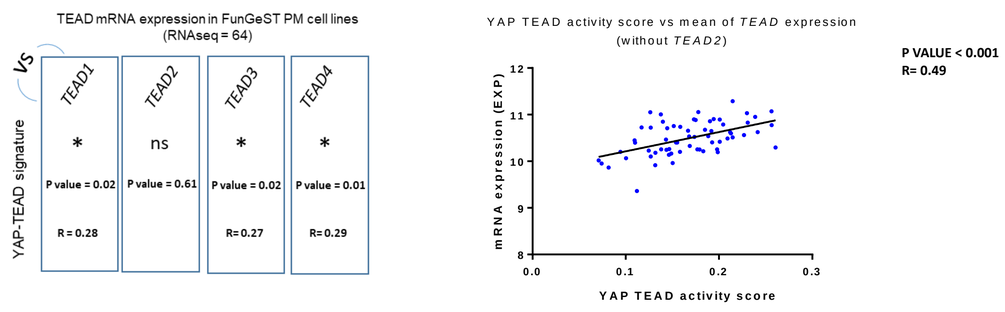


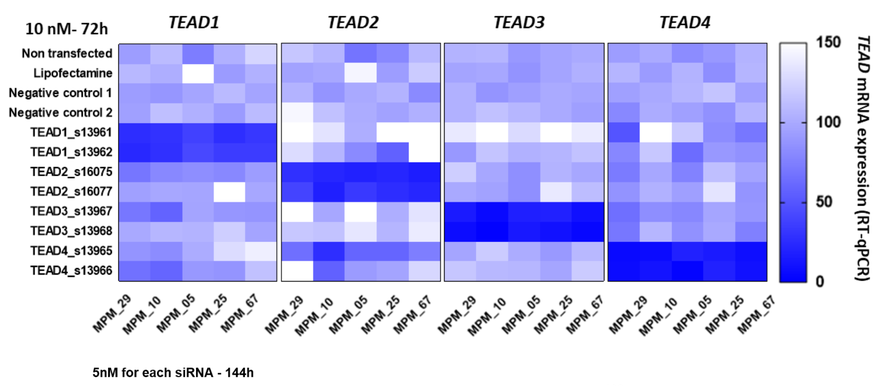
C



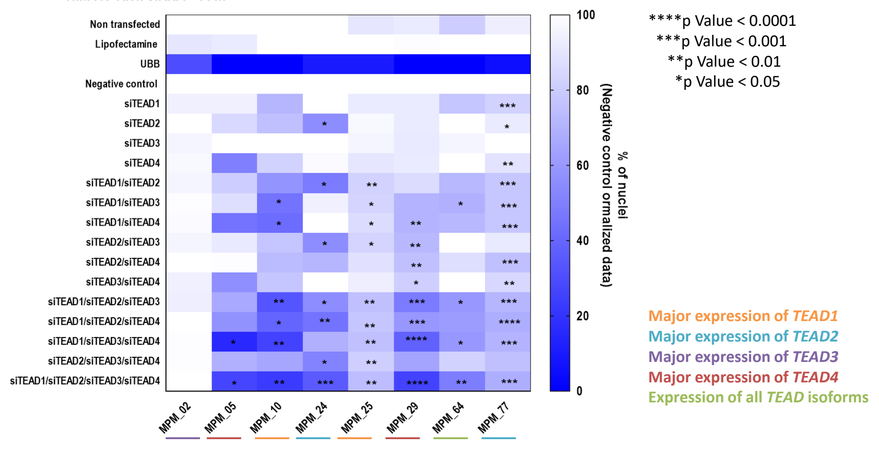
RNA-seq data showed a higher expression of *TEAD1*, compared to the other isoforms (**A**) and only *TEAD2* and *TEAD3* gene expression are correlated (data not shown). No strong correlation between *TEAD* expression and clinical features was observed (data not shown). However, we noted a higher expression of *TEAD1* for *BAP1* muted cell lines and tumours in all series (**B**). Interestingly, a correlation was observed between a YAP-TEAD activity signature *(Calvet et al 2022)* and *TEAD1*, *TEAD3* and *TEAD4* gene expression (**C**).

TEAD downregulation

A



B



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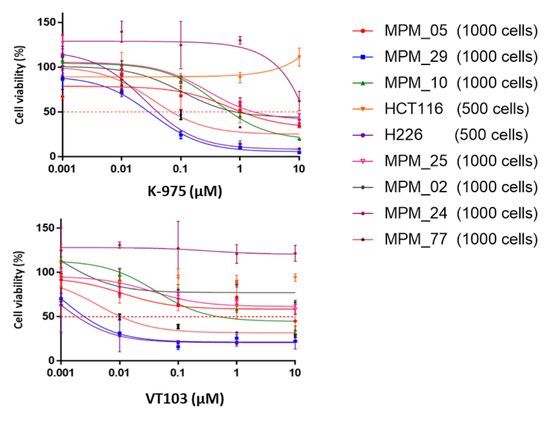
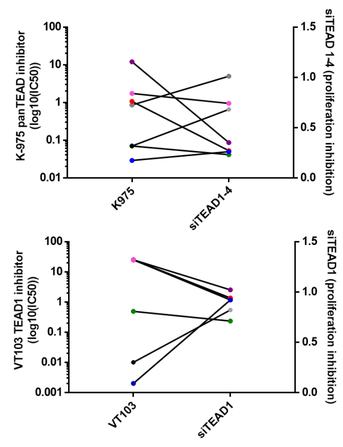


D



TEAD siRNAs induced a strong and specific inhibition of the mRNA expression (**A**), but did not change the expression of TEAD target genes (data not shown). Furthermore, single KD had no impact on cell proliferation of PM cell lines. However, KD combining several TEAD isoforms decreased significantly cell proliferation in a large number of cell lines when targeting three or four TEAD isoforms together (**B**). Inhibitory effect of a given TEAD KD is related to its gene expression level.

Pharmacological TEAD inhibition using K-975 and VT103 inhibitors shows a variable sensitivity in PM cell lines (**C**), not consistent with the inhibitory effect of KD in all cell lines (**D**).

Conclusion

The specific KD of a single *TEAD* does not seem to affect the global TEAD transcriptional activity and the cell proliferation of PM cells. Targeting a combination of *TEAD* isoforms is more effective. Additional analyses to determine the contribution of each *TEAD* isoform are ongoing by generating a knock-out models using CRISPR-Cas9 in primary PM cell lines.

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