Predicting cancer cell response to TEAD auto-palmitoylation inhibitor using bulk RNA-seq data and a random-forest based algorithm

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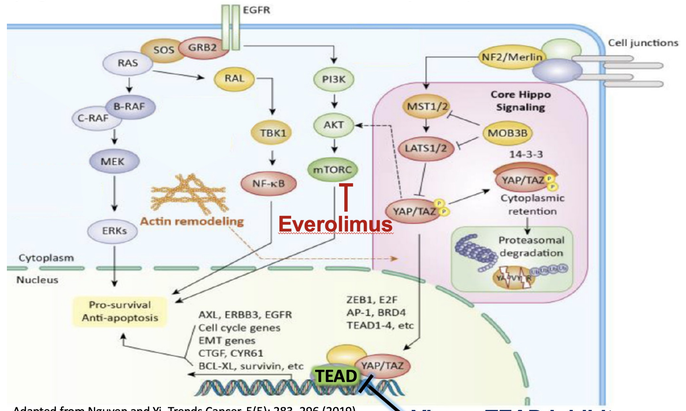
Poster #5403

Abstract

TEAD transcription factors are the major effectors of the Hippo-YAP/TAZ pathway essential in controlling organ size and maintaining tissue homeostasis. It has been shown that auto-palmitoylation is required for TEAD interaction with YAP/TAZ coactivator and hence activation of transcriptional activity. Potent small molecule TEAD auto-palmitoylation inhibitors have been reported (Tang *et al*, 2021)[1]. These TEAD inhibitors disrupt YAP/TAZ-TEAD protein interaction, suppress TEAD transcriptional activity, and selectively block proliferation of *NF2*-deficient mesothelioma *in vitro* and inhibit *NF2* mutant xenograft tumor growth *in vivo*. Although genetic alterations of pathway components (such as *NF2*) leading to TEAD activation have been reported in a variety of human malignancies, these alterations are infrequent in most cancers. In order to find potential responders to treatment with TEAD inhibitors within cancers without Hippo-YAP pathway mutations, we developed a custom data-processing and normalization pipeline to process publicly available RNA-seq datasets and train a random-forest based classifier using *in vitro* efficacy data from cell line screens. The algorithm showed early promise by classifying a model with *in vivo* efficacy as the most likely to respond.



Adapted from Nguyen and Yi, Trends Cancer. 5(5): 283–296 (2019)

1. Tang et al. Mol Cancer Ther. 2021;20(6):986-998.
2. Qiu *et al*, 2019, *Cancer Cell* 36:179–193.

Methods and Datasets

Our random-forest classifier was originally trained on the public bulk RNA-seq pan-cancer *in vitro* dataset available in the Cancer Cell Line Encyclopedia (CCLE), using privately obtained TEADi response data for a subset of 252 CCLE cell lines. Binary response labels (responder/non-responder) for each cell line were obtained by thresholding efficacy measures (IC50). Afterwards, from our screen of 50 patient-derived Chinese liver cancer (CLC) cell models (Qiu *et al*, 2019)[2], both as single agent and in combination with mTOR inhibitor everolimus, we found that TEAD inhibitors (TEADi) were efficacious in several of these liver cancer models. This allowed us to verify the classifier without any re-training on an independent dataset (CLC). Having established efficacy of predictions on both *in vitro* datasets, we re-trained the classifier using both CCLE and CLC datasets as input to achieve maximum predictive performance, we predicted probability of response for 2056 patient-derived xenograft models and tested the top candidate *in vivo*.

Definition: %Inhibition = {1 - [(Lsample - medium control) - (T0 - medium control)]/[(vehicle control - medium control) - (T0 - medium control)]}×100%. Lsample = the reading of drug treatment group; vehicle control = the mean value of solvent treatment group; medium control = the mean value of blank control; T0 = the day compound was added. IC50 = compound centration at 50%inhibition.

Summary and Conclusions

- **TEAD inhibitors show great promise in blocking proliferation of NF2-deficient mesothelioma.**
- **In order to explore indications beyond mesothelioma, a random-forest classifier was trained using normalized expression data from the CCLE and CLC datasets together with response data for a subset of the cell lines. The model showed efficacy of predictions with an AUC of 0.8 and 0.82, respectively.**
- **The model was retrained using both datasets together and used to predict probability of response for 2056 xenograft models.**
- **The top candidate was tested *in vivo* and showed significant response to treatment. It is relevant to mention, however, that the tested candidate had already shown response as a cell line, which the bioinformatician performing the analysis was not blinded to.**
- **Although these results are encouraging, in order to get a statistically significant result it would be necessary to test more models from the pool of top candidates *in vivo*.**

Sensitivity of cancer cell lines to TEAD inhibitors

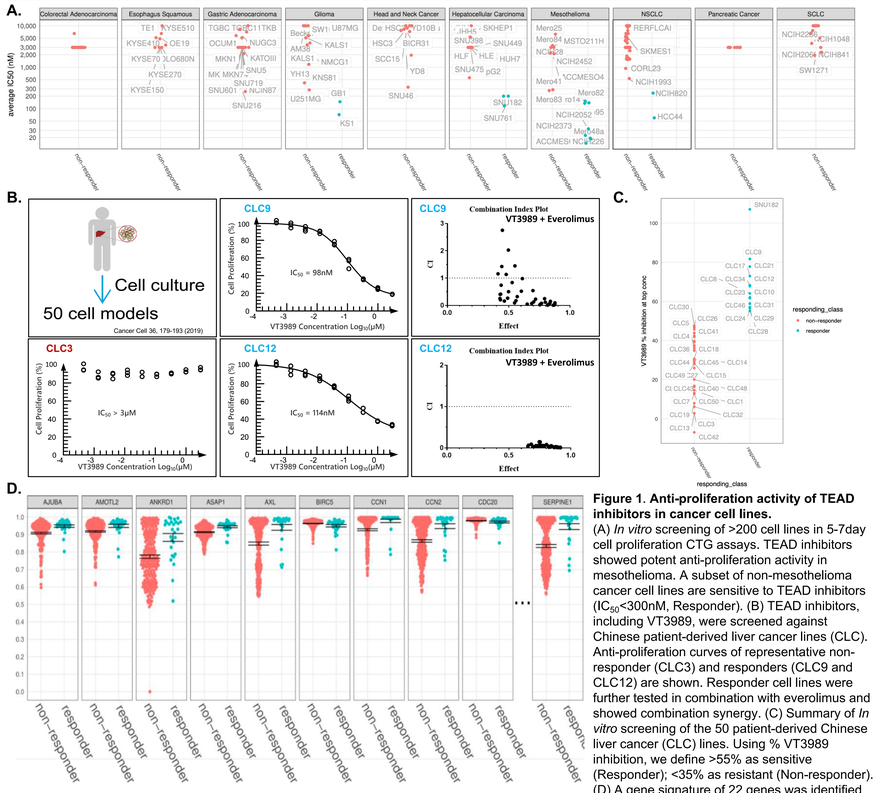


Figure 1. Anti-proliferation activity of TEAD inhibitors in cancer cell lines.
(A) *In vitro* screening of >200 cell lines in 5-7day cell proliferation CTG assays. TEAD inhibitors showed potent anti-proliferation activity in mesothelioma. A subset of non-mesothelioma cancer cell lines are sensitive to TEAD inhibitors (IC_{50}<300nM, Responder). (B) TEAD inhibitors, including VT3989, were screened against Chinese patient-derived liver cancer lines (CLC). Anti-proliferation curves of representative non-responder (CLC3) and responders (CLC9 and CLC12) are shown. Responder cell lines were further tested in combination with everolimus and showed combination synergy. (C) Summary of *In vitro* screening of the 50 patient-derived Chinese liver cancer (CLC) lines. Using % VT3989 inhibition, we define >55% as sensitive (Responder); <35% as resistant (Non-responder). (D) A gene signature of 22 genes was identified using RNA-seq data and defined drug sensitivity.

Note: Previously, silence of Hippo [SOH] signature consisting of 610 genes was shown to be significantly associated with poor prognosis in human HCC (Sohn *et al.* 2016. Clin Cancer Res.22(5): 1256–1264).

Drug response algorithm

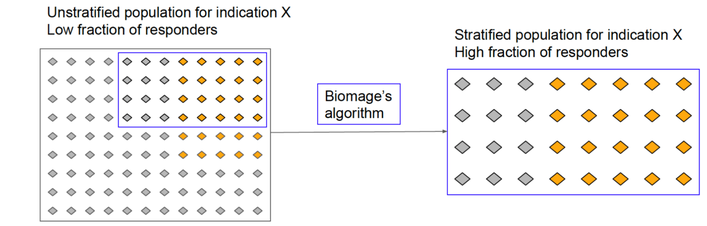


Figure 2. BioMage's drug response algorithm
In an unstratified population for the studied indication it is expected that fewer individuals will respond to the treatment. The goal of the algorithm was to stratify the population increasing the overall fraction of responders. Even though there might be a potential loss of total responders in the stratification process, the expectation is that the probability of selecting those individuals from the stratified population will be higher.

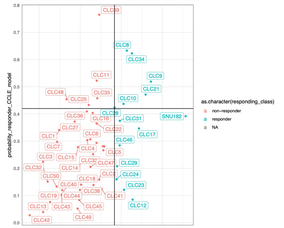
Algorithm predictions on Chinese Liver Cancer (CLC) data

A. Original response ratio

(TP + FN)/(#Samples) = 27%

Biomage response ratio

TP/(TP+FP) = 55%



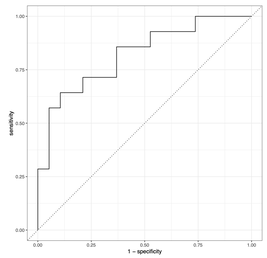
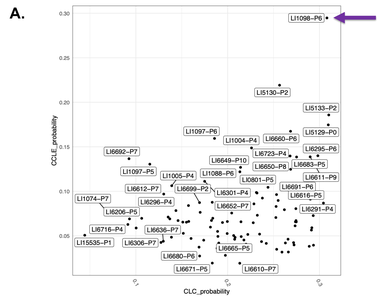


Figure 3. Prediction of TEAD inhibitor efficacy in cancer cell lines.
(A) A random forest classifier was trained using the CCLE dataset with response data obtained from 252 cell lines subjected to the treatment. The response variable was defined by the IC_{50} values in anti-proliferation assays (IC_{50}<300nM = Responder). The classifier was then applied to 51 cell lines of the CLC dataset and probability of response was calculated. The response variable was defined as probability of VT3989 % inhibition > 55%. (B) A ROC curve was generated for the Random Forest model, showing that our model outperforms random chance with an AUC of 0.82.

Efficacy demonstrated in a HCC patient-derived xenograft model



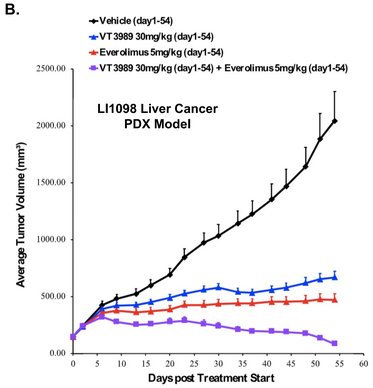


Figure 4. Validating prediction model with in vivo study
(A) A new model was then trained using both the CCLE and CLC training datasets and applied to gene expression data obtained from the Crown Bioscience portal for PDX models. (B) The top candidate was selected and tested *in vivo*. VT3989 showed significant anti-tumor efficacy in the selected model. In addition, combination with everolimus showed enhanced efficacy.

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