Mesothelioma biology (including pre-clinical models) and novel targets

PO-162

Signaling pathways analysis highlight YAP and TAZ contribution in pleural mesothelioma

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Background

- Pleural Mesothelioma (PM) → strong need to uncover mechanisms contributing to the development/maintenance of PM in order to find new therapeutic targets.
- Signaling pathways → crucial for tumor growth and interesting targets for therapy.

Aim: Investigate signaling pathways involved in PM proliferation according to their histological and molecular characteristics.

Methodology

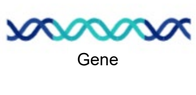
- Functional screens using siRNA knockdown (KD) on proliferation of PM low-passage patient-derived cell lines
- Proliferation evaluated by counting the number of nuclei with a high content screening (HCS) system.
- Transcriptomic changes induced by siRNA KD analyzed by RT-qPCR and 3' RNA-seq.
- Effects on cell cycle and apoptosis evaluated using an EdU test on a HCS system and annexin V and propidium iodide labeling by flow cytometry, respectively.

First cell proliferation screen

19 pathways

MAPK | PI3K/Akt/mTor | Hippo | Wnt/β-catenin | TGFβ | Jak/Stat | Hedgehog | SAPK/JNK | Src | Notch | FAK | TP53 | AMPK | PKC | NRF2 | NF-κB | Cell Cycle | Autophagy | Adherens Junction + Oncogenes linked to S.score

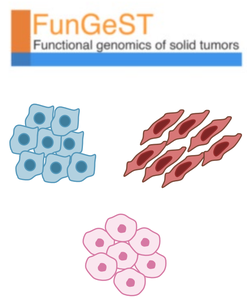
56 candidate target genes X 22 cell lines



Knockdown of **single gene** by 3 independent siRNA

→ 6 candidate target genes with **only moderate effect on proliferation**

Biobank of **cell lines** representative of heterogeneity:



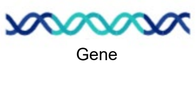
- Clinical annotations
- Histological type
- Mutational Status (targeted DNA-seq)
- Transcriptomic data (RNA-seq)
- Transcriptomic subtypes C1/C2[1]
- Histo-molecular gradient (E/S.score)[2]

1. de Reynies et *al.* (2014)
2. Blum *et al.* (2019)

Second cell proliferation screen

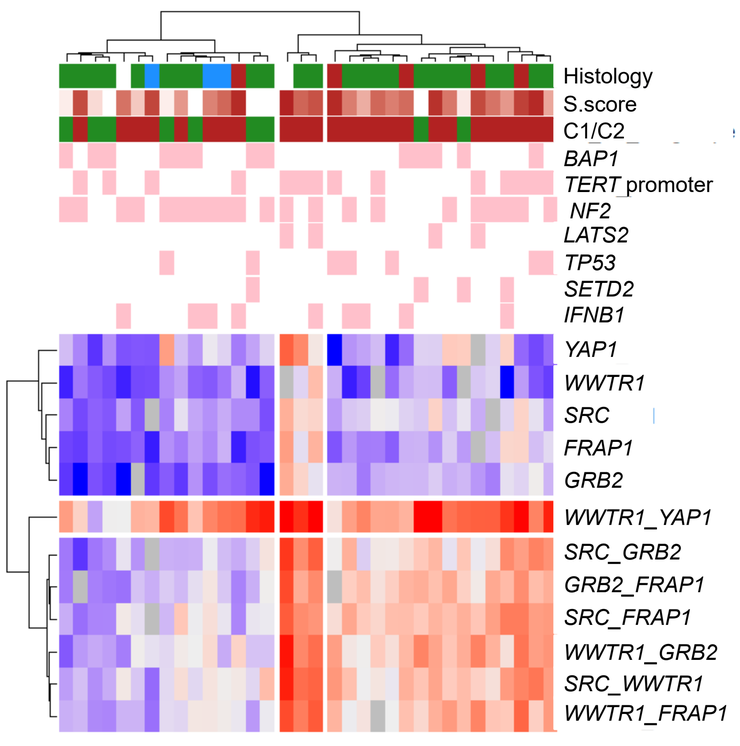
Pathway	Gene
Src	*SRC*
Hippo	*YAP1*
	WWTR1
MAPK	*GRB2*
mTor	*mTOR (FRAP1)*

5 candidate target genes X 34 cell lines



Knockdown of **two different genes** by 2 independent siRNA

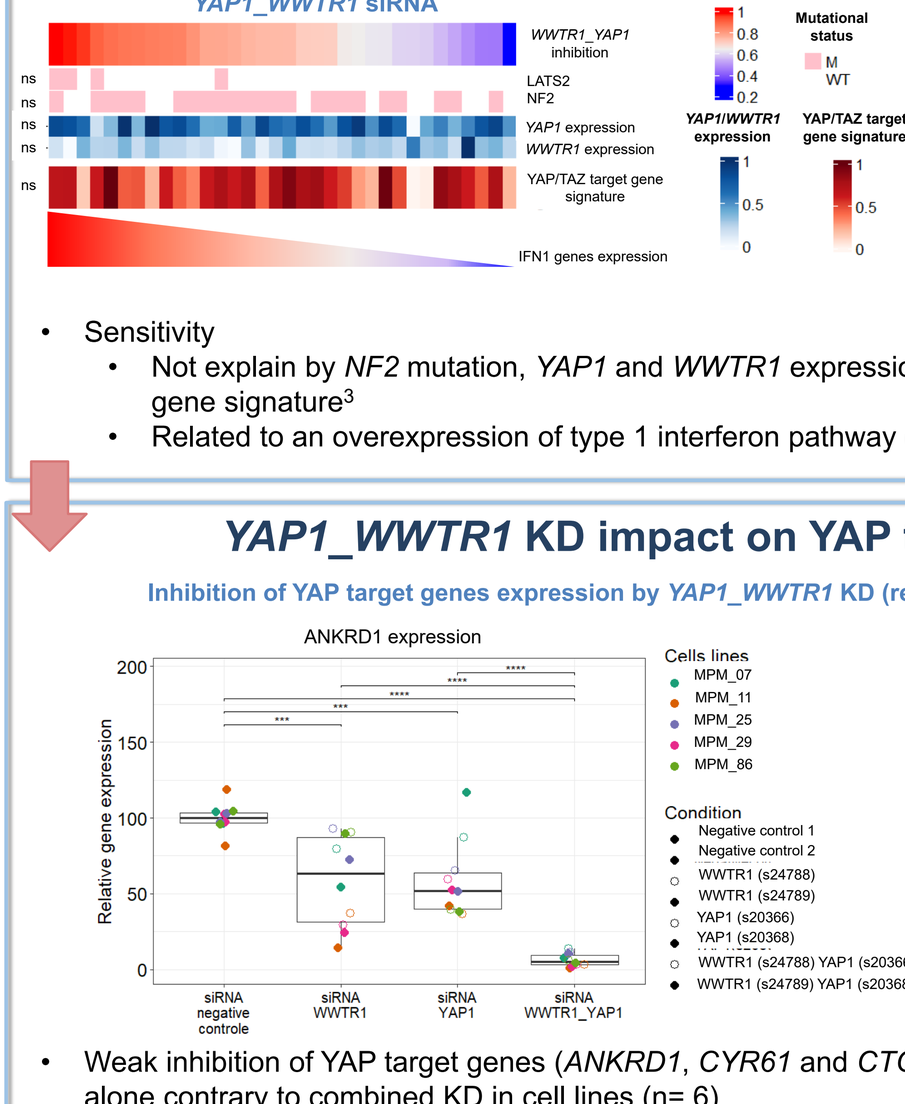
Unsupervised hierarchical clustering of the proliferation inhibition data



- siRNA in combination → better proliferation inhibition than siRNA alone.
- Two clusters of cell lines based on their sensitivity : C2 cell lines more sensitive than C1 (Fisher test, p.value = 0.025).
- **siRNA-*YAP1_WWTR1*** targeting YAP and TAZ transcription cofactors → strongest overall inhibition of proliferation and broadest sensitivity spectrum

Prediction of proliferation inhibition by *YAP1_WWTR1* KD

Cell lines ordered depending of the sensitivity to *YAP1_WWTR1* siRNA



RNA-seq data → 334 genes: overexpressed in sensitive → Enrichment type 1 interferon pathways

- Sensitivity
  - Not explain by *NF2* mutation, *YAP1* and *WWTR1* expression or YAP/TAZ target gene signature[3]
  - Related to an overexpression of type 1 interferon pathway (IFN1) genes

3. Yumeng Wang et *al.* (2018)

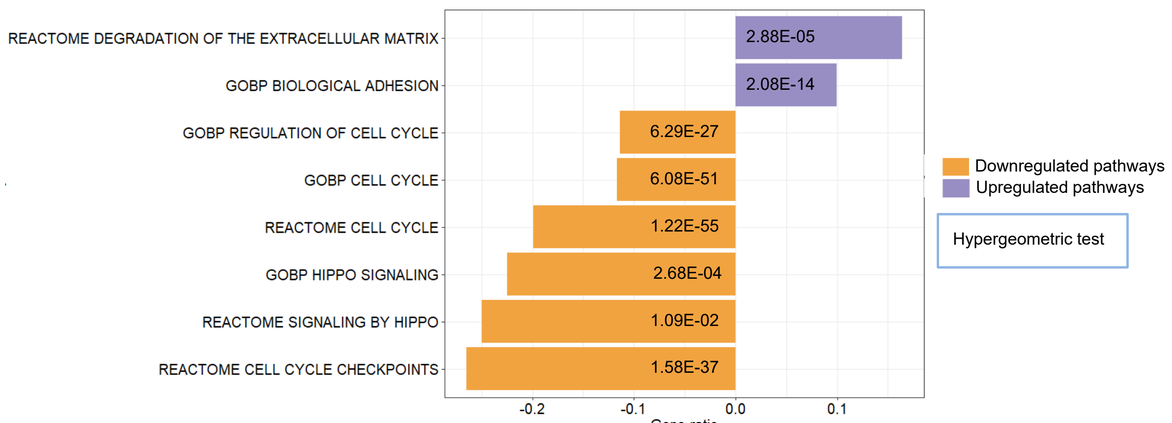
YAP1_WWTR1 KD impact on YAP target genes

Inhibition of YAP target genes expression by *YAP1_WWTR1* KD (representative experiment)

- Weak inhibition of YAP target genes (*ANKRD1*, *CYR61* and *CTGF*) by *YAP1* or *WWTR1* KD alone contrary to combined KD in cell lines (n= 6)
- **Need to target both to strongly inhibit YAP target genes**

Transcriptomic changes induced by *YAP1_WWTR1* KD

Pathway enrichment



- Decrease of cell cycle pathways by *YAP1_WWTR1* KD

GOBP: Gene ontology biological process

Effect on cell cycle/apoptosis of *YAP1_WWTR1* KD

Cell cycling test

Anova test
p.value = 1.35E-12

Tukey test
* p.value < 0.05
** p.value < 0.01
*** p.value < 0.001
**** p.value < 0.0001

Cell mortality test

Anova test
p.value = 9.00E-03

Tukey test
* p.value < 0.05
** p.value < 0.01
*** p.value < 0.001
**** p.value < 0.0001

- Inhibition of cell in cycle and induction of apoptosis by *YAP1_WWTR1* KD

Conclusion

Our findings:

- Confirm the contribution of the Hippo pathway and the downstream transcriptional cofactors YAP and TAZ in PM proliferation.
- Highlight that inhibition of both YAP and TAZ is more effective in inhibiting PM proliferation.
- Show the downregulation of the cell cycle and the upregulation of apoptosis by targeting YAP and TAZ.
- Support the redundancy between YAP and TAZ in PM.