Genome-wide CRISPR/Cas9 screen for the identification of novel YAP1/TAZ modulators

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INTRODUCTION

- Yes-associated protein 1 (YAP1) and the WW domain containing transcription regulator 1 (TAZ/WWTR1) are developmentally regulated Hippo-pathway effectors. Their activity is essential for the growth of whole organs, amplification of tissue-specific progenitor cells during tissue renewal and regeneration and cell proliferation.
- In cancer, YAP1/TAZ are frequently aberrantly activated through Hippo pathway mutations, focal amplification or perturbations in other upstream regulators. In the nucleus, YAP1/TAZ interact with transcription factors including TEADs, activating the expression of genes involved in oncogenic signaling.
- To identify novel regulators of YAP1/TAZ, we established a novel screening system monitoring YAP1/TAZ activity in Hippo pathway mutant MDA-MB-231 breast cancer cells modified to express luciferase (luc) under control of TEAD promoter binding-sites.
- We performed a pooled genome-wide CRISPR/Cas9 knockout screen to identify novel YAP1/TAZ regulating genes. Functional characterization of the novel candidate YAP1/TAZ modulators will aid to the further understanding of YAP1/TAZ biology in health and disease

METHODS

- MDA-MB-231 breast cancer cells were modified to express a YAP1/TAZ dependent TEAD-luciferase reporter that can be detected using a newly developed flow cytometry-based assay (Fig. 1A, B). Fixed and permeabilized cells were stained for firefly luciferase expression using an anti-firefly luciferase antibody (EPR17790, Abcam) followed by staining with goat anti-rabbit Alexa Fluor 488 secondary antibody (Thermo). Luciferase activity was measured using the Dual-Glo Luciferase Assay System (Promega).
- Treatment of cells with Cytochalasin D caused dose-dependent inactivation of the luciferase reporter (Fig. 1C). This was detected at comparable sensitivity by both luciferase activity assay and flow cytometry, indicating that flow cytometry-based detection of luciferase protein expression is a viable method to quantify luciferase activity (Fig. 1C).

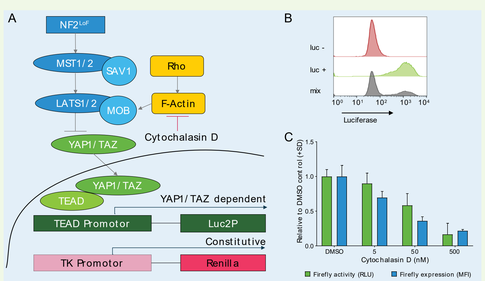

Fig. 1: Establishment of flow cytometry-based detection of luciferase protein to enable a pooled CRISPR screening approach

- To detect YAP1/TAZ translocation, cells were fixed, permeabilized and stained with anti-YAP1/TAZ antibody (Santa Cruz, sc-101199) and Alexa Fluor® 488 Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-546-062).

RESULTS

Pooled whole genome CRISPR/Cas9 screening strategy

- MDA-MBA-231 cells were transduced with a three-module whole genome 150k single guide RNA (sgRNA) CRISPR knockout lentiviral library (Cellecta) targeting the entire human genome at a multiplicity of infection of 0.3 (Fig. 2). Cells were selected for viral integration using puromycin.
- After 14 days of knockout generation, cells were harvested, stained for luciferase expression and cells with the highest and lowest (10%) luciferase expression were collected by flow cytometry sorting. Genomic DNA was isolated and subjected to next-generation sequencing to identify integrated sgRNAs.

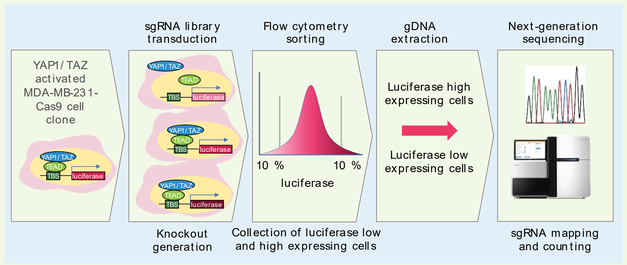

Fig.2: Strategy for pooled whole genome CRISPR/Cas9 YAP1/TAZ pathway screen

Whole genome CRISPR/Cas9 screen identifies known and novel YAP1/TAZ regulators

- The pooled whole genome CRISPR screening identified positive and negative regulator genes for YAP1/TAZ activity, including previously known ones such as TAOK1, AJUBA, PTPN14, LATS2, RHOA, RAC1 and TEAD1 (Fig. 3A, B).

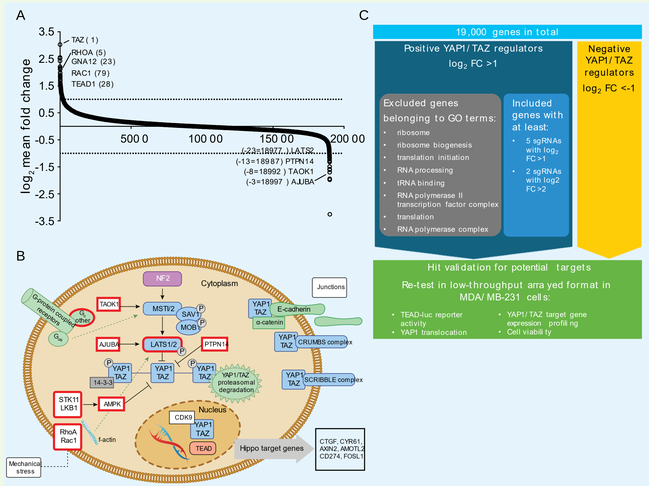

Fig.3: Pooled whole genome CRISPR/Cas9 screen identifies known and novel YAP1/TAZ regulators A) Mean log2 fold changes of sgRNAs targeting genes found in sorted cells with "low" vs "high" expression of luciferase. Annotated are known regulators of YAP1/TAZ signaling from the hit list. B) Signaling pathways regulating YAP1/TAZ activity. C) workflow for the selection and validation of candidates from the screen.

Validation of whole genome CRISPR/Cas9 screen hit results

- YAP1/TAZ cellular localization, TEAD-Luciferase activity and endogenous YAP1/TAZ target gene measurements of individual CRISPR/Cas9 knock-outs or siRNAs verified YAP1/TAZ modulation of known YAP1/TAZ regulators such as activators RHOA, TAZ and GNA12 as well as inhibitors such as AJUBA, PTPN14 and LATS2.

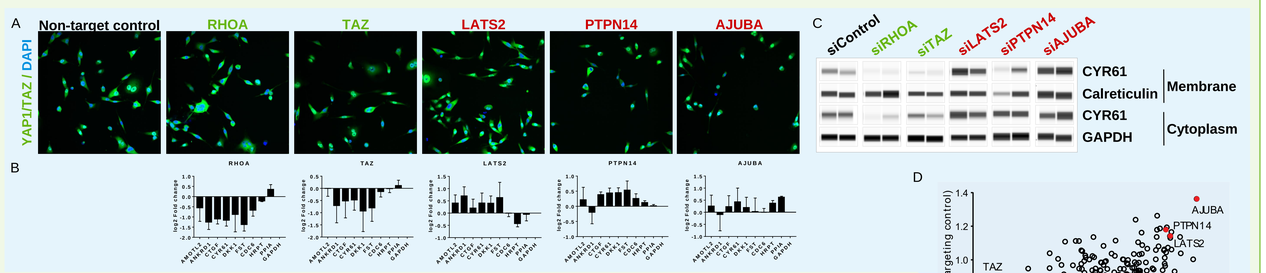

Fig. 4 : Effects of knockout / knock-down of hits on YAP1/TAZ activity, cellular YAP1/TAZ localization and YAP1/TAZ target gene expression. A) Image analysis of knockout effects of selected known YAP1/TAZ regulators on YAP1/TAZ translocation in the MDA-MB-231. B-C) siRNA mediated knockdown followed by qPCR and Protein (capillary electrophoresis) measurement of endogenous YAP1/TAZ target genes. D) Correlation of effects of target knockout on TEAD-luciferase activity and YAP1/TAZ cellular localization.

Novel potential positive and negative regulators of YAP1/TAZ

Gene name	TEAD-Luc activity (fold of control)	Nuclear YAP1/TAZ (fold of control)
TAZ	0,17	0,90
SLC7A6OS	0,17	0,83
RHOA	0,25	0,45
CSDE1	0,33	0,95
MARK2	0,33	0,70
NR2F2	0,38	0,78
RAC1	0,43	0,67
RBM14	0,45	0,79
SEC61A1	0,46	0,50
GNA12	0,48	0,66
PTK2	0,51	1,01
ELMO2	0,52	0,81
ARHGEF12	0,53	0,97
SEC61B	0,53	0,59
PPP4C	0,54	0,91
MAPK14	0,55	1,11
CNBD2	0,60	0,65
NFS1	0,60	0,83
UBE2D3	0,60	0,80
ILK	0,60	0,88
MED12	0,61	1,11
KTI12	0,63	0,95
EHMT1	0,64	0,98
NOP9	0,65	1,06
VEZF1	0,65	0,95
PLAGL2	0,65	0,84
TRIM28	0,65	1,05
NCKAP1	0,66	0,84
MLLT1	0,67	1,03
SRP14	0,69	1,00
ITGB5	0,69	0,73
SEC62	0,70	0,65
GPN2	0,71	0,87
CDC27	0,71	0,87
ARF4	0,71	0,94
MED26	0,71	0,88
DNMT3B	0,71	1,02
MAPK1	0,72	1,14
UVRAG	0,72	0,83
TP53INP2	0,72	0,88
CCNC	0,72	1,02
PTPN1	0,73	0,79
TAF13	0,73	0,93
WDR92	0,73	0,91
FDX1	0,73	0,69
HM13	0,73	0,95
C3orf17	0,75	0,90

Gene name	TEAD-Luc activity (fold of control)	Nuclear YAP1/TAZ (fold of control)
PTPN14	1,15	1,18
DPM1	1,15	1,04
GTF3C4	1,17	1,14
LATS2	1,17	1,14
CAND1	1,18	1,17
KCTD10	1,18	1,06
UBE2I	1,20	0,93
PTPN12	1,23	1,18
YEATS4	1,24	1,04
EPC2	1,31	1,00
AJUBA	1,33	1,36
CUL3	1,53	1,23
ZC3HAV1	1,54	0,92
FNTB	1,64	1,05

Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging

- In addition to previously known genes, additional genes with functions in actin cytoskeleton signaling, Integrin signaling, ER stress and protein transport, amongst others, were identified as potential novel YAP1/TAZ regulators.
- Knock-out or knock-down of MARK2, a member of the Par-1 family of serine/threonine protein kinases important for regulation of cell polarity consistently inhibited YAP1/TAZ activity in all assays tested. The highly related MARK4 protein has previously been shown to be an activator of YAP1/TAZ.
- Knock-out or knock-down of breast cancer tumor suppressors PTPN12 (Protein Tyrosine Phosphatase, Non-Receptor Type 12) or CUL3 (Cullin 3) consistently induced YAP1/TAZ activity in all assays tested:
  - PTPN12 interference suggests a conserved function with PTPN14, a known YAP1/TAZ inhibitory protein that sequesters YAP1/TAZ in the cytoplasm.
  - CUL3 is the core scaffolding protein of the CUL3–RING ubiquitin ligase complex which targets proteins for degradation by the proteasome.
  - The detailed mechanisms of how PTPN12 and CUL3 regulate YAP1/TAZ are currently being explored.

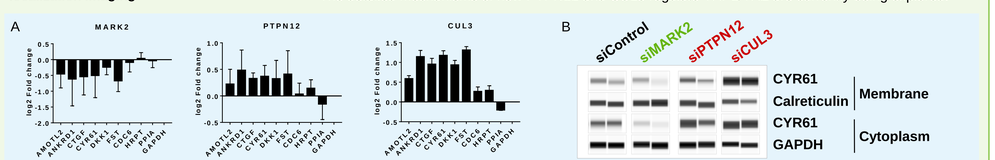

Fig. 5 : Effects of knockout of hits on YAP1/TAZ target gene expression. A,B) siRNA mediated knockdown followed by qPCR and Protein (capillary electrophoresis) measurement of endogenous YAP1/TAZ target genes.

CONCLUSIONS

- A pooled whole genome CRISPR/Cas9 screen identified previously known as well as novel positive and negative regulators of YAP1/TAZ in MDA-MB-231 breast cancer cells
- Functional characterization of the novel potential YAP1/TAZ modulators will aid to the further understanding of YAP1/TAZ biology in health and disease

ACKNOWLEDGMENTS

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