

Simplifying cancer: binary pan-cancer superclasses stratified by opposite YAP/TEAD effects

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ABSTRACT

The inherent complexity of cancer complicates treatment. Identifying higher-order principles that govern cancer biology can circumvent this problem and pinpoint broadly applicable treatment options. We recently found that opposite expression and pro- versus anti-cancer activity of a single transcriptional complex functionally stratifies cancer into binary superclasses.

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Mutational diversity, clonal heterogeneity, and epigenetic plasticity complicate cancer therapy. Precision medicine seeks to define and target the bewildering array of genetic factors that drive the growth of each cancer clone. A complementary approach is to uncover higher order rules of cancer behavior to identify broadly applicable therapeutic opportunities. Genomic and proteomic studies have identified some cancer groups that surpass tissue type,^{1–3} but these groupings remain numerous and their molecular drivers are largely unclear. The ability of cancers to change classes through lineage switching compounds this complexity.^{4,5} Thus, while overarching rules for cancer classification hold considerable appeal, the extent to which the disease can be simplified remains unclear.

The paralogues Yes1 associated transcriptional regulator (YAP1, better known as YAP) and WW domain transcriptional regulator 1 (WWTR1, better known as TAZ) are Hippo pathway effectors, and are well-characterized oncogenes in many solid cancers.⁶ These transcriptional co-activators primarily bind the TEA domain transcription factor (TEAD) family of DNA binding proteins where they co-operate with activator protein 1 (AP1) dimers at remote enhancers to drive cell cycle genes and promote oncogenesis.⁷ Combining transcriptomics, extensive gain- and loss-of-function studies, genome-wide binding assays and functional genomics, we identified an unanticipated tumor suppressor role for YAP and TAZ in many cancers, and further demonstrate that cancers can be functionally stratified into binary superclasses based on opposite pro- or anti-oncogenic activity of YAP/TAZ (Figure 1).⁸

Our work began in the childhood intraocular cancer, retinoblastoma. Knockout of *Yap*, *Taz* or both genes progressively increased tumorigenesis in a low-penetrance murine retinoblastoma model, demonstrating tumor suppressor activity. Moreover, we found that human retinoblastoma cell lines and tumors are YAP/TAZ-negative, while the benign variant,

retinoma, is YAP/TAZ-positive. Furthermore, ectopic YAP or TAZ expression induces cytostasis in retinoblastoma cell lines. The neuroendocrine cancer, small cell lung cancer (SCLC), was reported to lack YAP.⁹ We confirmed and extended these findings demonstrating that forced YAP/TAZ expression suppresses SCLC *in vitro* and *in vivo*, while *Yap/Taz* knockout accelerated SCLC initiation *in vivo*. Thus, YAP/TAZ are tumor suppressors in retinoblastoma and SCLC.

Extending these insights, transcriptomic data from thousands of cell lines and primary tumors revealed a striking on/off pattern of YAP/TAZ expression. Further, unbiased principal component analysis identified a broad group of YAP/TAZ-negative cancers and the stratification of cancers into YAP/TAZ-negative (YAP^{off}) and -positive (YAP^{on}) superclasses based on binary expression of *YAP*, *TAZ*, and ~80 co-regulated integrin/adhesion/extracellular matrix genes. The YAP^{off} class consisted of virtually all blood cancers (leukemia, lymphoma, and myeloma), many neural cancers (retinoblastoma, low-grade glioma, neuroblastoma) as well as small cell and well-differentiated neuroendocrine cancers from many tissues, including lung, prostate, breast and the gastrointestinal tract. These YAP^{off} cancers could be further parsed into two sub-groups consisting of either the liquid (blood cancers) or solid (neural/neuroendocrine) YAP^{off} cancers. YAP^{off} cancers exhibited elevated activity of the MYC proto-oncogene (liquid) or MYCN proto-oncogene (solid), and YAP^{off} solid cancers were enriched for amplification of *MYCN* and *MYCL* and loss of the RB transcriptional corepressor 1 (*RBI*) tumor suppressor. Mining the Cancer Dependency Map (DepMap) demonstrated that, while YAP/TAZ are essential in most YAP^{on} cell lines, they are dispensable in YAP^{off} lines. Further analysis of DepMap and drug sensitivity databases identified YAP^{on} and YAP^{off}-specific genetic and therapeutic vulnerabilities, emphasizing the clinical relevance of these classes and highlighting novel therapeutic opportunities to treat YAP^{off} cancers, such as

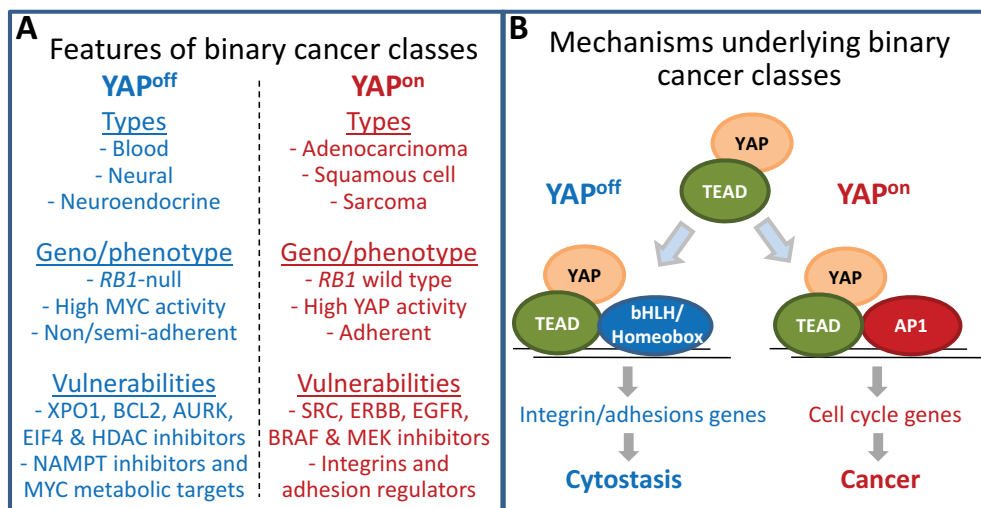


Figure 1. Features and mechanisms underlying binary YAP^{on} and YAP^{off} cancer superclasses. (a) Features of YAP/TAZ-deficient (YAP^{off}) and YAP/TAZ-positive (YAP^{on}) cancer classes, including the types, genetic and phenotypic characteristics, and key vulnerabilities. (b) Opposite anti- versus pro-oncogenic YAP/TEAD functions in YAP^{off} and YAP^{on} cancers are mediated through distinct enhancer engagement and target gene induction. YAP: Yes1 associated transcriptional regulator; YAP^{off}: YAP-deficient cancers; YAP^{on}: YAP-positive cancers; *RB1*: RB transcriptional corepressor 1; XPO1: Exportin 1; BCL2: BCL2 apoptosis regulator; AURK: Aurora Kinases; EIF4: Eukaryotic Translation Initiation Factor 4; HDAC: Histone Deacetylase; NAMPT: Nicotinamide Phosphoribosyltransferase; SRC: SRC proto-oncogene; ERBB: Erb-B2 Receptor Tyrosine Kinase; EGFR: Epidermal Growth Factor Receptor; BRAF: B-Raf proto-oncogene; MEK: Mitogen-Activated Protein Kinase (MAP2K, better known as MEK); TEAD: TEA domain transcription factor; bHLH: basic helix-loop-helix; AP1: activator protein 1.

Nicotinamide Phosphoribosyltransferase (NAMPT), BCL2 apoptosis regulator (BCL2)-family, Exportin 1 (XPO1), Eukaryotic Translation Initiation Factor 4 (EIF4), Aurora Kinases (AURK) and Histone Deacetylase (HDAC) inhibitors. Critically, we observed that the therapy-driven conversion of lung or prostate adenocarcinoma to drug-resistant neuroendocrine cancer is a YAP^{on} to YAP^{off} class switch.

To mechanistically dissect opposite oncogenic and tumor suppressor activities of YAP in binary classes, we performed structure-function experiments, transcriptomics, genome-wide binding assays and CRISPR screens. This revealed that YAP suppresses YAP^{off} cancers through TEAD-family proteins, which also mediate YAP oncogenic activity in YAP^{on} cancers. However, YAP/TEAD complexes target distinct enhancers in YAP^{on} and YAP^{off} cancers, resulting in the regulation of unique transcriptional targets. In contrast to YAP^{on} cancers, where YAP/TEAD are recruited to AP1-containing enhancers to induce cell cycle genes, YAP/TEAD engages enhancers occupied with homeobox and basic helix-loop-helix factors to induce integrin, extracellular matrix, and other adhesion genes in YAP^{off} cancers. Matching contrasting expression of adhesion-related genes, we found that YAP^{off} cancers are either non- or semi-adherent in culture, opposite to adherent YAP^{on} cancers, and ectopic expression of YAP induces adhesion of YAP^{off} cells. Finally, we identified the YAP-regulated integrin pair, Integrin- α V/ β 5, as a key mediator of YAP-induced tumor suppression.

The demonstration that cancers can be functionally stratified into binary YAP^{on}/YAP^{off} superclasses with distinct therapeutic and genetic vulnerabilities opens a new view on cancer diagnosis and treatment. Several pressing questions remain. For example, we showed that YAP silencing is epigenetic, but the mechanism needs to be deduced, especially since reactivating this program causes cytoskeleton-mediated tumor suppression in YAP^{off} cancers. How do YAP^{off} cancers divide in the absence of YAP given its critical cell cycle role in other cancers? Elevated MYC/MYCN activity and frequent *RB1*-loss provide

a logical answer, but requires further investigation. It will be critical to deduce the underlying mechanisms that drive YAP^{on}-to-YAP^{off} lineage switching, and determine how to thwart this lethal conversion. Whether YAP/TAZ-silencing is functionally required during this transition is unknown, but YAP induction is essential for transition of pulmonary neuroendocrine cells to non-neuroendocrine cells during the regenerative response to naphthalene injury,¹⁰ suggesting that the opposite may also be true. Our functional work focused on solid neural/neuroendocrine YAP^{off} cancers, and there is now considerable work needed to deduce whether and how YAP counters leukemia and lymphoma, the vast majority of which appear to be YAP^{off} cancers.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

- Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, Duyvesteyn K, Haidari S, Van Hoeck A, Onstenk W, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature*. 2019;575:210–216. doi:10.1038/s41586-019-1689-y.

2. Chen F, Zhang Y, Gibbons DL, Deneen B, Kwiatkowski DJ, Ittmann M, Creighton CJ. Pan-cancer molecular classes transcending tumor lineage across 32 cancer types, multiple data platforms, and over 10,000 cases. *Clin Cancer Res.* 2018;24:2182–2193. doi:10.1158/1078-0432.CCR-17-3378.
3. Chen F, Chandrashekar DS, Varambally S, Creighton CJ. Pan-cancer molecular subtypes revealed by mass-spectrometry-based proteomic characterization of more than 500 human cancers. *Nat Commun.* 2019;10:5679. doi:10.1038/s41467-019-13528-0.
4. Lee J-K, Lee J, Kim S, Kim S, Youk J, Park S, An Y, Keam B, Kim D-W, Heo DS, et al. Clonal history and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. *J Clin Oncol.* 2017;35:3065–3074. doi:10.1200/JCO.2016.71.9096.
5. Beltran H, Hruszkewycz A, Scher HI, Hildesheim J, Isaacs J, Yu EY, Kelly K, Lin D, Dicker A, Arnold J, et al. The role of lineage plasticity in prostate cancer therapy resistance. *Clin Cancer Res.* 2019;25:6916–6924. doi:10.1158/1078-0432.CCR-19-1423
6. Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. *Cancer Cell.* 2016;29:783–803. doi:10.1016/j.ccell.2016.05.005.
7. Zanconato F, Forcato M, Battilana G, Azzolin L, Quaranta E, Bodega B, Rosato A, Bicciato S, Cordenonsi M, Piccolo S. Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. *Nat Cell Biol.* 2015;17:1218–1227. doi:10.1038/ncb3216.
8. Pearson JD, Huang K, Pacal M, McCurdy SR, Lu S, Aubry A, Yu T, Kim W, Zhang L, Wang T, et al. Binary pan-cancer classes with distinct vulnerabilities defined by pro- or anti-cancer YAP/TEAD activity. *Cancer Cell.* 2021;39:1115–1134.e12. doi:10.1016/j.ccell.2021.06.016.
9. Rudin CM, Poirier JT, Byers LA, Dive C, Dowlati A, George J, Heymach JV, Johnson JE, Lehman JM, MacPherson D, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nat Rev Cancer.* 2019;19:289–297. doi:10.1038/s41568-019-0133-9.
10. Shue YT, Drainas AP, Li NY, Pearsall SM, Morgan D, Sinnott-Armstrong N, Hipkins S, Coles GL, Lim JS, Oro AE, et al. A conserved YAP/Notch/REST network controls the neuroendocrine cell fate in the lungs. *Nature Communications (In Revision).*