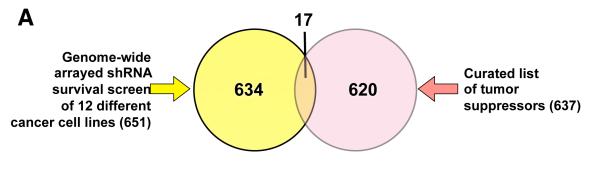
Identification of DISE-inducing shRNAs by monitoring cellular responses

Monal Patel^a and Marcus E. Peter^{a,b}

Supplementary data



-	-
_	_

	E. W. sama	TSG for Cancer	Kd lethal for # of cell lines	AVG H
The TS-D genes	Full name Platelet-activating factor	HCC [1]	lines	score
PAFAN IB I/LIS I	acetylhydrolase 1b, regulatory subunit 1 (45kDa)		12	80
NGFR	Nerve growth factor receptor	HCC [2], retinoblastoma [3], prostate cancer [4], bladder cancer [5]	11	89
ITGAV/CD51	Integrin Alpha V	Ovarian cancer [6], SCC [7]	11	76
DPP4/CD26	Dipeptidyl-peptidase 4	NSCLC [8]	10	94
EFNA5	Ephrin-A5	Glioma [9]	10	80
TMEFF1	Transmembrane protein with EGF-like and two follistatin-like domains 1	Brain cancers [10]	10	84
CHEK1	Checkpoint kinase 1	BrCa [11], Multiple cancers [12]	10	55
PTCH2	Patched 2	Basal cell carcinoma [13]	9	91
ARMC10/SVH	Armadillo repeat containing 10	Osteosarcoma [14], Leukemia [15]	9	84
BECN1/ATG6	Beclin 1	Breast cancer [16, 17, 18]	9	84
FASLG	Fas ligand	Multiple cancers [19]	9	84
DDX3X/DBX	DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked	HCC [20, 21], cutaneous squamous cell carcinoma [21]	9	80
THY1/CD90	Thy-1 cell surface antigen	Nasopharyngeal carcinoma [22], ovarian cancer [23, 24]	9	87
РНВ	Prohibitin	Prostate cancer [25], liver cancer [26]	9	84
SOCS3	Suppressor of cytokine signaling 3		9	82
ZNF366/DC-SCRIPT	Zinc finger protein 366	Breast cancer [28]	9	80
MAPKAPK5/MK5/PRAK	Mitogen-activated protein kinase- activated protein kinase 5	Colon cancer [29], skin cancer [30]	9	68
TGFBR2	Transforming growth factor, beta receptor II (70/80kDa)	HCC [31], breast cancer [32, 33]	9	58

Figure S1: Identification of TS genes among 651 survival genes. (A) Venn diagram showing the overlap of the 651 putative survival genes we identified in 12 genome-wide shRNAs screens with a list of 637 putative tumor suppressors (http://bioinfo.mc.vanderbilt.edu/TSGene). (B) A list of the 17 genes that are putative tumor suppressors and were identified in our lethality screen. The genes are ranked first according to the number of lethality screens in which these genes were found to be survival genes, and second according to the average H score. Higher counts are indicated by darker colors. HCC, hepatocellular carcinoma. FASLG is also shown for comparison. [1], ¹; [2], ²; [3], ³; [4], ⁴; [5], ⁵; [6], ⁶; [7], ⁷; [8], ⁸; [9], ⁹; [10], ¹⁰; [11], ¹¹; [12], ¹²; [13], ¹³; [14], ¹⁴; [15], ¹⁵; [16], ¹⁶; [17], ¹⁷; [18], ¹⁸; [19], ¹⁹; [20], ²⁰; [21], ²¹; [22], ²²; [23], ²³; [24], ²⁴; [25], ²⁵; [26], ²⁶; [27], ²⁷; [28], ²⁸; [29], ²⁹; [30], ³⁰; [31], ³¹; [32], ³²; [33], ³³. http://bioinfo.mc.vanderbilt.edu/TSGene ³⁴.

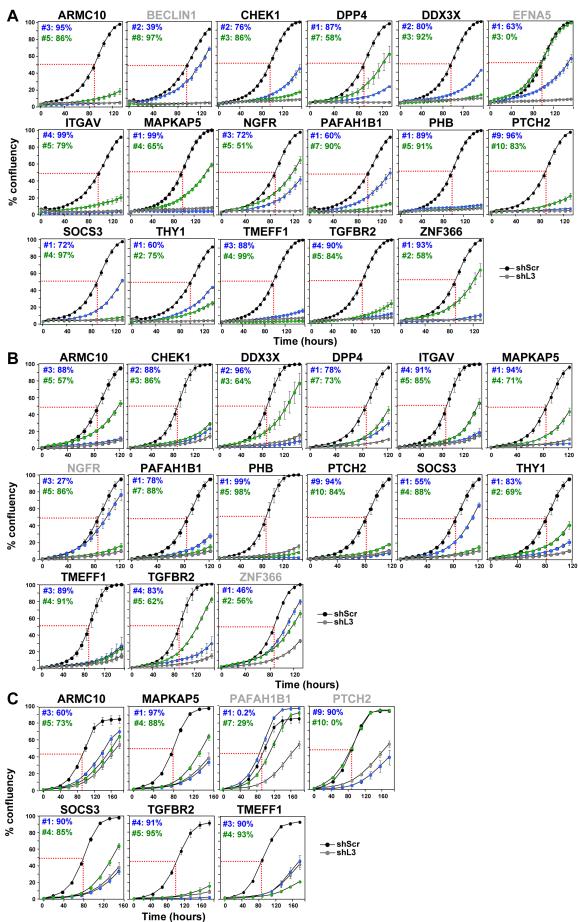


Figure S2. Toxic shRNAs cause growth reduction in T98G, HCT116 and Hap1 cells.

Percent cell confluency over time of T98G (A), HCT116 (B) and Hap1 (C) cells infected with shScr, shL3, and two shRNAs derived from each TS gene. The curves for cells infected with two independent shRNA for each TS gene and their specific ID number and respective growth reduction caused by each shRNA are shown in blue and green. Percent growth reduction values (as shown in Table 1) were calculated using STATA1C software when cells infected with shScr reached half maximal confluency as indicated by the red dotted line. Names of genes for which only one of the two shRNAs reduced growth more than 50% are shown in grey.

Supplementary references

- 1. Xing Z, Tang X, Gao Y, Da L, Song H, Wang S, et al. The human LIS1 is downregulated in hepatocellular carcinoma and plays a tumor suppressor function. Biochem Biophys Res Commun 2011; 409:193-9.
- 2. Yuanlong H, Haifeng J, Xiaoyin Z, Jialin S, Jie L, Li Y, et al. The inhibitory effect of p75 neurotrophin receptor on growth of human hepatocellular carcinoma cells. Cancer Lett 2008; 268:110-9.
- 3. Dimaras H, Gallie BL. The p75 NTR neurotrophin receptor is a tumor suppressor in human and murine retinoblastoma development. Int J Cancer 2008; 122:2023-9.
- 4. Khwaja F, Tabassum A, Allen J, Djakiew D. The p75(NTR) tumor suppressor induces cell cycle arrest facilitating caspase mediated apoptosis in prostate tumor cells. Biochem Biophys Res Commun 2006; 341:1184-92.
- 5. Tabassum A, Khwaja F, Djakiew D. The p75(NTR) tumor suppressor induces caspasemediated apoptosis in bladder tumor cells. Int J Cancer 2003; 105:47-52.
- 6. Kaur S, Kenny HA, Jagadeeswaran S, Zillhardt MR, Montag AG, Kistner E, et al. {beta}3integrin expression on tumor cells inhibits tumor progression, reduces metastasis, and is associated with a favorable prognosis in patients with ovarian cancer. Am J Pathol 2009; 175:2184-96.
- 7. McCarty JH, Barry M, Crowley D, Bronson RT, Lacy-Hulbert A, Hynes RO. Genetic ablation of alphav integrins in epithelial cells of the eyelid skin and conjunctiva leads to squamous cell carcinoma. Am J Pathol 2008; 172:1740-7.
- 8. Wesley UV, Tiwari S, Houghton AN. Role for dipeptidyl peptidase IV in tumor suppression of human non small cell lung carcinoma cells. Int J Cancer 2004; 109:855-66.
- 9. Li JJ, Liu DP, Liu GT, Xie D. EphrinA5 acts as a tumor suppressor in glioma by negative regulation of epidermal growth factor receptor. Oncogene 2009; 28:1759-68.
- 10. Gery S, Yin D, Xie D, Black KL, Koeffler HP. TMEFF1 and brain tumors. Oncogene 2003; 22:2723-7.
- 11. Sinha S, Singh RK, Bhattacharya N, Mukherjee N, Ghosh S, Alam N, et al. Frequent alterations of LOH11CR2A, PIG8 and CHEK1 genes at chromosomal 11q24.1-24.2 region in breast carcinoma: clinical and prognostic implications. Mol Oncol 2011; 5:454-64.
- 12. Lam MH, Liu Q, Elledge SJ, Rosen JM. Chk1 is haploinsufficient for multiple functions critical to tumor suppression. Cancer Cell 2004; 6:45-59.
- 13. Smyth I, Narang MA, Evans T, Heimann C, Nakamura Y, Chenevix-Trench G, et al. Isolation and characterization of human patched 2 (PTCH2), a putative tumour suppressor gene inbasal cell carcinoma and medulloblastoma on chromosome 1p32. Human Mol Gen 1999; 8:291-7.
- 14. Zhou X, Yang G, Huang R, Chen X, Hu G. SVH-B interacts directly with p53 and suppresses the transcriptional activity of p53. FEBS Lett 2007; 581:4943-8.
- 15. Curtiss NP, Bonifas JM, Lauchle JO, Balkman JD, Kratz CP, Emerling BM, et al. Isolation and analysis of candidate myeloid tumor suppressor genes from a commonly deleted segment of 7q22. Genomics 2005; 85:600-7.
- Furuya N, Yu J, Byfield M, Pattingre S, Levine B. The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. Autophagy 2005; 1:46-52.
- 17. Liang XH, Yu J, Brown K, Levine B. Beclin 1 contains a leucine-rich nuclear export signal that is required for its autophagy and tumor suppressor function. Cancer Res 2001; 61:3443-9.
- Aita VM, Liang XH, Murty VV, Pincus DL, Yu W, Cayanis E, et al. Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. Genomics 1999; 59:59-65.

- 19. Muschen M, Warskulat U, Beckmann MW. Defining CD95 as a tumor suppressor gene. J Mol Med (Berl) 2000; 78:312-25.
- Shih JW, Tsai TY, Chao CH, Wu Lee YH. Candidate tumor suppressor DDX3 RNA helicase specifically represses cap-dependent translation by acting as an eIF4E inhibitory protein. Oncogene 2008; 27:700-14.
- 21. Chao CH, Chen CM, Cheng PL, Shih JW, Tsou AP, Lee YH. DDX3, a DEAD box RNA helicase with tumor growth-suppressive property and transcriptional regulation activity of the p21waf1/cip1 promoter, is a candidate tumor suppressor. Cancer Res 2006; 66:6579-88.
- 22. Lung HL, Cheung AK, Cheng Y, Kwong FM, Lo PH, Law EW, et al. Functional characterization of THY1 as a tumor suppressor gene with antiinvasive activity in nasopharyngeal carcinoma. Int J Cancer 2010; 127:304-12.
- 23. Abeysinghe HR, Pollock SJ, Guckert NL, Veyberman Y, Keng P, Halterman M, et al. The role of the THY1 gene in human ovarian cancer suppression based on transfection studies. Cancer Genet Cytogenet 2004; 149:1-10.
- 24. Abeysinghe HR, Cao Q, Xu J, Pollock S, Veyberman Y, Guckert NL, et al. THY1 expression is associated with tumor suppression of human ovarian cancer. Cancer Genet Cytogenet 2003; 143:125-32.
- 25. Dart DA, Spencer-Dene B, Gamble SC, Waxman J, Bevan CL. Manipulating prohibitin levels provides evidence for an in vivo role in androgen regulation of prostate tumours. Endocr Relat Cancer 2009; 16:1157-69.
- 26. Ko KS, Tomasi ML, Iglesias-Ara A, French BA, French SW, Ramani K, et al. Liver-specific deletion of prohibitin 1 results in spontaneous liver injury, fibrosis, and hepatocellular carcinoma in mice. Hepatology 2010; 52:2096-108.
- 27. Barclay JL, Anderson ST, Waters MJ, Curlewis JD. SOCS3 as a tumor suppressor in breast cancer cells, and its regulation by PRL. Int J Cancer 2009; 124:1756-66.
- 28. Ansems M, Hontelez S, Karthaus N, Span PN, Adema GJ. Crosstalk and DC-SCRIPT: expanding nuclear receptor modulation. Biochim Biophys Acta 2010; 1806:193-9.
- 29. Kress TR, Cannell IG, Brenkman AB, Samans B, Gaestel M, Roepman P, et al. The MK5/PRAK kinase and Myc form a negative feedback loop that is disrupted during colorectal tumorigenesis. Mol Cell 2011; 41:445-57.
- 30. Sun P, Yoshizuka N, New L, Moser BA, Li Y, Liao R, et al. PRAK is essential for rasinduced senescence and tumor suppression. Cell 2007; 128:295-308.
- 31. Riehle KJ, Campbell JS, McMahan RS, Johnson MM, Beyer RP, Bammler TK, et al. Regulation of liver regeneration and hepatocarcinogenesis by suppressor of cytokine signaling 3. J Exp Med 2008; 205:91-103.
- 32. Forrester E, Chytil A, Bierie B, Aakre M, Gorska AE, Sharif-Afshar AR, et al. Effect of conditional knockout of the type II TGF-beta receptor gene in mammary epithelia on mammary gland development and polyomavirus middle T antigen induced tumor formation and metastasis. Cancer Res 2005; 65:2296-302.
- 33. Shida N, Ikeda H, Yoshimoto T, Oshima M, Taketo MM, Miyoshi I. Estrogen-induced tumorigenesis in the pituitary gland of TGF-beta(+/-) knockout mice. Biochim Biophys Acta 1998; 1407:79-83.
- 34. Zhao M, Sun J, Zhao Z. TSGene: a web resource for tumor suppressor genes. Nucleic Acids Res 2013; 41:D970-6.