Supplementary information S1 | Ras superfamily GEFs implicated in human cancer

GEF	Alteration in cancer	References
Ras GEFs		
CNrasGEF	RNAi Knockdown in melanoma cell lines increased proliferation	1
RasGEF1A	mRNA overexpressed in patient samples and RNAi knockdown decreased proliferation in cholangiocarcinoma tumor cells	2
RasGRF2	One missense and one nonsense mutation in colon cancer screen of 35 tumors	3
	Hypermethylation and decreased mRNA expression in NSCLC cell lines and patient tumors.	4
RasGRP1	Targeted overexpression in mouse keratinocytes leads to squamous cell papillomas and carcinomas	5
RasGRP2	Deletion accelerated the T-cell lymphoma formation caused by a Vav1 deficiency	6
RasGRP4	Transforming gene expressed in patient-derived acute myeloid leukemia encoding a missense mutation	7
Sos1	Gain-of-function mutations in Noonan syndrome	8, 9
	Three possible missense mutations found in the analyses of 810 tumors or cell lines	10
	mRNA and protein overexpression in prostate cancer patient tumors. RNAi knockdown in cell lines reduced proliferation, migration and invasion	11
RapGEFs		
C3G	DNA amplification, mRNA and protein overexpressed in NSCLC patient samples	12
DOCK4	Several missense mutations found in a variety of cancer cell lines, restoration of wild-type but not mutant into an osteosarcoma cell line missing DOCK4 reduced anchorage-independent growth and invasion. This study described DOCK4 as a Rap activation, although later studies showed DOCK4 activation of Rac, which is more consistent with its sequence homology to other DOCK family proteins 13, 14.	15
PLCε	Null mice have reduced incidence of carcinogen-induced skin tumors	16
RalGEFs		
RalGDS	Null mice have reduced incidence and size of carcinogen-induced skin tumors	17
	One Missense mutation found in 11 colon cancers	18
RGL1	Two missense mutations in 35 breast cancers	18
	W272*, G314R mutation in skin cancer	COSMIC
RGL2	Protein overexpressed in pancreatic cancer patient samples; knockdown reduces anchorage-independent growth and invasion in pancreatic cancer cell lines	19
RGR	N-terminal deletion splice variants in T-cell lymphoma patient samples	20
RhoGEFs		
ARHGEF10	Two missense mutations in colorectal cancer screen of 35 tumors	3
ASEF/ARHGEF4	Identified as APC tumor suppressor binding protein and activated by mutant APC to	21, 22

	promote cell migration.	
	One missense mutation identified in 11 breast cancers	18
	Activated by wild type and not mutant APC and knockdown in colorectal cancer cell lines with mutant APC support tumor suppressor function; breast cancer-associated missense mutation causes loss of RhoGEF activity.	23
	ASEF deficiency in the Apc(Min/+) background have reduced size and number of tumors and reduced tumor angiogenesis	24
	Loss in mice prevents growth and angiogenesis of xenograft tumors	25
Asef2	ASEF2 deficiency reduced migration of APC-mutant colorectal cancer cells	26
	Mouse ASEF2 knockouts in the Apc(Min/+) background have reduced size and number of tumors and tumor angiogenesis	24
β-Pix	mRNA and protein overexpression in breast cancer patient samples	27
Dbs	RNAi suppression of endogenous Dbs expression reduced breast cancer cell line motility	28
Ect2	High mRNA and protein expression in a variety of cancer cell lines	29
	mRNA and protein overexpressed in glioblastoma patient samples, associated with decreased survival	30
	mRNA and protein expressed in glioblastoma patient samples, associated with decreased survival; RNAi suppression reduced proliferation and invasion	31
	mRNA and protein overexpressed in NSCLC and esophageal carcinomas patient samples; knockdown reduces proliferation	32
	mRNA overexpressed in pancreatic cancer patient samples	33
	Genomic amplification and mRNA overexpression in NSCLC patients samples; knockdown decreased anchorage-independent growth and invasion	34
GEF-HI/ARHGEF2	Identified as a mutant p53-regulated gene and mRNA overexpression in mutant p53 tumor cells. RNAi suppression reduced proliferation in mutant p53 tumor lines.	35
	One missense and one nonsense mutation in breast cancer screen of 35 tumors	3
LARG	Truncated and fusion to MLL in acute myelogenous leukemia	36
Net-1	mRNA overexpressed in gastric cancer patient samples and cell lines; RNAi suppression reduced proliferation and invasion	37
	Coexpression of NET1 and $\alpha 6\beta 4$ integrin protein in node-positive breast cancer patients was associated with high risk for distant metastasis.	38
Obscurin	Missense, nonsense, deletion mutations in primary breast (4 of 35) and colon (7 of 35) cancers	18
P-Rex1	Upregulated in mouse brain tumors;	39
	Protein overexpressed in metastatic prostate cancer patient samples and cell lines; knockdown decreased invasion of metastastic cell lines; overexpression of wild-type but	40

	not GEF-dead promoted invasion and metastasis of non-metastatic cell lines	
P-Rex2a	Three-fold higher mRNA expression in PTEN wild type breast tumors. P-Rex2a identified as a PTEN-binding protein and RNAi suppression of PREX2 decreased AKT activation and decreased proliferation of PTEN wild type but not mutant breast carcinoma cell lines.	41
PDZ-RhoGEF	SNPs modulate lung cancer risk in Mexican-Americans	42
TEM4/ARHGEF17	mRNA overexpressed in tumor endothelium, during angiogenesis	43
Tiam1	Identified in a functional screen for T-cell invasion and metastasis inducing genes	44
	Mutated (A441G) in renal cell carcinoma lines and tumors	45
	Protein overexpressed in both pre-neoplastic high-grade prostatic intraepithelium neoplasia and prostate carcinoma patient samples	46
	Protein overexpression in a subset of colorectal cancer patient samples; RNAi suppression promoted anoikis and decreased migration	47
	Null mice form less carcinogen-induced *HRAS* mutation-associated skin tumors, but the tumors that arose were more metastatic	48
	Two missense mutations in human colorectal cancer screen of 35 tumors	3
	mRNA significantly higher in tumour tissue from patients who died from breast cancer when compared with those who survived.	49
	Protein expression decreased with the progression of breast carcinomas	50
Trio	mRNA overexpressed in glioblastoma patient samples,and RNAi suppression reduced invasion and proliferation	31
	Alternative gene splicing and expression in a patient with T-cell leukaemia of a truncated protein with transforming activity	51
	mRNA and protein overexpressed in breast cancer patient tumor when compared with normal tissue, and associated with poor predictive outcome	49
Vav1	Protein overexpressed in pancreatic cancer patient samples and cell lines, and RNAi suppression reduced anchorage-independent growth and xenograft growth in mice.	52
	Protein overexpressed in a subset of neuroblastomas, no correlation with stage or outcome of disease	53
	Decreased mRNA expression in breast cancer patient tumors associated with poorer survival	49
	Protein overexpressed in lung cancer cell lines and patient tumors, and RNAi suppression reduced anchorage-independent growth and tumor xenograft growth in mice	54
	Deletion in mice accelerates T-cell lymphoma formation; not seen with Vav2 and/or Vav3 -/- mice.	6
Vav2	RNAi suppression impaired CXCL12 chemokine-stimulated melanoma cell line invasion in vitro.	55

	Elevated protein phosphorylation in head and neck squamous cell carcinoma cell lines and RNAi suppression reduced invasion in vitro.	56
Vav3	mRNA and protein overexpressed in androgen-independent prostate cancer cell lines, regulated androgen receptor expression by GEF-independent mechanism.	57
	mRNA overexpressed in glioblastoma patient samples, knockdown reduced invasion	31
	Protein overexpressed in breast cancer patient tumor but not normal tissue; RNAi suppression in breast carcinoma cell lines reduced estrogen-independent and dependent proliferation, regulated estrogen receptor expression	58
DOCK1/DOCK180	Protein overexpressed in glioblastoma patient samples, and RNAi suppression reduced invasion.	59
DOCK2	Two missense mutations identified in discovery genomic DNA sequencing screen of 24 pancreatic cancers	60
DOCK3	NEDD9 overexpression in metastatic melanoma cells causes DOCK3 activation of Rac and promoted mesenchymal-type cell movement.	61
DOCK8	Decreased mRNA expression in 62/71 primary lung cancers (mixed types) when compared to normal lung tissue and decreased in 58/61 lung cancer cell lines (mixed types)	62
Dock10	RNAi suppression in melanoma cell lines redumced Cdc42 activation, but enhanced Rac activation and enhanced invasion in vitro; concurrent inhibition of Rac inhibited invasion	63
ArfGEFs		
GEP100/BRAG2	Protein overexpressed in breast cancer patient tumors and cell lines, frequently together with EGFR overexpression. RNAi suppression reduced invasion	64
ARFGEF2/BIG2	Two missense mutations in breast cancer screen of 35 tumors	3

Abbreviations: NSCLC, non-small cell lung carcinoma;

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Supplementary information S2 | Ras superfamily GAPs implicated in human cancer

GAP	Alteration in cancer	References
Ras family GAPs		
C3G/RapGAP1	DNA amplification and protein overexpression in a subset of NSCLC tumors	1
DAB2IP	Loss causes metastatic prostate cancer in an orthotopic mouse model through RasGAP dependent and independent ways. Protein is lost in human prostate patient samples, and its expression negatively correlates with tumor grade and patient prognosis	2
IQGAP1	Protein overexpressed in colorectal cancer patient tumor tissue when compared to normal tissue and is associated with invasive fronts of tumors	3
	High protein expression in glioblastoma patient samples correlated with poor prognosis,	4
	Elevated protein expression in breast tumor when compared to match normal tissue; RNAi suppression in a breast cancer cell line reduced proliferation and anchorage-independent growth, whereas ectopic overexpression enhanced xenograft tumor growth in mice.	5
IQGAP2	Null mice developed higher frequency of hepatocellular carcinoma that was associated with increased IQGAP1 protein expression; concurrent IQGAP1 deficiency reduced tumor development.	6
	Reduced protein expression and hypermethylation in gastric cancer patient tumors and cell lines. RNAi suppression promoted invasion	7
Neurofibromin	Single base changes (frameshift, missense), deletions, in neurofibromatosis type 1 patients.	COSMIC
	Somatic nonsense, splice site, missense changes, frameshift mutations and deletions found in glioblastoma tumors.	8, 9
	Single copy genomic loss in glioblastoma and reduced protein expression by proteosomal degradation in patient samples and cell lines; restoration of degradation-resistant NF1 reduced anchorage-independent growth and xenograft growth in mice	10
Plexin-B1	Missense mutations in 56/115 prostate cancers, mRNA and protein overexpression in patient samples; mutations hinder R-RasGAP activity, resulting in an increase in cell invasion	11
	Reduced mRNA and protein expression in renal cell carcinoma patient samples, ectopic restoration reduced proliferation in vitro.	12

	Lowered mRNA and protein levels in estrogen receptor-positive breast cancer tumors and loss correlates with poor prognosis	13
Plexin C1	Significant loss of mRNA and protein expression in metastatic melanoma compared with primary melanoma and cell lines	14, 15
Rap1GAP	Lowered protein expression in invasive pancreatic cancer patient samples, restoration reduced proliferation, invasion, and tumor progression and metastasis in an orthotopic mouse model	16
	Protein expression correlated with MMP9 expression in head and neck squamous cell carcinoma patient tumors and in vitro tumor cell line analyses found MMP9-dependent Matrigel invasion by overexpressed Rap1GAP.	17
	Lowered protein expression and hypermethylation in melanoma patient samples and cell lines, restoration of expression reduced proliferation of cell lines	18
	Decreased protein expression correlated with increased Rap-GTP levels and invasiveness of prostate tumor cell lines. Ectopic expression decreased whereas RNAi suppression increased Matrigel invasion. Ectopic Rap1 activation increased metastasis in vivo.	19
RASAL1	Lowered mRNA and protein expression in advanced wild-type Ras colorectal cancer patient samples, restoration reduced proliferation, RNAi suppression reduced tumor xenograft growth in mice	20
RASAL2	One missense and one nonsense mutation identified in 35 breast cancers	21
Sipa1	Amino acid polymorphism lowers RapGAP activity and associated with increased risk of breast cancer metastasis in mice, mRNA overexpression correlates with human prostate cancer metastasis, knockdown reduced, while overexpression promoted lung metastasis in a mammary breast cancer cell line	22
	Germline DNA polymorphisms are associated with breast cancer lymph node metastasis and estrogen receptor negative status	23
Tuberin-Harmartin	Base changes, insertions, deletions in nearly all exons of *TSC1* or *TSC2* in tuberous sclerosis patients	COSMIC
	The Eker rat strain that develops renal cell carcinoma contains a germline retrotransposon insertion inactivation mutation of one *TSC2* allele	24, 25
	TSC1 heterozygous null mice develop kidney cystadenomas and	26

	liver hemangiomas	
Rho family GAPs		
ARHGAP5/p190-B RhoGAP	Gene amplification and protein overexpressed in hepatocellular carcinoma cell lines, RNAi suppression reduced migration.	27
	Haploinsufficiency (p190-B +/-) in the mouse epithelium impaired MMTV-Neu induced mammary tumor progression, associated with reduced tumor angiogenesis	28
ARHGAP8	mRNA overexpression in colorectal cancer patient samples.	29
ARHGAP22	Knockdown suppresses mesenchymal-like invasion of melanoma cells	30
ARHGAP25	One missense and one deletion mutation in breast cancer screen of 35 tumors	31
Bcr	Fused to the Abl kinase in chronic myelogenous leukemia, fusion protein lacks the RhoGAP domain	32
DLC-1	Genomic deletion or reduced mRNA and expression by promoter methylation in non-small cell lung carcinoma, hepatocellular carcinoma, breast, colon, ovarian, uterine, gastric, pancreatic, prostate, renal, nasopharyngeal, multiple myeloma, lymphoma, and ALL	33-43
	Restoration of DLC1 in DLC1 negative prostate, NSCLC, breast, HCC, nasopharyngeal, esophageal, menangiomas, multiple myeloma, and renal cancer cell lines inhibits proliferation, anchorage-indepent growth, invasion or tumorigenicity or metastasis in mice	35, 38, 39, 41, 43-52
	Downregulation of endogenous DLC1 cooperates with Myc to form HCC in mice	53
	Two missense mutations identified in discovery genomic DNA sequencing screen of 24 pancreatic cancers	54
DLC-2/STARD13	Deletion or reduced mRNA expression in NSCLC, HCC, ovarian, renal, gastric, colorectal, kidney, breast and uterine cancers.	33, 55
	Restoration of DLC2 in HCC cells inhibits proliferation and migration	56
DLC-3/STARD8	Two missense mutations in 35 breast cancers	21
	Reduced mRNA expression in prostate, kidney, NSCLC, ovarian, uterine and breast cancer patient samples.	57
	Restoration of DLC3 in prostate cancer cells inhibited proliferation in vitro	57
GRAF/ARHGAP26	Chromosome translocation and formation of fusion protein with MLL	58, 59

	in one myelomonocytic leukemia and one AML patient. Additionally, missense mutation or DNA insertions were found in three AML patients.	
RalBP1	Protein overexpression in bladder, colorectal and ovarian cancer	60-62
	RNA suppression reduced melanoma, NSCLC, colorectal, prostate and renal cancer cell xenograft growth in mice	63-66
	RNAi suppression promoted lung cancer cell anti-cancer drug sensitivity	67, 68
Arf familyGAPs		
AGAP2/PIKE-A/GGAP2	Gene amplification and mRNA overexpression in glioblastoma cell lines and tumors, knockdown promoted apoptosis	69, 70
	Protein overexpression in prostate cancer patient samples and cell lines	71
	Mutations in cell lines of various cancer types enhance AKT activation.	72
	Overexpression promoted proliferation in a glioblastoma cell lines	73
ASAP1/DDEF1/AMAP1	Protein overexpressed in invasive breast cancer patient samples and cell lines, knockdown reduced invasion	74
	DNA amplification and mRNA protein overexpression in uveal melanoma patient samples.	75
	mRNA and protein overexpressed in metastatic prostate cancer patient samples and cell lines. Knockdown reduced invasion.	76
ASAP3/DDEFL1/UPLC1	mRNA overexpression in hepatocellular carcinoma patient samples.	77

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