Table 2. Genes showing downregulation or upregulation scores of 5 or more in clear cell RCCs

Gene	Chromosome	Entrez GeneID	Downregulation score[1]
(a) Genes showing reduced mRNA expression associated with DNA hypemethylation in their 5′-regions			
CLCNKB	1	1,188	24
SCNN1A	12	6,337	24
RAB25	1	57,111	22
TMEM213	7	155,006	22
ATP6V0A4	7	50,617	22
NR0B2	1	8,431	21
KCNJ1	11	3,758	21
GGT6	17	124,975	21
CLDN8	21	9,073	20
CLDN19	1	149,461	19
MUC15	11	143,662	16
RANBP3L	5	202,151	15
HRG	3	3,273	14
TSPAN8	12	7,103	14
RGS7	1	6,000	11
PTH1R	3	5,745	11
CWH43	4	80,157	11
F11	4	2,160	11
IRX2	5	153,572	11
EHF	11	26,298	11
CBLC	19	23,624	11
ATP6V1B1	2	525	10
LRRC2	3	79,442	10
CLDN16	3	10,686	10
EGF	4	1,950	10
WISP3	6	8,838	10
PHYHD1	9	254,295	10
FLJ45983	10	399,717	10
WIT-AS	11	51,352	10
ACSF2	17	80,221	10
ALDOB	9	229	9
ANKRD2	10	26,287	9
WT1	11	7,490	9
SOST	17	50,964	9
CYP4F3	19	4,051	9
COL18A1-AS1	21	378,832	9
BSND	1	7,809	8
TACSTD2	1	4,070	8
SLC44A4	6	80,736	8
KHDRBS2	6	202,559	8
VWC2	7	375,567	8
CHRM1	11	1,128	8
COL4A6	X	1,288	8
XPNPEP2	X	7,512	8
PROM2	2	150,696	7
ACPP	3	55	7
CKMT2	5	1,160	7
NEFM	8	4,741	7
KCNA4	11	3,739	7
FLRT1	11	23,769	7
OLFM4	13	10,562	7
SERPINA4	14	5,267	7
STRA6	15	64,220	7
CRABP1	15	1,381	7
SLC7A10	19	56,301	7
CSDC2	22	27,254	7
VWA5B1	1	127,731	6
LAD1	1	3,898	6
SYN2	3	6,854	6
SLC22A13	3	9,390	6
ABHD14A	3	25,864	6
UPK1B	3	7,348	6
KCTD8	4	386,617	6
SFRP1	8	6,422	6
GATA3	10	2,625	6
DAO	12	1,610	6
TMPRSS3	21	64,699	6
CHD5	1	26,038	5
PRELP	1	5,549	5
PLD5	1	200,150	5
MAL	2	4,118	5
ENTPD3	3	956	5
TNNC1	3	7,134	5
ANK2	4	287	5
PART1	5	25,859	5
SVOPL	7	136,306	5
DMRT2	9	10,655	5
AMBP	9	259	5
RBP4	10	5,950	5
SLC22A12	11	116,085	5
PDZRN4	12	29,951	5
PROZ	13	8,858	5
RHCG	15	51,458	5
KLK6	19	5,653	5

Table 2. Genes showing downregulation or upregulation scores of 5 or more in clear cell RCCs (Continued)

Gene	Chromosome	Entrez GeneID	Downregulation score[1]
BEX1	X	55,859	5
ZCCHC16	X	340,595	5
Gene	**Chromosome**	**Entrez GeneID**	**Up-regulation score[2]**
(b) Genes showing increased mRNA expression associated with DNA hypomethylation in their 5′-regions.			
CA9	9	768	25
C3	19	718	23
CP	3	1,356	22
NNMT	11	4,837	21
FABP7	6	2,173	11
REG1A	2	5,967	10
UBD	6	10,537	8
ENPP3	6	5,169	8
MCHR1	22	2,847	7
FCGR3A	1	2,214	6
FGG	4	2,266	6
PMCHL1	5	5,369	6
CPA6	8	57,094	6
SAA2	11	6,289	6
SAA1	11	6,288	6
DNAJB13	11	374,407	6
VWF	12	7,450	6
FGF11	17	2,256	6
SPAG4	20	6,676	6
CHI3L2	1	1,117	5
FCRL3	1	115,352	5
TIGIT	3	201,633	5
APOLD1	12	81,575	5
CCL18	17	6,362	5
CARD14	17	79,092	5
LILRA2	19	11,027	5
CXorf36	X	79,742	5
SH2D1A	X	4,068	5

[1]If the probe of the Infinium array was designed in the 5′-region of the gene, if $\Delta\beta$ ($\beta_T - \beta_N$) was 0.2 or more (DNA hypermethylation) and if ΔE ($E_T - E_N$) based on the expression microarray was −4 or less (reduced expression) in one paired sample (T and N), then a gene downregulation score of 1 was assigned.
[2]If the probe of the Infinium array was designed in the 5′-region of the gene, if $\Delta\beta$ ($\beta_T - \beta_N$) was −0.2 or less (DNA hypomethylation) and if ΔE ($E_T - E_N$) based on the expression microarray was 4 or more (overexpression) in one paired sample (T and N), then a gene upregulation score of 1 was assigned.

Among genes showing frequent genetic aberrations (genetic aberration score of 4 or more in Table 1), *GCN1L1* has recently been reported to be associated with the *CDK8* mediator complex, which includes *CDK8*, cyclin C (also known as *CCNC*), *MED12* and *MED13*.[25] *CDK8* directly regulates β-catenin-driven transcription[25] and human *CDK8* is known to be an oncogene that is amplified in a subset of colon cancers.[26] In addition, our quantitative RT-PCR analysis revealed a tendency for down regulation of β-catenin after knockdown of CDK8 by siRNA in RCC cell lines A-498 and ACHN (Supporting Information Fig. S4). These results are consistent with those of previous studies showing that knockdown of CDK8 in the human colon cancer cell line HCT116[27] and the human gastric cancer cell line SNU-638[28] resulted in significant reduction of β-catenin, indicating correlations between *CDK8* and the Wnt/β-catenin pathway.

The fly *MED12* and *MED 13* homologs, kohtalo and skuld, respectively activate Wnt/β-catenin target genes through direct interaction with the Wnt pathway component Pygopus.[29] However, *let-19* and *doy-22*, homologs of human *MED12* and *MED13*, respectively, in *Caenorhabditis elegans*, suppress the transcription of Wnt/β-catenin target genes.[30] Frequent mutation of human *MED12* has been reported in human uterine leiomyomas.[31] Deletion of the *CCNC* gene is frequently detected in human lymphoid malignancies[32] and sarcomas.[33] Wnt/β-catenin signaling is constitutively active in RCCs and activates their cell growth and metastasis.[34] However, unlike other human carcinomas, the incidence of mutation of exon 3 of the β-catenin gene is not so high in RCCs.[34] Analogously with other members of the *CDK8* mediator complex, mutations of *GCN1L1* may participate in renal carcinogenesis *via* Wnt/β-catenin signaling.

All 5 amino acid substitutions of the *GCN1L1* occurred within or near to Huntingtin protein, eEF3, protein phosphatase 2A and TOR (HEAT) repeats, which are crucial for protein-protein interaction[35] (Supporting Information Fig. S5). In addition, SIFT and PolyPhen-2 software predicted that amino acid substitutions due to mutations of the *GCN1L1* gene result in dysfunction of GCN1L1 protein (Table 1). The present study demonstrated not only a genetic aberration score of 5 for *GCN1L1*, but also a genetic aberration score of 3 for *MED12* and *CCNC* (Table 1). SIFT and PolyPhen-2 analyses have predicted that amino acid substitutions due to mutations of the *MED12* and *CCNC* genes also result in dysfunction of the proteins (Table 1). Taken together, the present data indicate that the function of the *CDK8* mediator complex may have been disturbed in 16% of the examined 67 RCCs. Genetic aberrations in members of the *CDK8* mediator complex may thus participate in the Wnt/β-catenin-related carcinogenetic pathway in clear cell RCCs.

MACF 1, a member of the plakin family of cytoskeletal linker proteins, regulates dynamic interactions between actin and microtubules to sustain directional cell movement.[36] *MACF1* is known to function in the Wnt signaling pathway through association with a complex containing axin, β-catenin, *GSK3β* and *APC* during mouse embryogenesis.[36] Somatic mutation of *MACF1* (Table 1) may also participate in the Wnt/β-catenin-related carcinogenetic pathway in clear cell RCCs. With respect

Table 3. Statistically significant GeneGo pathway maps revealed by MetaCore pathway analysis

		Involved genes		
Pathway	*P*-value	Genes	Entrez Gene ID	Multilayer-omics scoring (exome, methylome and transcriptome)
Cell adhesion_tight junctions	9.98×10^{-4}	*CLDN8*	9073	Downregulation score 20
		CLDN16	10686	Downregulation score 10
		CLDN19	149461	Downregulation score 19
Blood coagulation	1.26×10^{-3}	*VWF*	7450	Upregulation score 6
		F11	2160	Downregulation score 11
		FGG	2266	Upregulation score 6
Translation_non-genomic (rapid) action of androgen receptor	1.36×10^{-3}	*MTOR*	2475	Genetic score 4
		PTEN	5728	Genetic score 3
		EGF	1950	Downregulation score 10
Signal transduction_PTEN pathway	2.04×10^{-3}	*MTOR*	2475	Genetic score 4
		PTEN	5728	Genetic score 3
		EGF	1950	Downregulation score 10
Development_EGFR signaling *via* PIP3	7.04×10^{-3}	*PTEN*	5728	Genetic score 3
		EGF	1950	Downregulation score 10
Protein folding and maturation_-Bradykinin/ Kallidin maturation	1.34×10^{-2}	*KLK6*	5653	Downregulation score 5
		XPNPEP2	7512	Downregulation score 8
Transcription_receptor-mediated HIF regulation	1.95×10^{-2}	*MTOR*	2475	Genetic score 4
		PTEN	5728	Genetic score 3
Serotonin modulation of dopamine release in nicotine addiction	2.24×10^{-2}	*PTEN*	5728	Genetic score 3
		CHRM1	1128	Downregulation score 8
Signal transduction_AKT signaling	2.34×10^{-2}	*MTOR*	2475	Genetic score 4
		PTEN	5728	Genetic score 3
cAMP/ Ca(2+)-dependent Insulin secretion	2.34×10^{-2}	*PLCE1*	51196	Genetic score 3
		RYR2	6262	Genetic score 3
Immune response_interleukin-4 signaling pathway	2.45×10^{-2}	*MTOR*	2475	Genetic score 4
		GATA3	2625	Downregulation score 6
Role of alpha-6/beta-4 integrins in carcinoma progression	2.55×10^{-2}	*MTOR*	2475	Genetic score 4
		EGF	1950	Downregulation score 10
G-protein signaling_regulation of cAMP levels by muscarinic acetylcholine receptor	2.55×10^{-2}	*PLCE1*	51196	Genetic score 3
		CHRM1	1128	Downregulation score 8
Development_PIP3 signaling in cardiac myocytes	2.77×10^{-2}	*MTOR*	2475	Genetic score 4
		PTEN	5728	Genetic score 3

Table 3. Statistically significant GeneGo pathway maps revealed by MetaCore pathway analysis (Continued)

Pathway	*P*-value	Involved genes		
		Genes	Entrez Gene ID	Multilayer-omics scoring (exome, methylome and transcriptome)
Some pathways of EMT in cancer cells	3.22×10^{-2}	*MTOR*	2475	Genetic score 4
		EGF	1950	Downregulation score 10
Development_beta-adrenergic receptors signaling *via* cAMP	3.34×10^{-2}	*RYR2*	6262	Genetic score 3
		TNNC1	7134	Downregulation score 5
Development_IGF-1 receptor signaling	3.34×10^{-2}	*MTOR*	2475	Genetic score 4
		PTEN	5728	Genetic score 3
Translation _regulation of EIF4F activity	3.45×10^{-2}	*MTOR*	2475	Genetic score 4
		EGF	1950	Downregulation score 10
G-protein signaling_RAP2B regulation pathway	3.81×10^{-2}	*PLCE1*	51196	Genetic score 3
DNA damage_DNA-damage-induced responses	4.87×10^{-2}	*ATM*	472	Genetic score 3

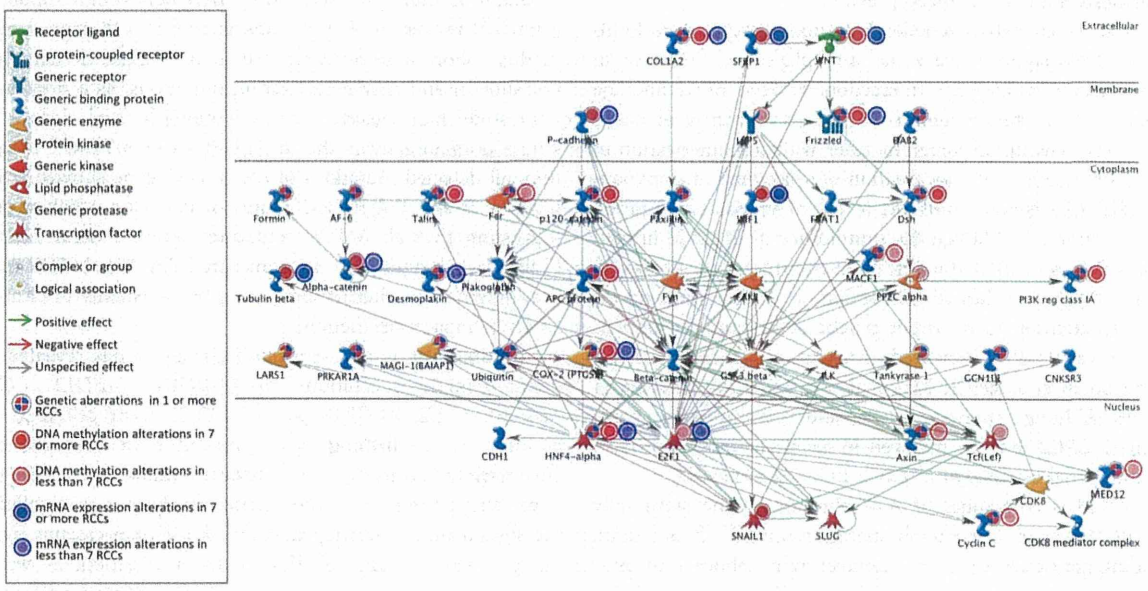

Figure 1. Genes for which a correlation with Wnt/β-catenin signaling was indicated by MetaCore pathway analysis. The numbers of genetic aberrations, DNA hyper- or hypo-methylation and/or increased or reduced mRNA expression (shown in Supporting Information Table S6) detected among the 67 examined RCCs are indicated schematically: legends are shown at the left of the panel. The 36 marked genes that showed genetic aberration, DNA methylation alterations and/or mRNA expression alterations in one or more RCCs were correlated with Wnt/β-catenin signaling.

to 29 RCCs for which transcriptome analysis was performed, mRNA expression levels of the targets genes of the Wnt/β-catenin signaling, such as *MYC*,[37] *MYCN*,[37] *IGF2*,[38] *POU5F1*,[39] *SOX9*,[40] *CYR61*,[41] *ENPP2*[42] and *MITF*,[43] tended to be higher in the 8 RCCs with mutations of any of the *GCN1L1, MED12, CCNC* and *MACF1* genes than in 21 RCCs without them (Supporting Information Table S10), indicating that such mutations may result in activation of Wnt/β-catenin signaling.

The downregulation score for the *SFRP1* gene was 6: reduced expression associated with DNA hypermethylation of *SFRP1* was frequent in clear cell RCCs. Members of the secreted frizzled-related protein (SFRP) family contain an N-terminal domain homologous to the cysteine-rich domain of the Wnt receptor Frizzled and lack a transmembrane region and the cytoplasmic domain required for transduction of signals into the cells.[44] This enables SFRPs to downregulate Wnt/β-catenin signaling by competing with Frizzled for Wnt binding *via* their cysteine-rich domain. Silencing of *SFRP1* due to DNA hypermethylation is known to result in activation of Wnt/β-catenin signaling.[44]

Since this study indicated possible alternative activation mechanisms (mutations of the *GCN1L1, MED12, CCNC* and *MACF1* genes and reduced expression of *SFRP1* due to DNA hypermethylation), we extensively examined Wnt/β-catenin signaling. MetaCore pathway analysis revealed that the 36 genes (marked in Fig. 1 and included in Supporting Information Table S6), which showed genetic aberration, DNA hypermethylation or hypomethylation and/or increased or reduced mRNA expression in one or more RCCs, are included in the Wnt/β-catenin signaling pathway. The present multilayer-omics analysis revealed that the Wnt/β-catenin signaling pathway may be of greater significance in renal carcinogenesis than was realized previously.

ERC2, which had a genetic aberration score of 4, is localized in presynaptic active zones and plays a critical role in neurotransmitter release.[45] Interaction between *ERC2* and the tandem PDZ protein syntenin-1, which is known to associate with many synaptic proteins, together with multimerization of *ERC2* both promote the localization of syntenin-1 at presynaptic *ERC2* clusters and contribute to the molecular organization of active zones.[45] Although the significance of *ERC2* in human cancers has remained unclear, frequent intragenic breaks in the *ERC2* gene indicated disruption of *ERC2* function in RCCs. In addition to recurrent genetic aberration, the present quantitative RT-PCR revealed frequent reduction of *ERC2* expression in clear cell RCCs relative to the corresponding N samples. Although frequent genetic and transcriptional inactivation of *ERC2* may be involved in renal carcinogenesis, further functional analysis of *ERC2* in RCCs is needed.

ABCA13 is a member of ATP-binding cassette sub-family A (*ABC1*) and a transmembrane transporter.[46] Xenobiotics, including anticancer drugs, are extensively metabolized by activation enzymes such as cytochromes *P450* and conjugation enzymes such as glutathione S-transferases or glucuronide transferases. Biotransformation represented by ABC transporters represents another important component of xenobiotic metabolism. In addition, ABC transporters play a crucial role in the development of resistance through efflux of anticancer agents from cancer cells.[46] The disease-free interval of patients with colorectal cancers treated by adjuvant chemotherapy is significantly shorter in patients with low *ABCA13* transcript levels.[47] In addition to recurrent genetic aberration (Table 1), the present quantitative RT-PCR revealed frequently reduced expression of *ABCA13* in RCCs relative to the corresponding N samples. Our findings suggest that it may be necessary to pay more attention to aberrations of *ABCA13* at both the genetic and expressional levels when deciding the indications for chemotherapy in patients with clear cell RCCs.

In Table 3 based on MetaCore pathway analysis, it is feasible that expression of *CLDNs* required for generating cation-selective paracellular channels[48] was reduced in clear cell RCCs, which lack the original absorptive function of the renal tubule. Moreover, *MTOR* mutations were highlighted as one of the major disrupters of multiple cell signaling during renal carcinogenesis: the *MTOR* gene participated in 10 (50%) of the 20 significant pathways in Table 3. The mammalian target of rapamycin (*mTOR*) encoded by the *MTOR* gene is a serine/threonine kinase that regulates cell growth, proliferation and autophagy.[49] *mTOR* inhibitors, such as rapamycin and its derivatives, are being introduced for targeted therapy of clear cell RCCs. Overactivation of *mTOR* is generally considered to be due to homozygous deletion of the *PTEN* tumor suppressor gene.[50] However, all 4 mutations of the *MTOR* gene detected in this cohort were located close to the kinase domain (data not shown) and may be activating mutations, as a previous *in vitro* study has suggested that mutations located close to the kinase domain activate the mutant form of *mTOR*.[50] In addition, all detected mutations of the *MTOR* gene showed a SIFT score of 0 and PolyPhen-2 scores of 0.998 or 0.999, strongly suggesting that all *MTOR* mutations affect protein function (Table 1 and Supporting Information Table S3). *MTOR* mutation may be a marker for predicting the sensitivity of clear cell RCCs to rapamycin therapy.

In summary, the present exome analysis has revealed frequent genetic aberrations of *GCN1L1, MED12, CCNC, MACF1, ERC2, ABCA13* and *MTOR* in clear cell RCCs. In addition to confirming the significance of aberrations of chromatin remodeling and histone modification-related proteins, the present multilayer-omics analysis has highlighted the significance of dysregulation of the Wnt/β-catenin signaling pathway including *CDK8* mediator function, as well as the need to pay closer attention to *MTOR* mutations, causing major disruption of cell signaling during renal carcinogenesis, in relation to chemosensitivity. Multilayer-omics analysis can be considered a powerful tool for revealing significant carcinogenetic pathways in human cancers.

References

1. Ljungberg B, Campbell SC, Choi HY, et al. The epidemiology of renal cell carcinoma. *Eur Urol* 2011;60: 615–21.
2. Dalgliesh GL, Furge K, Greenman C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature* 2010;463:360–3.
3. van Haaften G, Dalgliesh GL, Davies H, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nat Genet* 2009;41:521–3.

4. Varela I, Tarpey P, Raine K, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature* 2011;469:539–42.
5. Baldewijns MM, van Vlodrop IJ, Vermeulen PB, et al. VHL and HIF signaling in renal cell carcinogenesis. *J Pathol* 2010;221:125–38.
6. Guo G, Gui Y, Gao S, et al. Frequent mutations of genes encoding ubiquitin-mediated proteolysis pathway components in clear cell renal cell carcinoma. *Nat Genet* 2012;44:17–9.
7. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. *Nat Rev Cancer* 2011;11:726–34.
8. Kanai, Y. Genome-wide DNA methylation profiles in precancerous conditions and cancers. *Cancer Sci* 2010;101:36–45.
9. Arai E, Ushijima S, Fujimoto H, et al. Genome-wide DNA methylation profiles in both precancerous conditions and clear cell renal cell carcinomas are correlated with malignant potential and patient outcome. *Carcinogenesis* 2009;30:214–21.
10. Arai E, Kanai Y, Ushijima S, et al. Regional DNA hypermethylation and DNA methyltransferase (DNMT) 1 protein overexpression in both renal tumors and corresponding nontumorous renal tissues. *Int J Cancer* 2006;119:288–96.
11. Arai E, Chiku S, Mori T, et al. Single-CpG-resolution methylome analysis identifies clinicopathologically aggressive CpG island methylator phenotype clear cell renal cell carcinomas. *Carcinogenesis* 2012;33:1487–93.
12. Sato Y, Yoshizato T, Shiraishi Y, et al. Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat Genet* 2013;45:860–7.
13. Eble JN, Togashi K, Pisani P. Renal cell carcinoma. World Health Organization classification of tumours. Pathology and genetics. Tumours of the urinary system and male genital organs. Lyon: IARC Press, 2004. 10–43.
14. Fuhrman SA, Lasky LC, Limas, C. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol* 1982;6:655–63.
15. Sobin LH, Gospodarowicz MK, Wittekind C, eds. International Union Against Cancer (UICC). TNM classification of malignant tumors, 7th edn. New York: Wiley, 2009.
16. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 2010;26:589–95.
17. Ye K, Schulz MH, Long Q, et al. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics* 2009;25: 2865–71.
18. Wang L, Tsutsumi S, Kawaguchi T, et al. Whole-exome sequencing of human pancreatic cancers and characterization of genomic instability caused by MLH1 haploinsufficiency and complete deficiency. *Genome Res* 2012;22:208–19.
19. Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. *Genome Res* 2002;12:436–46.
20. Hicks S, Wheeler DA, Plon SE, et al. Prediction of missense mutation functionality depends on both the algorithm and sequence alignment employed. *Hum Mutat* 2011;32:661–8.
21. Sim NL, Kumar P, Hu J, et al. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res* 2012;40:W452–7.
22. Van Loo P, Nordgard SH, Lingjærde OC, et al. Allele-specific copy number analysis of tumors. *Proc Natl Acad Sci USA* 2010;107:16910–5.
23. Li A, Liu Z, Lezon-Geyda K, et al. GPHMM: an integrated hidden Markov model for identification of copy number alteration and loss of heterozygosity in complex tumor samples using whole genome SNP arrays. *Nucleic Acids Res* 2011;39: 4928–41.
24. Nagashio R, Arai E, Ojima H, et al. Carcinogenetic risk estimation based on quantification of DNA methylation levels in liver tissue at the precancerous stage. *Int J Cancer* 2011;129: 1170–9.
25. Firestein R, Hahn WC. Revving the Throttle on an oncogene: CDK8 takes the driver seat. *Cancer Res* 2009;69:7899–901.
26. Firestein R, Bass AJ, Kim SY, et al. CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. *Nature* 2008;455:547–51.
27. He SB, Yuan Y, Wang L, et al. Effects of cyclin-dependent kinase 8 specific siRNA on the proliferation and apoptosis of colon cancer cells. *J Exp Clin Cancer Res* 2011;30:109.
28. Seo JO, Han SI, Lim SC. Role of CDK8 and beta-catenin in colorectal adenocarcinoma. *Oncol Rep* 2010;24:285–91.
29. Carrera I, Janody F, Leeds N, et al. Pygopus activates Wingless target gene transcription through the mediator complex subunits Med12 and Med13. *Proc Natl Acad Sci USA* 2008;105:6644–9.
30. Yoda A, Kouike H, Okano H, et al. Components of the transcriptional mediator complex are required for asymmetric cell division in C. elegans. *Development* 2005;132:1885–93.
31. Mäkinen N, Mehine M, Tolvanen J, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. *Science* 2011;334:252–5.
32. Jackson A, Carrara P, Duke V, et al. Deletion of 6q16-q21 in human lymphoid malignancies: a mapping and deletion analysis. *Cancer Res* 2000; 60:2775–9.
33. Ohata N, Ito S, Yoshida A, et al. Highly frequent allelic loss of chromosome 6q16-23 in osteosarcoma: involvement of cyclin C in osteosarcoma. *Int J Mol Med* 2006;18:1153–8.
34. Banumathy G, Cairns P. Signaling pathways in renal cell carcinoma. *Cancer Biol Ther* 2010;10: 658–64.
35. Andrade MA, Petosa C, O'Donoghue SI, et al. Comparison of ARM and HEAT protein repeats. *J Mol Biol* 2001;309:1–18.
36. Chen HJ, Lin CM, Lin CS, et al. The role of microtubule actin cross-linking factor 1 (MACF1) in the Wnt signaling pathway. *Genes Dev* 2006;20:1933–45.
37. Karim R, Tse G, Putti T, et al. The significance of the Wnt pathway in the pathology of human cancers. *Pathology* 2004;36:120–8.
38. Heaton JH, Wood MA, Kim AC, et al. Progression to adrenocortical tumorigenesis in mice and humans through insulin-like growth factor 2 and β-catenin. *Am J Pathol* 2012;181:1017–33.
39. Li J, Li J, Chen B. Oct4 was a novel target of Wnt signaling pathway. *Mol Cell Biochem* 2012; 362:233–40.
40. Blache P, van de Wetering M, Duluc I, et al. SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes. *J Cell Biol* 2004;166:37–47.
41. Li ZQ, Ding W, Sun SJ, et al. Cyr61/CCN1 is regulated by Wnt/β-catenin signaling and plays an important role in the progression of hepatocellular carcinoma. *PLoS One* 2012;7:e35754.
42. Zirn B, Samans B, Wittmann S, et al. Target genes of the WNT/beta-catenin pathway in Wilms tumors. *Genes Chromosomes Cancer* 2006; 45:565–74.
43. Syed DN, Afaq F, Maddodi N, et al. Inhibition of human melanoma cell growth by the dietary flavonoid fisetin is associated with disruption of Wnt/β-catenin signaling and decreased Mitf levels. *J Invest Dermatol* 2011;131:1291–9.
44. Suzuki H, Watkins DN, Jair KW, et al. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. *Nat Genet* 2004;36:417–22.
45. Ko J, Yoon C, Piccoli G, et al. Organization of the presynaptic active zone by ERC2/CAST1-dependent clustering of the tandem PDZ protein syntenin-1. *J Neurosci* 2006;26:963–70.
46. Cole SP, Bhardwaj G, Gerlach JH, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 1992;258:1650–4.
47. Hlavata I, Mohelnikova-Duchonova B, Vaclavikova R, et al. The role of ABC transporters in progression and clinical outcome of colorectal cancer. *Mutagenesis* 2012;27:187–96.
48. Angelow S, El-Husseini R, Kanzawa SA, et al. Renal localization and function of the tight junction protein, claudin-19. *Am J Physiol Renal Physiol* 2007;293:166–77.
49. Populo H, Lopes JM, Soares P. The mTOR signalling pathway in human cancer. *Int J Mol Sci* 2012;13:1886–918.
50. Sato T, Nakashima A, Guo L, et al. Single amino-acid changes that confer constitutive activation of mTOR are discovered in human cancer. *Oncogene* 2010;29:2746–52.

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Druggable Oncogene Fusions in Invasive Mucinous Lung Adenocarcinoma

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Translational Relevance

Oncogene fusions, such as the *ALK*, *RET*, and *ROS1* fusions, have recently been revealed as therapeutic targets in lung adenocarcinoma. We identified multiple druggable oncogene fusions, including those involving the *NRG1*, *ERBB4*, and *BRAF* genes, in invasive mucinous adenocarcinoma (IMA), a malignant type of lung adenocarcinoma. The fusions occurred mutually exclusively with *KRAS* mutations, a common driver oncogene aberration in IMA. These fusions represent potentially clinically relevant targets for treatment of IMAs that lack *KRAS* mutations.