

The role of mTORC1 pathway in intestinal tumorigenesis

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Activation of the mTORC1 pathway has been implicated in many types of cancer, and several mTORC1 inhibitors are currently under clinical trials for treating various cancer patients. Notably, Temsirolimus has recently been approved for treatment of advanced renal cell carcinoma. However, the role of mTORC1 pathway in colorectal tumorigenesis remains largely unknown. We have recently found that the mTORC1 pathway is activated in intestinal adenomas of *Apc* mutant mice, accompanied by an elevated level of mTOR protein, and that treatment with RAD001, an mTORC1 inhibitor, suppresses the growth of these polyps. Our results suggest an important role of mTORC1 pathway in colorectal cancer, as well as a therapeutic possibility for mTORC1 inhibitors in its treatment.

mTORC1 and Cancer

Activation of mTORC1 in cancer and anti-tumor effects of mTORC1 inhibitors. The mammalian target of rapamycin complex 1 (mTORC1) signaling plays key roles in cell growth and metabolism in response to environmental changes. The mTORC1 activity is regulated by upstream signals from growth factors, amino acids, stresses and energy state, and its activation induces phosphorylation of S6 kinase and 4EBP1, leading to enhanced translation of a subset of mRNAs that are important for cell growth and metabolism.¹ Recent studies have revealed that the PI3K-Akt pathway, MEK-Erk pathway² and AMPK signaling³ represent major upstream regulators of the mTORC1 pathway. Activation of Akt or Erk, or inhibition of AMPK leads to decreased activity of TSC1/2 complex as a GTPase-activating

protein (GAP) toward the small GTPase Rheb.⁴ GTP-bound Rheb then activates mTORC1 by antagonizing FKBP38, an endogenous mTOR inhibitor (Fig. 1).⁵

Activation of the mTORC1 pathway has been observed in various types of cancer.⁶ Since activating mutations of mTOR itself have not been reported so far, dysregulation of the upstream signaling is likely to be responsible for the mTORC1 activation in cancer. Consistently, aberrations of the upstream signaling molecules are frequently observed in cancer cell lines, as well as in clinical samples from cancer patients.^{6,7} Such aberrations include deletion or inactivating mutations of PTEN, and activating mutations of PIK3CA or K-Ras, which lead to constitutive activation of the PI3K-Akt pathway. Mutations of TSC2 also increase the risk of renal cell carcinoma.⁸ Essential roles of the mTORC1 activation in tumorigenesis induced by these upstream mutations are supported by studies using mouse models. Namely, mTORC1 activation has been observed in tumors that developed in transgenic mice carrying constitutively active allele of Akt or K-ras,⁹⁻¹² in *Pten* knockout mice,^{13,14} and in mice with prostate-specific Rheb-overexpression.¹⁵ Importantly, tumors in these mice were all sensitive to treatment with rapamycin or its derivatives (except Rheb transgenic mice that were not tested). Accordingly, clinical trials are ongoing with mTORC1 inhibitors for glioblastoma, lung cancer, renal cell carcinoma, and other cancers.⁶ Notably, *Temsirolimus* (CCI-779) has been approved by FDA for treatment of advanced renal cell carcinoma, and a recent report has shown that *Everolimus* (RAD001) is effective for treatment of patients with metastatic renal cell carcinoma in a phase III trial.¹⁶

Key words: mTOR, Wnt, APC, β -catenin, colorectal cancer

Abbreviations: mTORC1, mammalian target of rapamycin complex 1; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; APC, adenomatous polyposis coli; PI3K, phosphoinositide 3-kinase; AMPK, 5' AMP-activated protein kinase; TSC, tuberous sclerosis complex; PTEN, phosphatase and tensin homolog; CDK, cyclin-dependent kinase; LRP, low density lipoprotein receptor-related protein; PRAS40, AKT1 substrate 1 (proline-rich)

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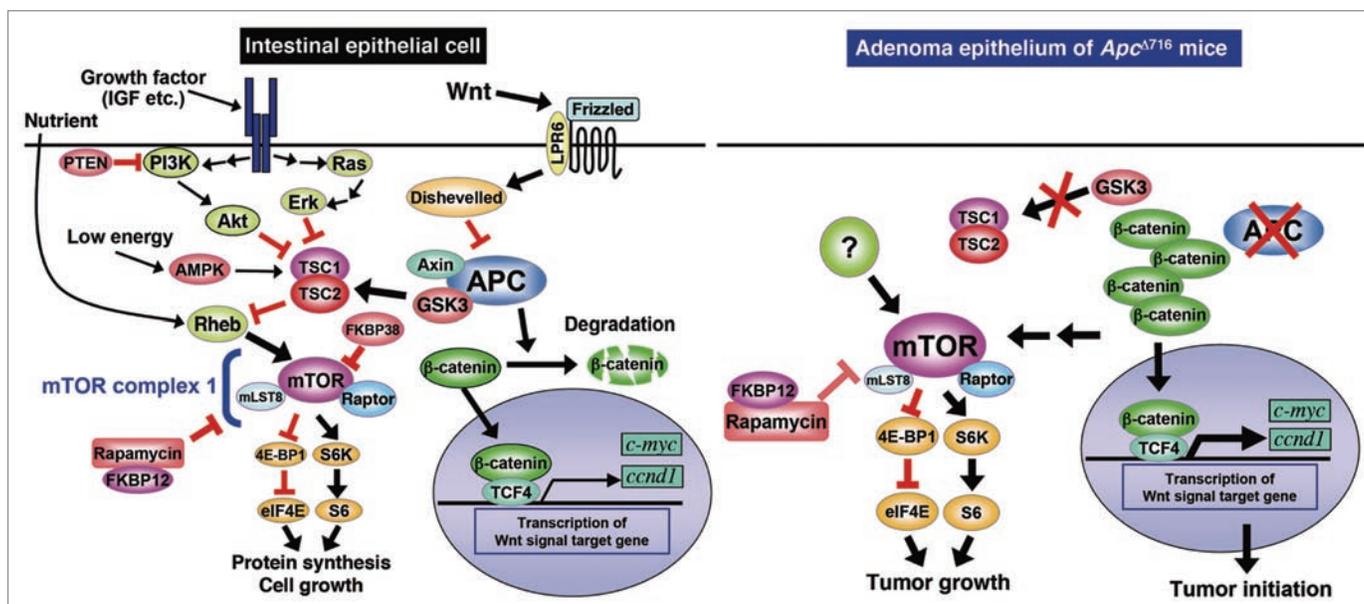


Figure 1. mTOR signaling in intestinal polyps of *Apc*^{Δ716} mice. (Left) In the normal epithelium of intestine, β-catenin is degraded by destruction complex containing APC, Axin and GSK3β. β-Catenin is finally destroyed through the ubiquitin-proteasome pathway. Activity of mTORC1 signaling is regulated by energy state, nutrients etc. (Right) In the adenoma epithelium of intestinal polyps, loss of heterozygosity (LOH) of *Apc* gene results in β-catenin stabilization and activation of the Wnt signaling, and triggers off polyp initiation. mTORC1 signaling is constitutively activated in polyps. This activation is not affected by nutrient deprivation or PI3K-Akt pathway inhibition. mTOR protein is increased in adenoma epithelium with activated Wnt signaling activation. Treatment with RAD001 inhibits adenoma cell growth.

mTORC1 and cell proliferation. Deregulated proliferation is one of the hallmarks of cancer cells. The mTORC1 signaling appears to be involved in cancer cell proliferation because mTORC1 inhibitors strongly suppressed tumor cell proliferation in mouse models described above, and delayed cell cycle progression in some cancer cell lines in culture. mTORC1 positively controls the level of cell cycle regulators, such as cyclin D, E and A,^{17,18} although the precise mechanism remains unclear. It is also reported that eIF4GI, which is regulated by mTORC1 indirectly, controls translation of Skp2, a ubiquitin ligase involved in the degradation of CDK inhibitor p27/Kip1.¹⁹ Activation of mTORC1 has also been shown to stimulate degradation of p27 through activation of serum/glucocorticoid regulated kinase (SGK).²⁰ Consistent with these findings, activation of mTORC1 was shown to accelerate cell cycle progression in colon cancer cell lines and in mouse enterocytes, causing their chromosomal instability.²¹

mTORC1 and angiogenesis. In addition to cell-autonomously regulated proliferation, angiogenesis also plays essential roles in tumor growth by supplying oxygen

and nutrients. Accordingly, angiogenesis can be a therapeutic target, and treatment with anti-vascular endothelial growth factor (VEGF) antibody combined with chemotherapy has been shown to increase the survival of patients with metastatic colorectal cancer.²² Interestingly, rapamycin inhibited tumor angiogenesis and caused a marked regression of tumor transplants derived from cancer cell lines that were rapamycin-insensitive in vitro.²³ Many pre-clinical studies have shown that mTORC1 inhibitors can inhibit angiogenesis by suppressing VEGF production or by directly suppressing the proliferation of angiogenic vessel cells.^{24,25} mTORC1 inhibitors are thus expected to target both tumor cell growth and tumor angiogenesis.

mTORC1 and Wnt Signaling in Intestinal Tumors

mTORC1 signaling in intestinal tumors. Genes encoding signaling molecules upstream of mTORC1 such as *PIK3CA* or *KRAS* are frequently mutated in colorectal cancer. It was reported that the mTORC1 pathway was activated in about 40% of colorectal cancer patients.²⁶

However, the efficacy of mTORC1 inhibitors has not been tested in colon cancer patients. In vitro studies have shown that the effect of mTORC1 inhibitors on proliferation of colon cancer cell lines varies among cell lines. Some colon cancer cell lines are sensitive to growth inhibition by rapamycin, while others are not. Thus, molecules or pathways responsible for their different sensitivity remain to be identified.^{26,27} Although *Pten* knockout mice and oncogenic *K-ras* (*K-ras*^{G12D}) transgenic mice develop colorectal cancer, their responsiveness to mTORC1 inhibitors has not been determined.^{28,29}

Wnt signaling in intestinal tumors. In normal physiology, canonical Wnt signaling is activated by binding of Wnt ligands to their receptor Frizzled and co-receptor LRP on the cell surface. Mediated by transducers including Dishevelled, the signaling inhibits the GSK3β-mediated phosphorylation of β-catenin complexed with APC and Axin, and thereby reduces degradation of β-catenin through the ubiquitin-proteasome system.³⁰ Stabilized β-catenin now moves into the nucleus, and forms a complex with TCF/LEF transcription factors, which results in transcriptional

activation of their target genes such as *c-myc* and *cyclin D1* (*CCND1*).

In most human colorectal cancers, somatic mutations are found that induce excessive activation of the Wnt signaling. Namely, *APC* gene is mutated in more than 60% of human colorectal cancer lesions,³¹ and many of those that do not carry *APC* mutations suffer from mutations in β -catenin at the GSK3 β phosphorylation sites. It appears that *APC* mutation-induced intestinal tumorigenesis also depends on the transcriptional corepressor C-terminal binding protein-1 (CtBP1) as suggested in zebrafish and human samples.³²

mTORC1 signaling in Wnt-induced intestinal tumors. Consistent with the role of *APC* in intestinal tumorigenesis, mice heterozygous for *Apc* mutations spontaneously develop a number of intestinal polyps.^{33,34} We determined the roles of mTORC1 signaling in the intestinal tumors of *Apc* ^{Δ 716} mouse, a model for familial adenomatous polyposis (FAP), developing ~300 polyps in the intestines caused by the loss of heterozygosity (LOH) at the *Apc* locus. We found that the mTORC1 signaling was strongly activated in the intestinal polyps of the *Apc* mutant mice as compared with their normal epithelia.³⁵ Most interestingly, treatment with RAD001 suppressed their polyp formation significantly. In particular, the number of polyps in the large size class (>2 mm in diameter) was reduced dramatically. Furthermore, most RAD001-treated *Apc* ^{Δ 716} mice lived longer than 1 year despite that the placebo-treated *Apc* ^{Δ 716} mice failed to survive beyond 30 weeks of age. We also found that expression of cyclin proteins, especially cyclin E, was reduced in the polyps of RAD001-treated *Apc* ^{Δ 716} mice compared with the placebo-treated controls. Consistently, BrdU uptake was also reduced significantly in the intestinal polyps of RAD001-treated *Apc* ^{Δ 716} mice. These results indicate that activation of mTORC1 signaling contributes to intestinal tumor cell proliferation.

Tumor angiogenesis is one of the targets of the mTORC1 inhibitors as mentioned above, and intestinal polyps of *Apc* ^{Δ 716} mice show angiogenic blood vessels,³⁶ suggesting that the mTORC1 inhibitor may also have affected tumor angiogenesis in

Apc ^{Δ 716} mice. As anticipated, treatment with the mTORC1 inhibitor significantly reduced the number of angiogenic vessels in the polyps. It caused disappearance of the endothelial cells, suggesting that the mTORC1 inhibitor directly suppresses the proliferation of angiogenic vessels in *Apc* ^{Δ 716} mouse polyps, although RAD001 treatment did not affect the level of VEGF in the angiogenic vessels. mTORC1 inhibitors can thus interfere with both proliferation of adenoma epithelial cells and tumor angiogenesis, by which intestinal polyp formation is likely to be inhibited in a synergistic manner. mTORC1 inhibition may be a safer strategy compared with VEGF pathway inhibition, because recent reports have shown that anti-angiogenic therapy using such inhibitors can accelerate malignant progression of the tumors.^{37,38} Namely, these inhibitors cause hypoxia in tumors, which induces accumulation of hypoxia inducible factor 1 (HIF-1) and subsequent expression of its target genes involved in cancer metastasis. mTORC1 inhibitors, in contrast, can suppress expression of HIF-1 α and its target genes.³⁹⁻⁴¹

Although the precise mechanism of mTORC1 activation in the intestinal polyps remains to be investigated, our data exclude the involvement of the PI3K-Akt and Erk pathways, AMPK and the nutrient status. Instead, we found that expression of mTOR at the mRNA and protein levels was increased significantly in the polyps of *Apc* ^{Δ 716} mice. In addition, knockdown of β -catenin in some human colorectal cancer cell lines carrying *APC* mutations reduced the mTOR protein levels. These results suggest that mTORC1 activation in intestinal tumors is mediated, at least partially, through the increased mTOR level induced by β -catenin accumulation (Fig. 1). Consistently, elevated levels of mTOR protein have been reported in clinical samples of colorectal cancer patients.⁴²

Two reports indicated crosstalks between Wnt signaling and mTORC1 pathway. Namely, GSK3 binds and phosphorylates TSC1/2 in the presence of Wnt, whereas Dishevelled can interact with TSC2 in the absence of Wnt, suggesting that Wnt signaling regulates the mTORC1 signaling.⁴³ On the other hand, mTORC1 signaling is activated by stimulation with

Wnt ligands through suppression of GSK3 activity in several types of cells including RIE, a rat intestinal epithelial cell line.⁴⁴ These results indicate that GSK3 complexed with Axin and Apc normally phosphorylates TSC2 and thereby activates TSC1/2 complex, and that inhibition of GSK3 activity by Wnt ligands liberates the mTORC1 from restriction by TSC1/2 complex. However, we have found no reduction in the kinase activity of GSK3 β in the *Apc* ^{Δ 716} polyps, compared with the normal intestinal epithelium, excluding the involvement of such a mechanism in the polyp adenoma. Taken together, we propose that accumulation of β -catenin caused by *Apc* LOH increases the level of mTOR protein in the intestinal polyps, which should enhance mTORC1 activation induced by still unidentified cues.

Perspectives

We have found important roles of mTORC1 signaling activation in the intestinal tumors of *Apc* ^{Δ 716} mice. However, questions remain as to what upstream signals stimulate mTORC1 and how the loss of *Apc* or mutation of β -catenin leads to enhanced mTOR expression.

Recently, several studies have identified new signaling molecules that are involved in controlling the mTORC1 activity. PRAS40, which has been known as an Akt substrate, can directly inhibit mTORC1, although the precise mechanism (i.e., binding to mTOR or competing with mTOR for substrate) appears controversial.⁴⁵⁻⁴⁷ The Rag GTPase can bind to mTORC1, and the binding is necessary for amino-acid induced mTORC1 activation.⁴⁸ On the other hand, it has been reported that FBXW7 (F-box and WD repeat domain-containing 7), an F-box protein component of SCF ubiquitin ligase, binds to mTOR protein and promotes its ubiquitylation and degradation thorough the proteasome pathway.⁴⁹ Interestingly, FBXW7 is inactivated in some colon cancer cell lines and patient lesions,⁵⁰ which may be responsible for stabilization of mTOR protein in colorectal cancer.

Our studies have indicated that mTORC1 inhibition suppresses intestinal tumorigenesis and reduces the mortality

of *Apc*^{Δ716} mice. We propose that targeting mTORC1 as a therapeutic strategy may be an effective armamentarium against colon cancer.

References

- Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006; 124:471-84.
- Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk: implications for tuberous sclerosis and cancer pathogenesis. *Cell* 2005; 121:179-93.
- Inoki K, Zhu T, Guan K-L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003; 115:577-90.
- Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 2003; 17:1829-34.
- Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y, et al. Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. *Science* 2007; 318:977-80.
- Easton JB, Houghton PJ. mTOR and cancer therapy. *Oncogene* 2006; 25:6436-46.
- Karakas B, Bachman KE, Park BH. Mutation of the PIK3CA oncogene in human cancers. *Br J Cancer* 2006; 94:455-9.
- Kwiatkowski DJ. Tuberous sclerosis: from tubers to mTOR. *Ann Hum Genet* 2003; 67:87-96.
- Majumder PK, Yeh JJ, George DJ, Febbo PG, Kum J, Xue Q, et al. Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: the MPAKT model. *Proc Natl Acad Sci USA* 2003; 100:7841-6.
- Rathmell JC, Elstrom RL, Cinalli RM, Thompson CB. Activated Akt promotes increased resting T cell size, CD28-independent T cell growth, and development of autoimmunity and lymphoma. *Eur J Immunol* 2003; 33:2223-32.
- Malstrom S, Tili E, Kappes D, Ceci JD, Tschlis PN. Tumor induction by an Lck-MyrAkt transgene is delayed by mechanisms controlling the size of the thymus. *Proc Natl Acad Sci USA* 2001; 98:14967-72.
- Wislez M, Spencer ML, Izzo JG, Juroskie DM, Balhara K, Cody DD, et al. Inhibition of mammalian target of rapamycin reverses alveolar epithelial neoplasia induced by oncogenic K-ras. *Cancer Res* 2005; 65:3226-35.
- Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, et al. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol* 1998; 8:1169-78.
- Freeman D, Lesche R, Kertesz N, Wang S, Li G, Gao J, et al. Genetic background controls tumor development in PTEN-deficient mice. *Cancer Res* 2006; 66:6492-6.
- Nardella C, Chen Z, Salmena L, Carracedo A, Alimonti A, Egia A, et al. Aberrant Rheb-mediated mTORC1 activation and Pten haploinsufficiency are cooperative oncogenic events. *Genes Dev* 2008; 22:2172-7.
- Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* 2008; 372:449-56.
- Takuwa N, Fukui Y, Takuwa Y. Cyclin D1 expression mediated by phosphatidylinositol 3-kinase through mTOR-p70S6K-independent signaling in growth factor-stimulated NIH 3T3 fibroblasts. *Mol Cell Biol* 1999; 19:1346-58.
- Decker T, Hipp S, Ringshausen I, Bogner C, Oelsner M, Schneller F, et al. Rapamycin-induced G₁ arrest in cycling B-CLL cells is associated with reduced expression of cyclin D3, cyclin E, cyclin A, and survivin. *Blood* 2003; 101:278-85.
- Ramírez-Valle F, Braunstein S, Zavadil J, Formenti SC, Schneider RJ. eIF4G links nutrient sensing by mTOR to cell proliferation and inhibition of autophagy. *J Cell Biol* 2008; 181:293-307.
- Hong F, Larrea MD, Doughty C, Kwiatkowski DJ, Squillace R, Slingerland JM. mTOR-raptor binds and activates SGK1 to regulate p27 phosphorylation. *Mol Cell* 2008; 30:701-11.
- Aoki K, Tamai Y, Horiike S, Oshima M, Taketo MM. Colonic polyposis caused by mTOR-mediated chromosomal instability in *Apc*^{Δ716} *Cdx2*^{-/-} compound mutant mice. *Nat Genet* 2003; 35:323-30.
- Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; 350:2335-42.
- Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002; 8:128-35.
- Riesterer O, Zingg D, Hummerjohann J, Bodis S, Pruschy M. Degradation of PKB/Akt protein by inhibition of the VEGF receptor/mTOR pathway in endothelial cells. *Oncogene* 2004; 23:4624-35.
- Viñals F, Chambard JC, Pouyssegur J. p70 S6 kinase-mediated protein synthesis is a critical step for vascular endothelial cell proliferation. *J Biol Chem* 1999; 274:26776-82.
- Nozawa H, Watanabe T, Nagawa H. Phosphorylation of ribosomal p70 S6 kinase and rapamycin sensitivity in human colorectal cancer. *Cancer Lett* 2007; 251:105-13.
- Shao J, Evers BM, Sheng H. Roles of phosphatidylinositol 3'-kinase and mammalian target of rapamycin/p70 ribosomal protein S6 kinase in K-Ras-mediated transformation of intestinal epithelial cells. *Cancer Res* 2004; 64:229-35.
- Lu TL, Chang JL, Liang CC, You LR, Chen CM. Tumor spectrum, tumor latency and tumor incidence of the Pten-deficient mice. *PLoS ONE* 2007; 2:1237.
- Calcagno SR, Li S, Colon M, Kreinest PA, Thompson EA, Fields AP, et al. Oncogenic K-ras promotes early carcinogenesis in the mouse proximal colon. *Int J Cancer* 2008; 122:2462-70.
- Taketo MM. Wnt signaling and gastrointestinal tumorigenesis in mouse models. *Oncogene* 2006; 25:7522-30. Review.
- Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, et al. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992; 359:235-7.
- Phelps RA, Chidester S, Dehghanizadeh S, Phelps J, Sandoval IT, Rai K, et al. A two-step model for colon adenoma initiation and progression caused by APC loss. *Cell* 2009; 137:623-34.
- Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated *Apc* gene. *Proc Natl Acad Sci USA* 1995; 92:4482-6.
- Taketo MM, Edelmann W. Mouse models of colon cancer. *Gastroenterology* 2009; 136:780-98.
- Fujishita T, Aoki K, Lane HA, Aoki M, Taketo MM. Inhibition of the mTORC1 pathway suppresses intestinal polyp formation and reduces mortality in *Apc*^{Δ716} mice. *Proc Natl Acad Sci USA* 2008; 105:13544-9.
- Seno H, Oshima M, Ishikawa TO, Oshima H, Takaku K, Chiba T, et al. Cyclooxygenase 2- and prostaglandin E₂ receptor EP₂-dependent angiogenesis in *Apc*^{Δ716} mouse intestinal polyps. *Cancer Res* 2002; 62:506-11.
- Páez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, et al. Antiangiogenic Therapy Elicits Malignant Progression of Tumors to Increased Local Invasion and Distant Metastasis. *Cancer Cell* 2009; 15:220-31.
- Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell* 2009; 15:232-9.
- Thomas GV, Tran C, Mellinshoff IK, Welsbie DS, Chan E, Fueger B, et al. Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat Med* 2006; 12:122-7.
- Averous J, Fonseca BD, Proud CG. Regulation of cyclin D1 expression by mTORC1 signaling requires eukaryotic initiation factor 4E-binding protein 1. *Oncogene* 2008; 27:1106-13.
- Shackelford DB, Vasquez DS, Corbeil J, Wu S, Leblanc M, Wu CL, et al. mTOR and HIF-1 α -mediated tumor metabolism in an LKB1 mouse model of Peutz-Jeghers syndrome. *Proc Natl Acad Sci USA* 2009; 106:11137-42.
- Xu G, Zhang W, Bertram P, Zheng XF, McLeod H. Pharmacogenomic profiling of the PI3K/PTEN-AKT-mTOR pathway in common human tumors. *Int J Oncol* 2004; 24:893-900.
- Mak BC, Kenerson HL, Aicher LD, Barnes EA, Yeung RS. Aberrant β -catenin signaling in tuberous sclerosis. *Am J Pathol* 2005; 167:107-16.
- Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* 2006; 126:955-68.
- Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* 2007; 9:316-23.
- Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, et al. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* 2007; 25:903-15.
- Oshiro N, Takahashi R, Yoshino K, Tanimura K, Nakashima A, Eguchi S, et al. The proline-rich Akt substrate of 40 kDa (PRAS40) is a physiological substrate of mammalian target of rapamycin complex 1. *J Biol Chem* 2007; 282:20329-39.
- Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 2008; 320:1496-501.
- Mao JH, Kim IJ, Wu D, Climent J, Kang HC, DelRosario R, et al. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science* 2008; 321:1499-502.
- Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, et al. Inactivation of hCDC4 can cause chromosomal instability. *Nature* 2004; 428:77-81.