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Cancer Cell
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# Rab1A Is an mTORC1 Activator and a Colorectal Oncogene

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http://dx.doi.org/10.1016/j.ccell.2014.09.008

## SUMMARY

**Amino acid (AA) is a potent mitogen that controls growth and metabolism. Here we describe the identification of Rab1 as a conserved regulator of AA signaling to mTORC1. AA stimulates Rab1A GTP binding and interaction with mTORC1 and Rheb-mTORC1 interaction in the Golgi. Rab1A overexpression promotes mTORC1 signaling and oncogenic growth in an AA- and mTORC1-dependent manner. Conversely, Rab1A knockdown selectively attenuates oncogenic growth of Rab1-overexpressing cancer cells. Moreover, Rab1A is overexpressed in colorectal cancer (CRC), which is correlated with elevated mTORC1 signaling, tumor invasion, progression, and poor prognosis. Our results demonstrate that Rab1 is an mTORC1 activator and an oncogene and that hyperactive AA signaling through Rab1A overexpression drives oncogenesis and renders cancer cells prone to mTORC1-targeted therapy.**

## INTRODUCTION

Cell growth is a process of assimilating extracellular nutrients into the cell mass, which requires the coordinated regulation of nutrient transport and protein synthetic capability. Signaling modules have evolved to transduce nutrient cues to cellular programs such as transcription and translation (Dechant and Peter, 2008; Jorgensen and Tyers, 2004; Zaman et al., 2008). mTOR is a conserved central growth controller in eukaryotes (Loewith and Hall, 2011; Sengupta et al., 2010). It forms two distinct kinase complexes, mTORC1 and mTORC2 (Loewith et al., 2002; Sarbassov et al., 2004). In response to nutrient signals, mTORC1 controls cellular growth and metabolic processes, and mTORC2 regulates survival through AKT phosphorylation.

Hyperactive mTORC1 signaling is a major cause of human diseases such as cancer (Tsang et al., 2007). Because mTORC1 is commonly hyperactivated in human tumors, it is a desirable target for cancer therapy (Bjornsti and Houghton, 2004; Zhang et al., 2011). Two rapamycin analogs (rapalogs), everolimus and temsirolimus, are Food and Drug Administration-approved drugs for advanced renal and breast carcinomas. However, the overall objective response rate remains low for rapalogs. Therefore, studying the regulation of mTORC1 is of considerable biological and clinical importance.

### Significance

AA is an essential nutrient and key chemical signal for cell growth and metabolism. This study shows that Rab1A, a small GTPase previously known for vesicular trafficking, has a crucial role in the Rag- and lysosome-independent activation of mTORC1 by AA and elucidates a Golgi-based mechanism by which Rab1A engages mTORC1 interaction with Rheb. It further demonstrates that aberrant hyperactivation of AA signaling through Rab1A overexpression is a common event driving oncogenic transformation and malignant growth. Moreover, Rab1A overexpression is correlated with rapamycin sensitivity, suggesting that it is a determinant for mTORC1-targeted cancer therapy. Further study of this process could have important implications in normal cell physiology and the pathobiology and therapy of human cancer.

AA is not only an essential nutrient but also a potent mitogen. AA rapidly activates mTORC1. Rag proteins are lysosomal/vacuolar membrane-bound small GTPases. Upon AA stimulation, Rag GTPases function as heterodimers that bind to and activate TORC1 (Kim et al., 2008; Sancak et al., 2008). In the presence of leucine, leucyl tRNA synthetase binds to Rags and promotes TORC1 signaling (Han et al., 2012). Rag is well conserved from yeast to humans (Sekiguchi et al., 2001). The yeast Rag homologs Gtr1 and Gtr2 have been shown recently to also mediate AA signaling to TORC1 (Binda et al., 2009; Bonfils et al., 2012). Because the importance of AA in cell growth and metabolism, however, Rag proteins are probably not the only sensors. The goal of this study is to identify the Rag-independent regulator of AA signaling and investigate the underlying mechanism and significance.

## RESULTS

### Ypt1 Is Essential for AA to Activate TORC1 in Yeast

Gtr1 and Gtr2, the yeast orthologs of RagA/RagB and RagC/RagD, respectively, function as a heterodimer to regulate TORC1 (Binda et al., 2009; Bonfils et al., 2012). Consistently, *gtr1Δ* and *gtr2Δ* mutants are hypersensitive to rapamycin (Figure 1A), indicative of their role in TORC1 signaling (Bertram et al., 1998). However, these mutants exhibit no apparent growth defect, and AA can still fully activate TORC1 in *gtr1Δ* and *gtr2Δ* mutants, as judged by phosphorylation of the TORC1 substrates Sch9 and Maf1 (Figure 1B) (Wei and Zheng, 2009; Wei and Zheng, 2010). Additionally, the dominant-active Gtr1-guanosine triphosphate (GTP) and Gtr2-guanosine diphosphate (GDP) do not affect TORC1 activity during AA starvation and restimulation (Figure 1C). The yeast vacuole (lysosome) anchors Gtr1 and Gtr2 signaling (Binda et al., 2009; Bonfils et al., 2012), but the growth and TORC1 signaling remain normal in vacuolar biogenesis mutants *vps16Δ*, *vps33Δ*, *pep3Δ*, and *pep5Δ* (Figures 1A and 1D). Together, these observations clearly show that GTR and the vacuole are dispensable for AA signaling to TORC1 in yeast.

We showed previously that genes in the TORC1 pathway display the rapamycin-sensitive phenotype (Bertram et al., 1998). Using this assay, we carried out a genomic screen and identified a large set of TORC1 signaling genes (Chan et al., 2000). Because most known mTORC1 activators are small GTPases (e.g., Rheb, Rag, and Rho1) (Inoki et al., 2003; Kim et al., 2008; Sancak et al., 2008; Stocker et al., 2003; Tee et al., 2003; Yan et al., 2012; Zhang et al., 2003), we focused our search for the Rag-independent TORC1 activator on small GTPases, particularly Rab, one of the largest small GTPase subfamilies (Hutagalung and Novick, 2011; Stenmark, 2009). Among the nine nonessential yeast Rab mutants, *ypt6Δ* and *ypt7Δ* are hypersensitive to rapamycin (Figure 1E). With the two essential Rab genes, when assayed under Tet-off conditions (Hughes et al., 2000), the *Tet-YPT1* strain, but not the *Tet-SEC4* strain, showed a rapamycin-hypersensitive phenotype (Figure 1F). The *ypt1-ts* mutant also displayed rapamycin hypersensitivity (Figure 1G). Therefore, Ypt1, Ypt6, and Ypt7 are involved in TORC1 signaling. However, depletion of *YPT1* (Figure 1H), but not *YPT6* and *YPT7*, blocks the activation of TORC1 by AA (Figures 1H and 1I), indicating that Ypt1 is essential for AA signaling. The precise role of Ypt6 and Ypt7 in TORC1 signaling is presently unknown.

Ypt1 is the yeast paralog of Rab1, a highly conserved Golgi membrane-bound GTPase previously known for ER-to-Golgi vesicular trafficking (Hutagalung and Novick, 2011; Stenmark, 2009). However, a large number of yeast mutants in ER-Golgi trafficking do not display a rapamycin-sensitive phenotype (Figure S1A available online), suggesting that the role of Rab1 in TORC1 signaling is not directly related to its trafficking function. Consistently, Ypt1 interacts with Tor1, which is a TORC1-specific component in yeast, in a GTP- and AA-dependent manner (Figure S1B), and AA stimulates GTP-binding by Ypt1 (Figures S1C and S1D). These results indicate that AA regulates Ypt1 and its GTP-dependent interaction with TORC1.

### AA Stimulates Rab1A Interaction with mTORC1 in a GTP- and Golgi-Dependent Manner in HEK293E cells

A recent RNAi screen revealed that *dRAB1* knockdown in *Drosophila* S2 cells strongly inhibits dS6K phosphorylation (Li et al., 2010), suggesting that the Rab1 function in TORC1 signaling is conserved. To further explore this, we knocked down Rab1A in human embryonic kidney 293E (HEK293E) cells with three distinct Rab1A small hairpin RNAs (shRNAs), all of which efficiently downregulate Rab1A expression and inhibit the phosphorylation of S6K1(T389) but not AKT(S473), indicating that Rab1A is specifically required for mTORC1 signaling (Figure 2A). Rab1A knockdown attenuates the activation of mTORC1 by AA (Figure 2B). In contrast, mTORC1 can still be stimulated by insulin after Rab1A knockdown (albeit the overall mTORC1 signaling level is decreased) (Figure 2C). The latter phenomenon is similar to that of Rag knockdown (Kim et al., 2008).Therefore, Rab1A function in AA signaling is conserved in humans. Of note, Rab1A knockdown does not cause cell death, as judged by lack of PARP cleavage (Figure 2A), which is consistent with the mTORC1-specific function for Rab1A.

As in yeast, endogenous Rab1A also interacts with mTORC1 (Figure 2D). Hemagglutinin (HA)-Rab1A is associated with mTOR and Raptor, not Rictor (Figure S2A), and is bound more with Myc-Raptor than Myc-mTOR (Figures S2B and S2C). Myc-Raptor, not Myc-mTOR, remained associated with HA-Rab1A after the immunocomplex was washed with 0.5% TX-100, a condition known to disrupt mTORC1 (Figure S2D), indicating that Raptor mediates the binding of Rab1A. Rab1A is a small GTP-binding protein anchored on ER and Golgi membranes through prenylation (Calero et al., 2003; Gomes et al., 2003). mTOR preferentially binds to Rab1A-GTP (Rab1A-Q70L, $Rab1A^{GTP}$) (Pind et al., 1994) compared with Rab1A-GDP (Rab1A-S25N, $Rab1A^{GDP}$) (Alvarez et al., 2003) or a $Rab1A^{C202,\ 203S}$ mutant ($Rab1A^{C2S}$) deficient of prenylation and ER/Golgi localization (Calero et al., 2003; Gomes et al., 2003) (Figure 2E).

Upon AA stimulation, there is a significant increase in the GTP-binding activity of Rab1A (Figures 2F and 2G). Moreover, AA regulates the Rab1A interaction with mTORC1 in a GTP-dependent manner (Figure 2H). Strikingly, $Rab1A^{GTP}$ binds persistently to mTORC1 and sustains mTORC1 signaling even during AA starvation (Figures 2H and 2I). In contrast, serum and insulin do not affect Rab1A GTP-binding (Figure 2J) or the Rab1A-mTORC1

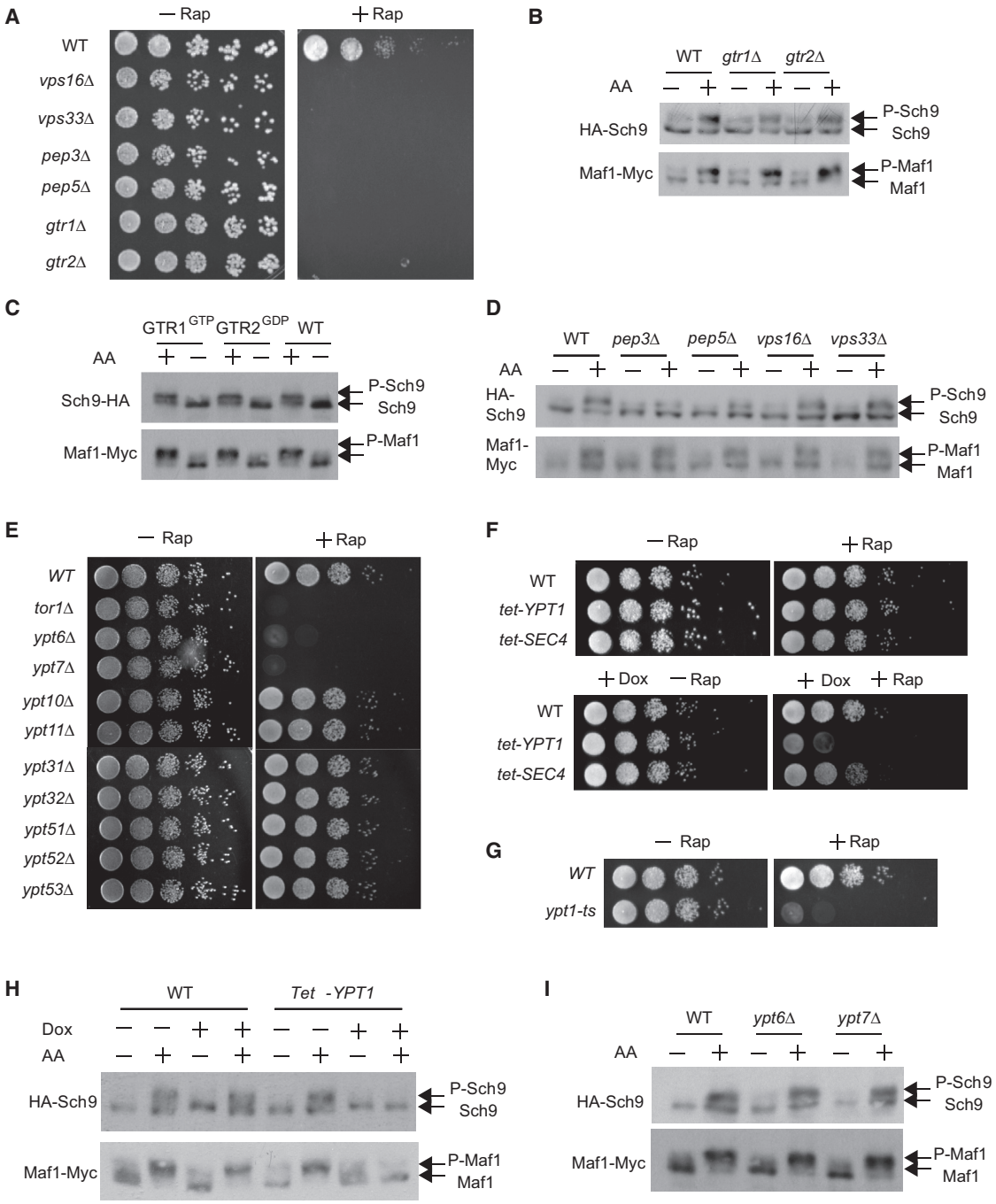


**Figure 1. Ypt1/Rab1 Is Crucial for AA to Activate TORC1 in Yeast**

(A) *GTR* wild-type (WT) and mutant yeast cells were assayed for rapamycin sensitivity by a spot assay (10-fold serial dilutions).

(B) WT, *gtr1Δ*, and *gtr2Δ* cells were starved of AA and restimulated. TORC1 signaling was measured by immunoblotting for phosphorylation of HA-Sch9 and Maf1-Myc.

(C) Yeast expressing Gtr1-GTP or Gtr2-GDP was starved of AA, restimulated, and assayed for TORC1 signaling as above.

(D) The WT and vacuolar biogenesis mutants were starved, restimulated with AA, and assayed for TORC1 signaling.

(E) Nonessential Rab gene deletion mutants were measured for rapamycin sensitivity. The *tor1Δ* strain was used as a positive control.

(F) The WT and Tet-off essential Rab mutant strains were assayed for rapamycin sensitivity with or without doxycycline (Dox).

(G) The WT and the *ypt1* temperature-sensitive (*ypt1-ts*) mutant were assayed for rapamycin sensitivity at a permissive temperature.

(H) WT and *Tet-YPT1* cells were starved and restimulated with AA in the presence or absence of Dox, and assayed for TORC1 signaling.

(I) WT, *ypt6Δ*, and *ypt7Δ* cells were starved, restimulated with AA, and assayed for TORC1 signaling.

See also Figure S1.

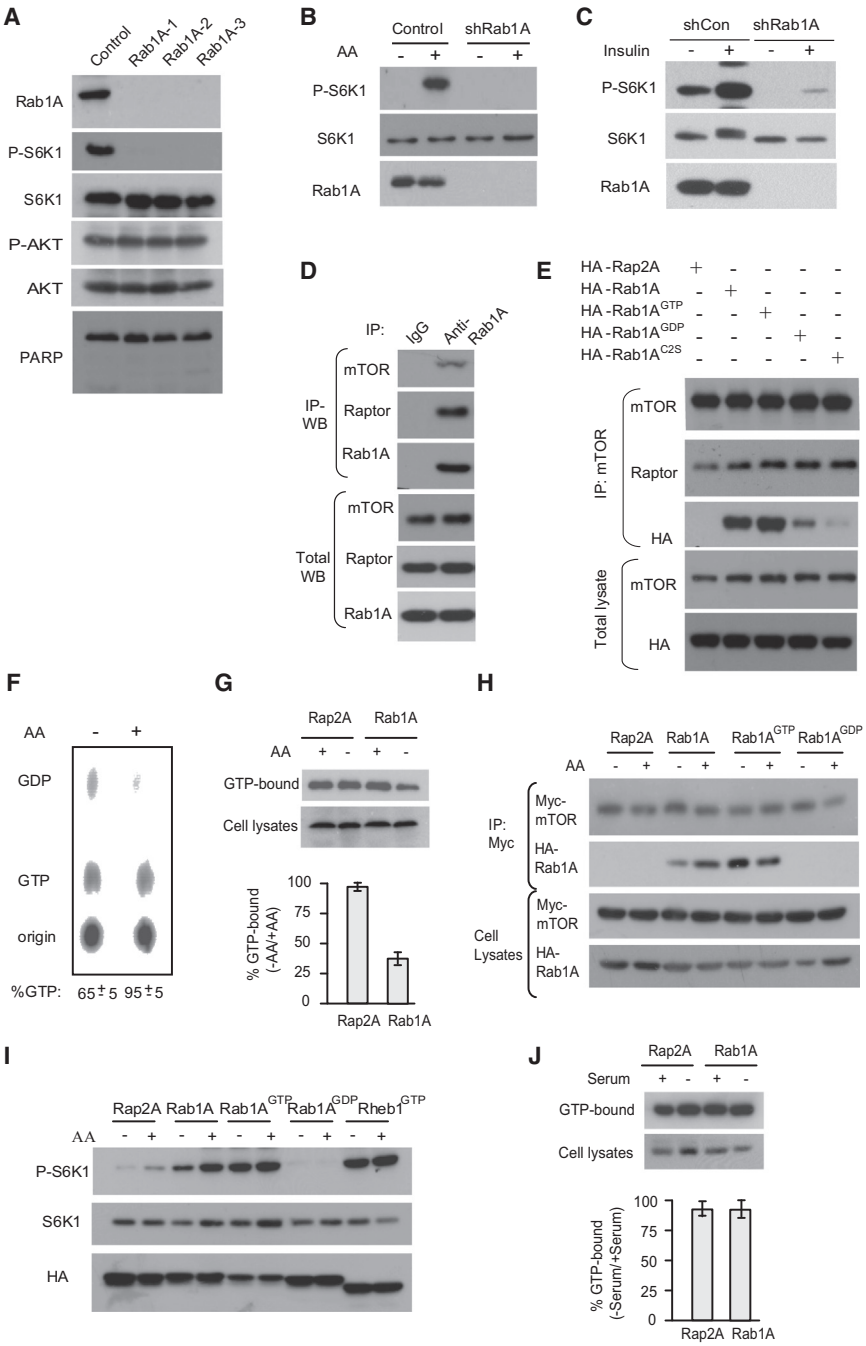


**Figure 2. Rab1A Is Essential for mTORC1 Activation by AA in Human Cells**

(A) HEK293E cells infected with lentiviral Rab1A shRNAs were analyzed for P-S6K1(T389), S6K, P-AKT(S473), AKT, and PARP cleavage.

(B) HEK293E cells infected with Rab1A shRNA were starved, restimulated with AA for 10 min, and analyzed for P-S6K1(T389).

(C) HEK293E cells were starved of serum (full complement of culture ingredients except serum), restimulated with 100 ng insulin for 10 min, and analyzed for P-S6K1(T389).

(D) Endogenous Rab1A was immunoprecipitated and analyzed for the presence of mTOR and Raptor.

(E) WT and mutant HA-Rab1A was transiently expressed in HEK293E cells. mTOR was immunoprecipitated and analyzed for its interaction with HA-tagged proteins and endogenous raptor. HA-Rap2A was a negative control.

(F) $^{32}$P-labeled HEK293E cells were starved and restimulated with AA. Rab1A was immunoprecipitated and analyzed for the bound $^{32}$P-labeled GTP and GDP by thin-layer chromatography. The numbers at the bottom show means ± SD of three independent experiments.

(G) HEK293E cells were starved and restimulated with AA. Extracts of cells were incubated with GTP-agarose beads and the binding of Rab1A were analyzed by western blot. The bottom panel shows means ± SD of three independent experiments.

(H) HEK293E cells expressing Myc-mTOR and WT or mutant HA-Rab1A proteins were starved and re-stimulated with AA. Myc-mTOR and HA-Rab1A were analyzed for interaction by co-IP. HA-Rap2A is a negative control.

(I) The same as Figure 2H, except mTORC1 signaling was analyzed by P-S6K1. Rheb was used as a positive control.

(J) The same as Figure 2G, except cells were starved and restimulated with serum. The bottom panel shows means ± SD of three independent experiments.

See also Figure S2.

interaction (Figure S2E). These observations indicate that Rab1A mediates AA signaling to mTORC1, which is a conserved phenomenon. Curiously, AA starvation disrupts the association of Ypt1$^{GTP}$ with TORC1 in yeast but not Rab1A$^{GTP}$ with mTORC1 in humans. This is likely due to the fact that nutrients such as AA play a more prominent growth regulatory role in single-celled organisms than in mammals. The latter is regulated by nutrients as well as polypeptide factors (e.g., cytokines and hormones). Consistent with this notion, starvation of HEK293E cells of both serum and AA also blocks the binding of Rab1A$^{GTP}$ to mTORC1 (data not shown).

## Rab1A Regulates the Formation of the Rheb-mTORC1 Complex in the Golgi

When ectopically expressed, Rab1A stimulates the level of P-S6K1 and P-4EBP1 but not P-AKT (Figures S3A and S3B), indicating that Rab1A overexpression specifically promotes mTORC1 signaling. The ability of Rab1A to enhance mTORC1 signaling is dependent on Rab1A GTP-binding and association with Golgi/ER membranes because such Rab1A$^{GDP}$ and Rab1A$^{C202,\ 203S}$ mutants fail to increase S6K1 phosphorylation (Figures S3A and S3B). To understand the underlying mechanism, we examined the functional relationship between Rab1A and two other major mTORC1 activators, Rheb and Rag. Rab1A knockdown strongly attenuates RagB/RagC-dependent mTORC1 activation by AA (Figure 3A). On the other hand,

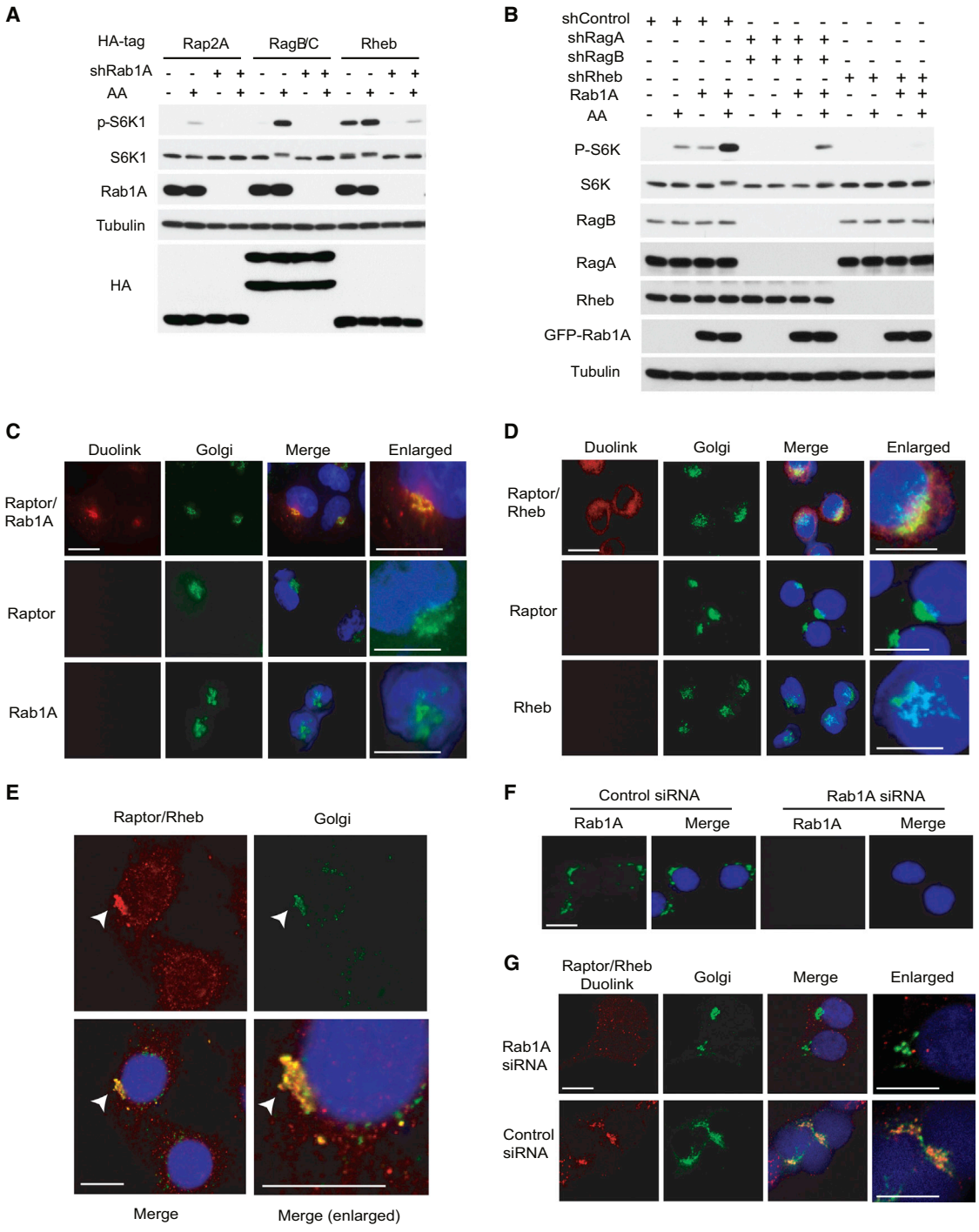


**Figure 3. Rab1A Stimulates mTORC1 Signaling and Regulates Rheb-mTORC1 Interaction in the Golgi**

(A) HEK293E cells infected with Rab1A shRNA or a control shRNA in the presence or absence of overexpressed RagB/RagC or Rheb were starved with AA (containing all culture ingredients except AA) and restimulated with AA for 10 min. The effect of Rab1A knockdown on P-S6K1 was analyzed by western blot.

(B) HEK293E cells overexpressing HA-Rab1A were infected with lentiviral shRNA against RagA/RagB or Rheb and then treated with AA starvation and restimulation. The effect on mTORC1 signaling was assayed by western blot.

(C) Duolink was used to detect the interaction of endogenous Rab1A and Raptor (red) in HEK293E cells transiently expressing GM130-GFP (Golgi, green). Shown is a representative image (n > 350). Scale bars, 10 μm.

(D) Duolink was used to detect the interaction of endogenous Rheb and Raptor (red) in HEK293E cells transiently expressing GM130-GFP (Golgi, green). Shown is a representative image (n > 350). Scale bars, 10 μm.

(E) The same as Figure 3E, except the images were analyzed by confocal microscopy. Shown is a Z section of the confocal images. Arrowheads indicate Golgi location. Scale bars, 10 μm.

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although downregulation of Rheb abolishes Rab1A-dependent mTORC1 activation by AA, RagA/RagB knockdown only has a partial effect (Figure 3B). Curiously, the dominant-active $RagB^{GTP}/RagC^{GDP}$ can partially rescue the loss of Rab1A (Figure S3C). These observations indicate that the regulation of mTORC1 by Rab1A is dependent on Rheb but that Rab1A and Rag are relatively independent of each other, with Rab1A having a more prominent role, which is consistent with the yeast results.

Although Rag GTPases regulate mTORC1 in lysosomes (Sancak et al., 2010), mTORC1 is widely distributed throughout the cell, including the ER, the Golgi, endosomes, mitochondria, and the nucleus (Drenan et al., 2004; Li et al., 2006; Liu and Zheng, 2007; Sancak et al., 2008; Schieke et al., 2006), suggesting that the regulation of mTORC1 signaling is more complex than currently thought, involving multiple subcellular systems and mechanisms. To locate where Rab1A engages mTORC1, we used Duolink, which allows the detection of protein-protein interactions in situ in intact cells and tissues (Söderberg et al., 2006), to analyze in situ Rab1A-mTORC1 interaction. Antibodies against Rab1A and Raptor or mTOR together, but not each individually or under the condition that one partner is knocked down, generated a strong signal in the Golgi (Figure 3C; Figures S3D–S3H). Therefore, Rab1A interacts with mTORC1 specifically in the Golgi, which is consistent with our earlier observation that Golgi localization is required for Rab1A to bind to and activate mTORC1 (Figure 2E; Figure S3A).

To further investigate the mechanism of mTORC1 regulation by Rab1A, we examined the localization of the Rheb-mTORC1 complex. Rheb-Raptor interaction prominently occurs in the Golgi, as judged by conventional and confocal microscopy (Figures 3D and 3E; Figure S3D). This result is consistent with the observation that both Rheb and mTORC1 are prominently localized in this organelle (Figure S3I) (Buerger et al., 2006; Drenan et al., 2004; Hanker et al., 2010; Liu and Zheng, 2007). In addition, Rheb interaction with mTORC1 has been mainly detected in the Golgi in live cells (Yadav et al., 2013). Raptor-Rheb15 is a fusion protein of Raptor and the C-terminal Rheb CAAX signal sequence. When expressed in cells, it causes constitutive activation of mTORC1 (Sancak et al., 2010). Because Rheb15 contains the CAAX motif that is sufficient to target Rheb to the Golgi (Buerger et al., 2006; Hanker et al., 2010), we investigated Raptor-Rheb15 subcellular localization and found that it is indeed predominantly found in the Golgi (Figures S3J and S3K). Moreover, Rab1A knockdown disrupts mTORC1 localization or Rheb-mTORC1 interaction in the Golgi (Figures 3F and 3G; Figure S3L) while not affecting mTORC1 localization or interaction with Rag in the lysosomes (Figures S3M-S3Q). Therefore, Rab1A controls mTORC1 signaling by regulating the formation of the Golgi Rheb-mTORC1 complex.

## Rab1A Is Overexpressed in Human Colorectal Cancer, Which Is Correlated with Hyperactive mTORC1 Signaling, Tumor Invasion, and Poor Prognosis

A previous microarray study revealed that Rab1A is overexpressed in 98% of human tongue squamous carcinomas (Shimada et al., 2005). To ask whether Rab1A is also aberrantly expressed in other malignancies, we performed immunohistochemistry (IHC) staining of Rab1A in primary human colorectal cancer (CRC) and adjacent normal tissues. Rab1A staining is much stronger (median H score, 255) in tumors than the matching normal tissues (median H score, 40) (Figures 4A and 4B). Rab1A is scored higher in approximately 80% tumors, although the intensity is markedly variable, with IHC scores 100-fold higher in tumors than in normal tissues (Figure 4C).

We next examined the relationship between Rab1A expression and mTORC1 signaling by staining Rab1A and P-S6K1 in consecutive tissue sections. Overall, Rab1A expression is strongly correlated with P-S6K1 staining (Figures 4D and 4E). In tumors with heterogeneous Rab1A expression, the P-S6K1 level still showed a striking correlation with the Rab1A level. In tumor nodules with high Rab1A expression, P-S6K1 staining is also stronger (Figure 4F). We further examined the relationship between Rab1A staining and different clinicopathologic parameters and found that high Rab1A-positive staining is statistically significantly correlated with poor survival (Figure 4G), increased tumor invasion, and advanced tumor stages (Table S1). In addition, high Rab1A-positive staining appears to be associated with lymph node metastasis, but the result is not statistically significant because of limited sample size (Table S1). Together, these results show that Rab1A is frequently overexpressed in CRC and that high Rab1A expression is correlated with hyperactive mTORC1 signaling, tumor invasion and progression, and poor prognosis.

## Rab1A Is Essential for the Oncogenic Growth of Rab1A-Overexpressing CRC Cells

In a panel of CRC cell lines, Rab1A expression is also highly variable, ranging from high (e.g., DLD-1 and CACO2) to low (e.g., RKO and COLO205) but is correlated with P-S6K1, not P-AKT (Figure 5A; Figures S4A and S4B). Therefore, these cell lines have similar characteristics as primary tumors and are good models for studying the significance of Rab1A expression. Surprisingly, P-S6K1 is not correlated with the expression of Rab1B, Rheb, RagA, RagB, RagC, and RagD (Figures S4C–S4H), suggesting that, unlike Rab1A, other known mTORC1 activators are not responsible for hyperactive mTORC1 signaling in CRC cells. When Rab1A is knocked down in three pairs of CRC cell lines (DLD-1 and CACO2, KM12 and HCT116, and RKO and COLO205, representative of high, moderate and low Rab1A expression in cells, respectively), mTORC1 signaling is abrogated (Figure 5B). However, there is a striking inverse correlation between growth inhibition and Rab1A expression.

(F) HEK293E cells were transfected with a Rab1A small interfering RNA (siRNA) or a control siRNA, and the Rab1A protein was analyzed by IF microscopy. Scale bar, 10 μm.
(G) HEK293E cells transiently expressing GM130-GFP (green) were analyzed for Rheb-Raptor interaction by Duolink (red) in the presence of Rab1A or control siRNA. Shown is a representative image (n > 350). Scale bars, 10 μm.
See also Figure S3.

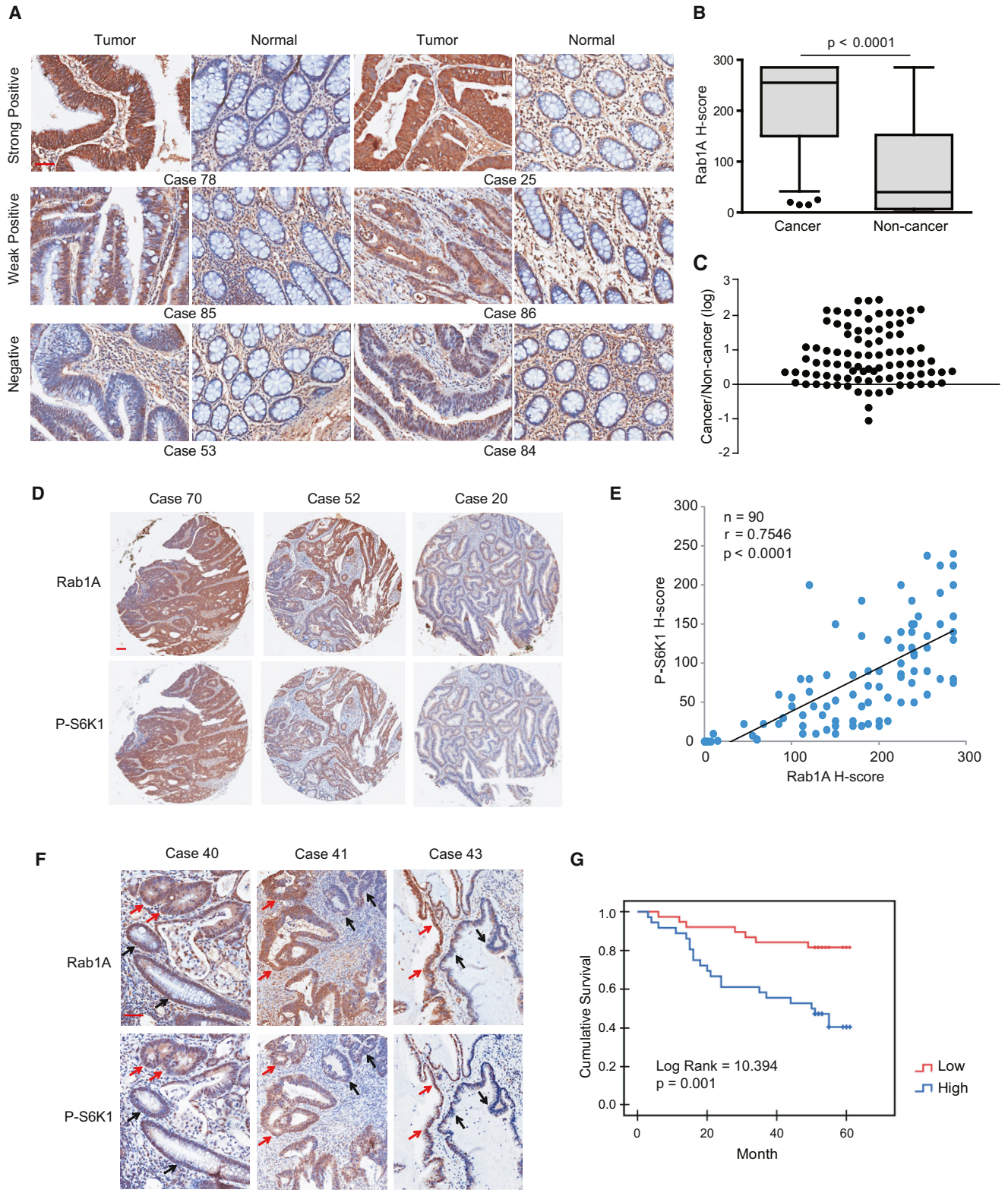


**Figure 4. Rab1A Is Frequently Overexpressed in CRC, Which Is Correlated with Hyperactive mTORC1 Signaling and Poor Survival**

(A) IHC staining of the primary human CRC tissue microarray and adjacent noncancerous tissues. Shown are stained tumor and noncancerous tissue sections representative of high, low, and negative Rab1A staining. Scale bar, 50 μm.

(B) Box plot graph showing a statistical analysis of Rab1A expression in CRC and adjacent noncancerous tissues.

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Growth and colony formation are strongly, moderately, and slightly inhibited, respectively, in the three cell pairs (Figures 5C–5H). Of note, Rab1A knockdown did not affect cell viability because no significant increase in apoptotic cell death was seen (data not shown), which is consistent with the results with HEK293E cells (Figure 2A). Furthermore, Rab1A knockdown attenuates mTORC1 signaling (P-S6K1 staining) and oncogenic growth (Ki67 staining and mitotic index) of DLD-1 xenograft tumors in nude mice (Figures 5I–5K). The fact that Rab1A knockdown preferentially inhibits CRC cells with high Rab1A suggests that Rab1A overexpression is a key driver for cancer growth.

### Rab1A Overexpression Rather Than Activated PI3K or MEK Is Essential for Hyperactive mTORC1 Signaling and Oncogenic Growth of CRC Cells

Phosphatidylinositol 3-kinase (PI3K) is thought to be a major upstream regulator of mTOR signaling. DLD1, HCT116, and RKO each carry a heterozygote-activating mutant *PIK3CA* allele. The P-S6K1 level is very low in RKO (Figure 5A), indicating that activated PI3K is insufficient to promote mTORC1 signaling in RKO cells. However, P-S6K is moderate or high in DLD1 and HCT116 (Figure 5A), presenting an interesting dilemma. Is Rab1A or PI3K responsible for elevated mTORC1 signaling and oncogenic growth? To address this question, we analyzed the isogenic DLD1 and HCT116 cell lines in which the $PIK3CA^{WT}$ or $PIK3CA^{mutant}$ allele is deleted (Samuels et al., 2005). Deletion of the $PIK3CA^{mutant}$ allele abolishes AKT but not S6K1 phosphorylation (Figures 6A; Figure S5A), which is in contrast to Rab1A knockdown, which abolishes mTORC1 signaling (Figure 6A). Moreover, Rab1A knockdown, rather than $PIK3CA^{mutant}$ deletion, attenuates CRC cell growth and colony formation (Figures 6B–6E). This result is consistent with a previous report showing that the activated PI3K mutant is responsible for enhanced survival but not growth of CRC cells (Samuels et al., 2005). The Ras/MEK/ERK pathway is another major mitogenic signaling pathway that is frequently mutated in CRC. However, MEK inhibition had little effect on mTORC1 signaling, cell growth, or colony formation of DLD1 and CACO2 cells that both have Rab1A overexpression and high ERK signaling (Figures S5B–S5D). These data show that Rab1A-AA signaling, rather than PI3K and the MEK pathway, is crucial for hyperactive mTORC1 signaling and growth of DLD1, HCT116, and CACO2 cells.

### CRC Cells with Rab1A Overexpression Are Addictive to AA for Growth

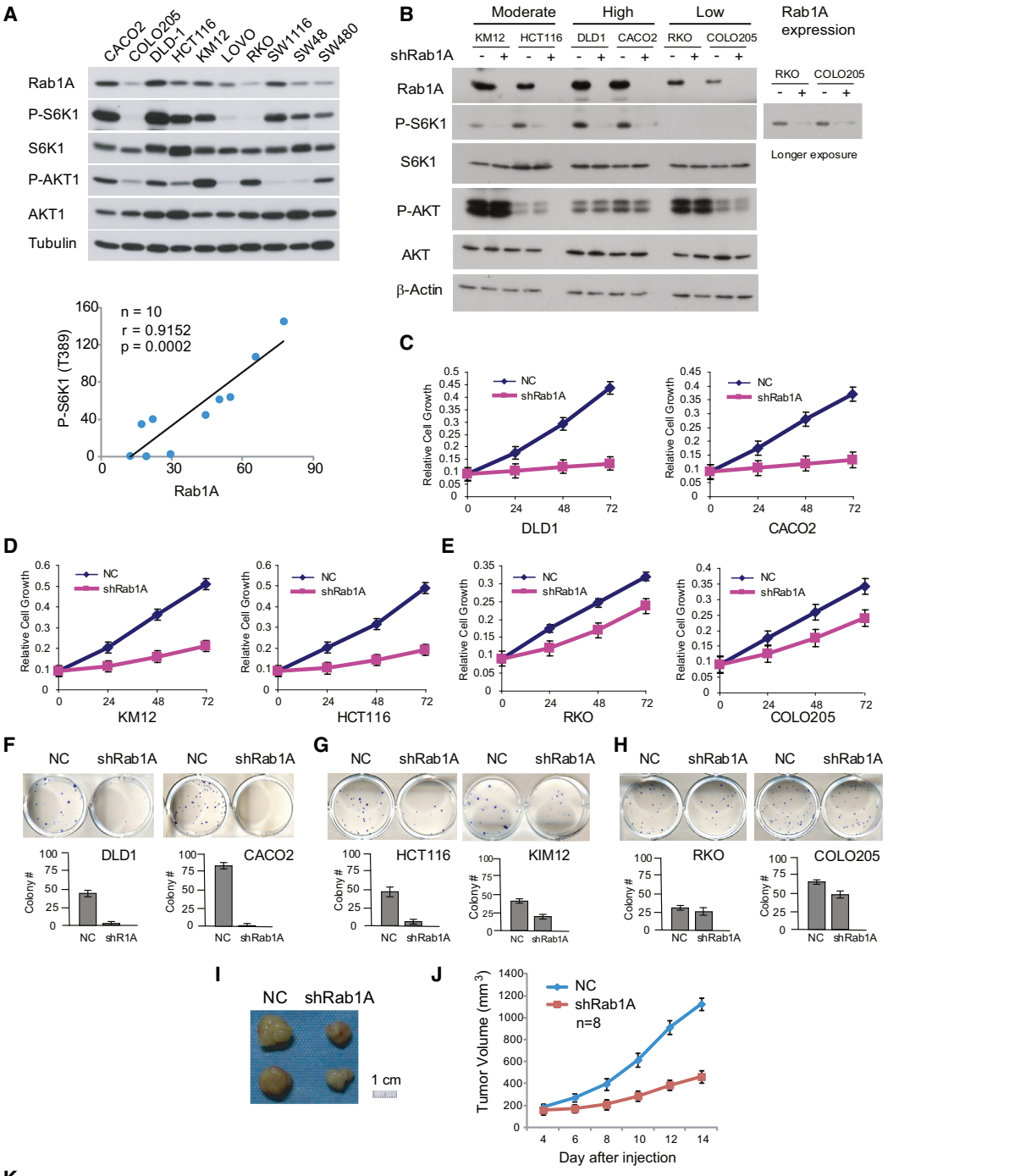
Because Rab1A mediates AA signaling to activate mTORC1, we investigated how AA modulates CRC oncogenic growth by analyzing the effect of AA restriction on three pairs of CRC cell lines with different Rab1A expression levels. There is a striking inverse relationship between Rab1A expression and cell growth during AA restriction. DLD1 and CACO2 cells (high Rab1A expression) are much more sensitive to AA restriction than RKO and COLO205 (low Rab1A expression), whereas HCT116 and KM12 (modest Rab1A expression) are intermediate (Figure 6F). For example, the growth of DLD1 and CACO2 cells is reduced by nearly 50% in culture medium supplied with 75% AA (Figure 6F). In contrast, that of RKO and COLO205 remains normal. On the other hand, no significant correlation is seen between Rab1A expression and the sensitivity to serum or glucose starvation (Figures 6G and 6H; Figure S5E). These observations suggest that CRC cells with Rab1A overexpression are highly addictive to AA for growth.

### Rab1A Overexpression Promotes Oncogenic Transformation and Malignant Growth

To evaluate the pathological consequence of Rab1A overexpression, we ectopically expressed GFP or GFP-Rab1A in NIH 3T3 cells. As observed in HEK293E cells, GFP-Rab1A stimulates S6K1 phosphorylation compared with GFP (Figure 7A) and the growth and colony formation of NIH 3T3 cells (Figures 7B and 7C; Figure S6A). These cells also form large foci in confluent culture and exhibit anchorage-independent growth (Figures 7D and 7E; Figure S6B). In contrast, the $H\text{-}Ras^{V12}$ mutant does not significantly affect mTORC1 signaling or promote oncogenic growth (Figures 7A–7E; Figures S6A and S6B). Of note, cells with GFP-Rab1A display similar oncogenic growth in the absence or presence of $H\text{-}Ras^{V12}$, indicating that Rab1A does not cooperate with $H\text{-}Ras^{V12}$ in oncogenic transformation. Compared with WT Rab1A, Rab1A-GTP further promotes, whereas Rab1A-GDP and Rab1A-C2S reduce, cell growth (Figure S6C), which is consistent with their mTORC1-activating activity.

We further evaluated the oncogenic potential of Rab1A in vivo by injecting nude mice with NIH 3T3 cells stably expressing GFP-Rab1A or GFP. Control cells have a low propensity to form tiny tumors in nude mice (6 out of 12) (Figures 7F and 7G), which is consistent with previously studies (Greig et al., 1985; Rong et al., 1994). In contrast, GFP-Rab1A cells form large tumors with 100% efficiency (12 out of 12) (Figures 7F and 7G). Histologically, GFP-Rab1A tumors display elevated S6K1 phosphorylation and malignant phenotypes (e.g., high cell density, mitotic index, and nuclear variability) (Figure 7H). These results indicate that Rab1A overexpression is sufficient to transform immortalized cells.

(C) Scatter plot showing the Rab1A staining level in individual tumors as a ratio of Rab1A staining in CRC versus paired non-cancerous tissue.
(D) Consecutive CRC tissue sections were stained for Rab1A and P-S6K1. Shown are representative cases with high, moderate, or negative Rab1A staining and P-S6K1 staining. Scale bar, 100 μm.
(E) Correlation plot of Rab1A and P-S6K1 IHC staining (arbitrary units). Correlation was evaluated by a nonparametric Spearman test. The number of cases (n), the coefficient of correlation (r), and the p value (p) are indicated.
(F) Correlation between Rab1A and P-S6K1 in cases with heterogeneous levels of Rab1A and P-S6K1. Red arrowhead, high Rab1A-positive/P-S6K1 staining; black arrowhead, low Rab1A-positive/P-S6K1 staining. Scale bar, 50 μm.
(G) Kaplan-Meier survival analysis of CRC cases separated into two groups by the median value for Rab1A-positive staining. The p value was calculated by log rank test.
See also Table S1.

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RKO is a CRC cell line with low Rab1A expression. GFP-Rab1A overexpression enhances S6K1 phosphorylation (Figure 7I) and promotes RKO cell growth and colony formation (Figures 7J and 7K) and xenograft tumor growth in nude mice (Figures 7L and 7M). RKO tumors expressing GFP-Rab1A show elevated P-S6K1 and an elevated mitotic index (Figure 7N). Therefore, Rab1A overexpression can also enhance the malignant growth of established tumors. To determine the dependence of Rab1A-mediated transformation on Rheb and Rag GTPases, we knocked down Rheb or RagA/RagB in RKO cells overexpressing GFP-Rab1A. Downregulation of Rheb and Raptor much more significantly attenuates Rab1A-stimulated mTORC1 signaling, cell growth, and colony formation than knockdown of RagA and RagB (Figures S6D–S6F). Therefore, Rab1A is primarily dependent on Rheb for stimulation of mTORC1 signaling and oncogenic growth.

### Rab1A Overexpression Promotes mTORC1-Dependent Oncogenic Growth and Determines Rapamycin Sensitivity in CRC

Successful targeted cancer therapy hinges on hyperactivation of the target signaling pathway (Shawver et al., 2002). Cancer cells become dependent on, or "addicted to" such "growth driver" pathway, rendering these cells prone to the targeted treatment. We therefore investigated the relationship between Rab1A overexpression and rapamycin sensitivity. Indeed, there is a strong correlation between Rab1A expression and growth inhibition in the panel of CRC cell lines, with the highest rapamycin sensitivity for DLD1 and CACO2 and the lowest for RKO and COLO205 (Figure 8A). To verify this finding in vivo, we generated DLD1 and RKO xenograft tumors, representing CRC with high and low Rab1A expression, respectively. After tumors were established, the animals were treated with rapamycin or a control vehicle. Rapamycin completely blocks the growth of DLD1 tumors but has no discernible effect on RKO tumors (Figure 8B), despite strong on-target inhibition of mTORC1 signaling by rapamycin (Figure 8C). Moreover, rapamycin abrogates the elevated mTORC1 signaling, growth, and colony formation of NIH 3T3 and RKO cells driven by Rab1A overexpression (Figures 8D and 8E), indicating that the oncogenic growth by Rab1A overexpression is indeed mediated by mTORC1. Together, these results demonstrate that Rab1A overexpression drives mTORC1-dependent oncogenic growth and determines drug sensitivity to mTORC1-targeted therapy in CRC.

## DISCUSSION

Rab1 is a small GTPase previously known for its role in vesicle transport from the ER to the Golgi. Here we describe a Rab1 function that mediates AA signaling to activate mTORC1. Downregulation of Rab1 inhibits mTORC1 activation by AA in yeast and humans, indicating that this Rab1 function is well conserved. Mechanistically, AA stimulates Rab1A GTP binding and the GTP-dependent interaction with mTORC1 in the Golgi. Rab1 does not directly activate mTORC1 kinase activity (data not shown). Instead, it regulates Rheb interaction with mTORC1 in the Golgi. Together, these observations show that Rab1 uses the Golgi as an anchor to regulate mTORC1 activation by Rheb in response to AA sufficiency.

Rab1A and Rag both interact with mTORC1 in response to AA sufficiency and promote the colocalization of mTORC1 with Rheb. However, they are anchored on two distinct endomembrane systems, with Rag on the lysosomes and Rab1A on the Golgi. Rab1A knockdown blocks Rheb-mTORC1 interaction on the Golgi but not the lysosomes (Figure 8F). Rab1A overexpression rescues the ability of AA to activate mTORC1 in the absence of RagA/RagB, whereas overexpression of the hyperactive $RagB^{GTP}/RagC^{GDP}$ complex partially restores AA activation of mTORC1 when Rab1A is knocked down. In addition, Rab1A requires Rheb, but not Rag, to stimulate mTORC1 signaling and oncogenic growth in CRC cells. These observations suggest that Rab1A and Rag operate as two independent axes of AA signaling to mTORC1. Interestingly, constitutively activated Rag GTPases in humans, but not in yeast, partially rescue mTORC1 activation by AA in the absence of Rab1A, suggesting that Rag gains a bigger role in AA signaling during evolution. Because AAs are essential for cell growth and metabolism, it makes sense for eukaryotic cells to add redundant signaling modules, Rab1 and Rag, to transduce AA signals, which ensures the reliable transmission of this crucial mitogenic signal. In addition, it is possible that the two signaling branches have distinct functions to provide finer control of the signaling process (e.g., engaging in external versus internal AA signals or a different type of AA).

Small GTPases such as Ras, Rho, Rac, and Ral are known for their roles in cancer initiation and development. Rab proteins constitute one of the largest subfamilies of small GTPases and are generally regarded as housekeeping proteins involved in intracellular membrane dynamics. To date, their roles in carcinogenesis remain obscure. Only until recently were select

**Figure 5. Rab1A Overexpression Is a Driver for CRC Growth**

(A) A panel of human CRC cell lines were analyzed for the level of Rab1A, P-S6K1(T389), and P-AKT(473) by immunoblot (top panel), and the correlation between the level of Rab1A and the level of P-S6K1(T389) was determined (bottom panel, shown as in Figure 2E).

(B) Rab1A was knocked down in three pairs of CRC cell lines representing high, moderate, and low Rab1A expression, as indicated, and the effect on P-S6K1(T389), S6K, P-AKT(S473), and AKT was analyzed by immunoblot.

(C–E) Rab1A was knocked down in human CRC cell lines expressing high (C), moderate (D), or low (E) levels of Rab1A, and the relative growth of these cells was analyzed by sulforhodamine B (SRB) assay. Data represent means ± SD of three independent experiments. NC, control shRNA.

(F–H) Rab1A was knocked down in human CRC cell lines expressing high (F), moderate (G), or low (H) levels of Rab1A, and their ability to form colonies was determined. Data represent means ± SD of three independent experiments (colony number/well in 12-well plates).

(I and J) Quantification results of tumor growth (I) and representative images of tumors dissected at the end of the study (J) showing the effect of Rab1A knockdown on the growth of DLD-1 xenograft tumors. Data represent means ± SD.

(K) DLD1 xenograft tumors were analyzed by hematoxylin and eosin (H&E) staining and by IHC as indicated. The mitotic index is expressed by the number of mitotic nuclei per high power field (HPF). Scale bar, 50 μm.

See also Figure S4.

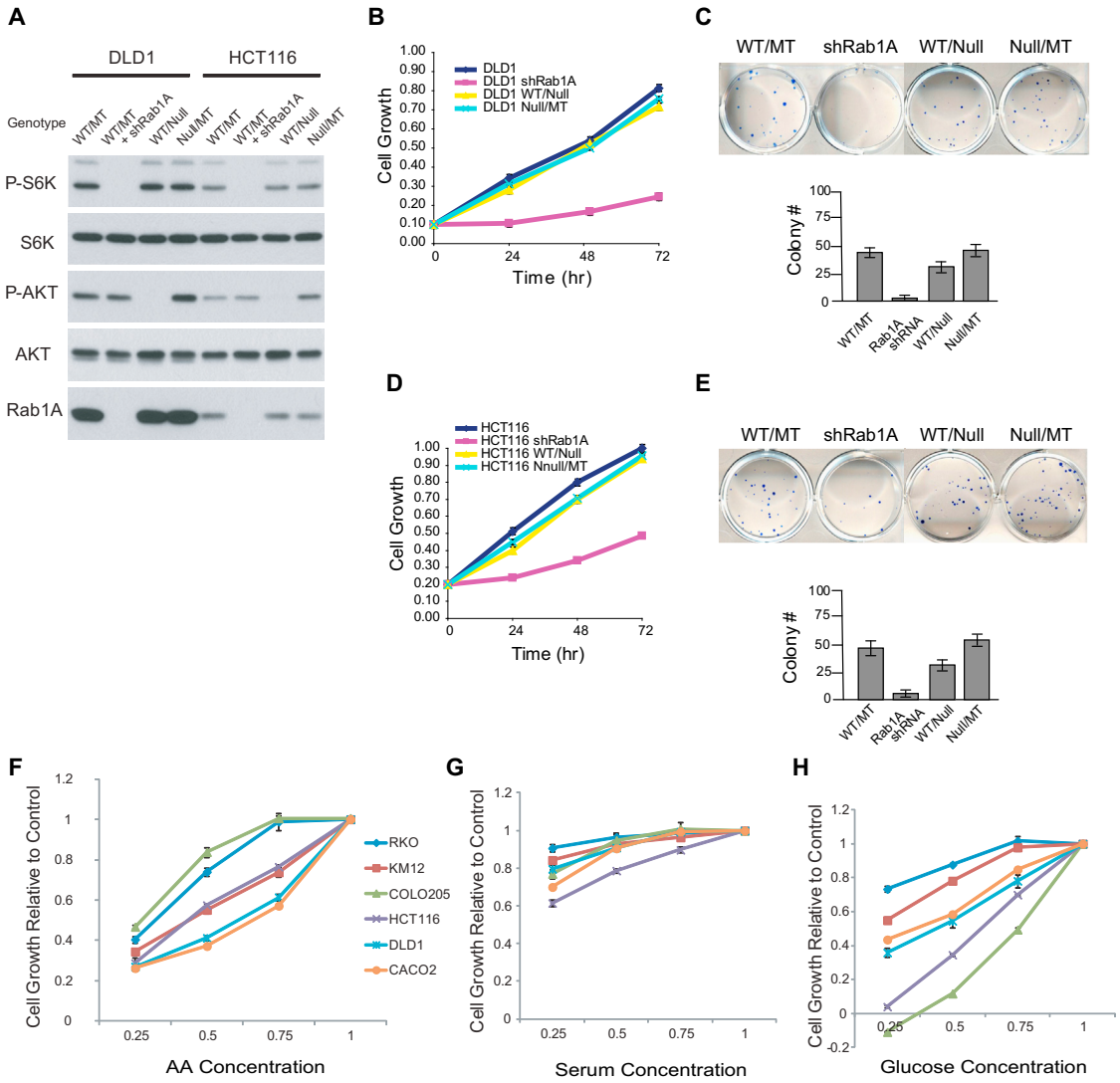


**Figure 6. Rab1A, Rather Than Activated PI3K or MEK, Is Crucial for mTORC1 Signaling and Oncogenic Growth in CRC**

(A) DLD1 and HCT116 parental cells, cells with Rab1A knockdown, or cells with deletion of the WT or mutant (MT) *PIK3CA* allele were analyzed for levels of P-S6K1, S6K1, P-AKT, and AKT.

(B–E) Parental, Rab1A knockdown, or WT or MT *PIK3CA* allele-deleted DLD1 (B and C) and HCT116 (D and E) cells were analyzed for cell growth (B and D) and colony formation (C and E). Data represent means ± SD of three independent experiments.

(F–H) The growth of CRC cell lines with differential Rab1A expression was assayed in culture media containing varied amounts of AA (F), serum (G), or glucose (H) (1×, 0.75×, 0.5×, or 0.25× normal). Data represent means ± SD of three independent experiments.

See also Figure S5.

members of this subfamily (e.g., Rab25) implicated in human cancer (Cheng et al., 2004). Interestingly, Rab1A has been reported previously to be highly overexpressed in human tongue squamous cell carcinomas (Shimada et al., 2005). Here we report that Rab1A is overexpressed in CRC. In addition, Rab1A is overexpressed in breast and liver tumors (unpublished data). These results indicate that aberrant Rab1A expression is a general phenomenon in human malignancies. Although PI3K acts upstream of mTORC1, surprisingly, activating PI3K mutations in CRC do not promote mTORC1 signaling or oncogenic growth. Moreover, the expression of several other mTORC1 activators such as Rheb and Rag is not correlated with mTORC1 activity. These observations suggest that tumors selectively activate Rab1A-dependent mTORC1 signaling through Rab1A overexpression to gain an oncogenic advantage.

Although Rab1A expression has been reported to be elevated in tongue cancer, the significance of Rab1 overexpression remained unclear. Here we show that Rab1A overexpression is sufficient to transform immortalized fibroblasts and promote malignant growth of established tumor cells, suggesting that Rab1A is capable of promoting both oncogenic transformation and growth. Although Rab1A is universally required for TORC1 signaling, only CRC cells with high Rab1A expression are strongly dependent on Rab1A for growth, indicating that

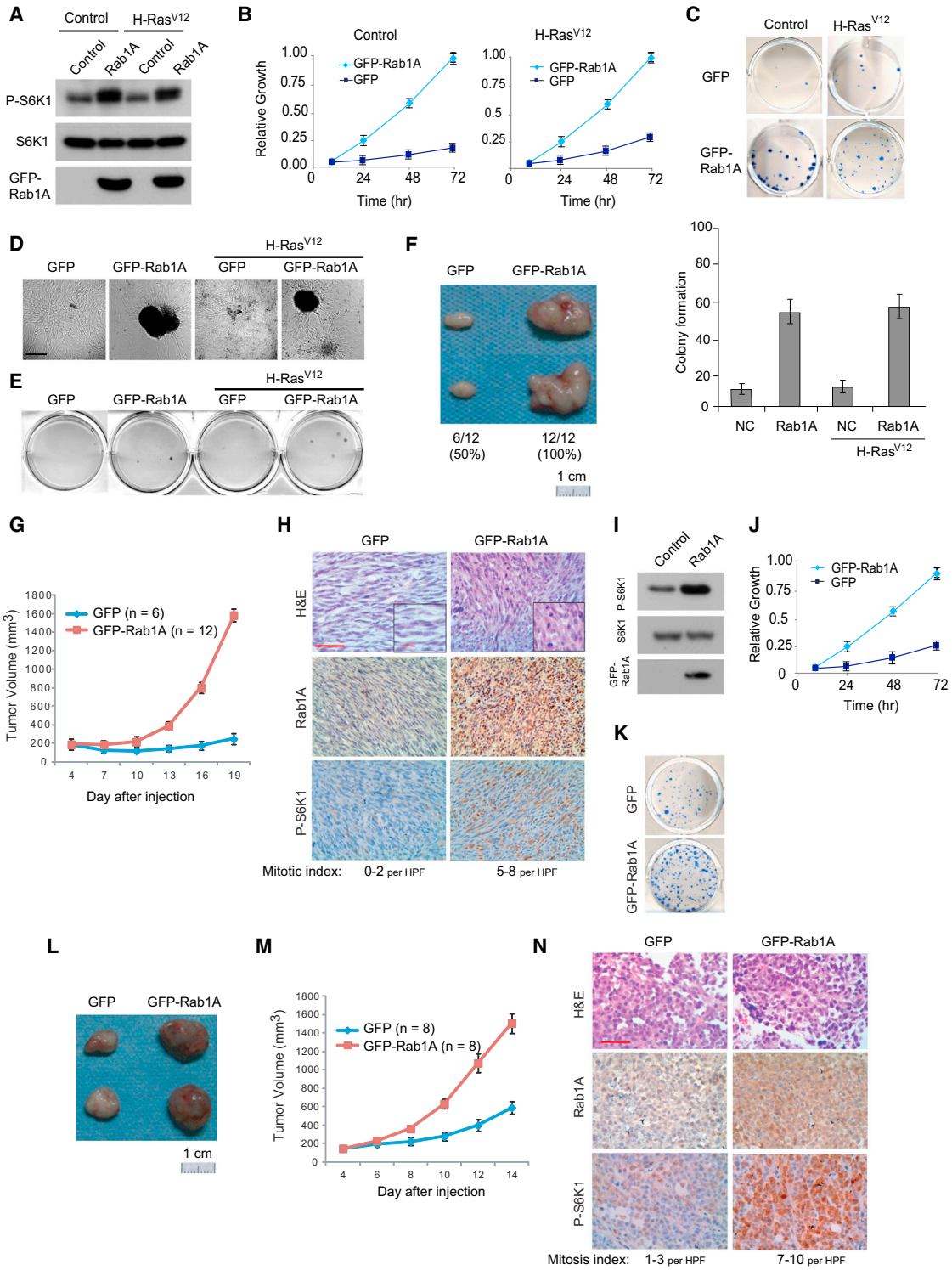


**Figure 7. Rab1A Overexpression Promotes Oncogenic Transformation and Oncogenic Growth**

(A) NIH 3T3 or NIH 3T3/H-Ras$^{V12}$ cells stably expressing GFP or GFP-Rab1A were analyzed for P-S6K1(T389).

(B) The growth of NIH 3T3 or NIH 3T3/H-Ras$^{V12}$ cells overexpressing Rab1A. Data represent means ± SD of three independent experiments. Shown are arbitrary units.

(C) Representative images (top) and quantification results (bottom) of colony formation of NIH 3T3 or NIH 3T3/H-Ras$^{V12}$ cells overexpressing Rab1A (means ± SD of three independent experiments). Shown is the number of colonies per well (12-well plate).

(D) Representative images of the focus formation assays of NIH 3T3 or NIH 3T3/H-Ras$^{V12}$ cells with or without overexpression of Rab1A. Scale bar, 100 μm.

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Rab1A overexpression is a driver for these CRC cells. Remarkably, CRC cells with Rab1A overexpression are highly dependent on AA, suggesting that elevated AA signaling renders cancer cells addictive to AA. AA signaling is increasingly recognized as a key mitogenic event, but its role in cancer remains relative obscure. A recent study implicated GATOR, the GTPase-activating protein (GAP) for Rag A/RagB as potential tumor suppressor (Bar-Peled et al., 2013). However, genomic mutation of GATOR components appears to be relatively rare. In contrast, Rab1A overexpression is widespread in human malignancies, suggesting that aberrant AA signaling as a result of Rab1 overexpression is a common mechanism to promote oncogenic transformation and growth.

mTOR is a major cancer therapeutic target (Bjornsti and Houghton, 2004; Tsang et al., 2007), with two rapalogs (temsirolimus and everolimus) presently used in the clinic. Although upstream regulators of mTORC1 such as PI3K and PTEN are commonly mutated in human tumors, such mutations have not correlated well with clinical responses (Don and Zheng, 2011). This is consistent with the observations that PI3K mutant is not responsible for mTORC1 activation and that it promotes survival rather than oncogenic growth in CRC (Samuels et al., 2005). Our data indicate that Rab1A overexpression is a driver for mTORC1-dependent growth, which is correlated with CRC sensitivity to rapamycin. Further research in this area could lead to a predictive biomarker for improving mTORC1-targeted therapy. In addition to cancer, Rab1A is upregulated in a dilated cardiomyopathy model, and heart-specific Rab1A transgenes are sufficient to cause cardiac hypertrophy in a gene dosage-dependent manner in mice (Wu et al., 2001). It is noteworthy that rapamycin is effective to regress established cardiac hypertrophy (McMullen et al., 2004). Our findings here provide a mechanistic explanation for the pathological role of Rab1A in cancer and cardiac hypertrophy.

## EXPERIMENTAL PROCEDURES

### Plasmids and Site-Directed Mutagenesis

The human Rab1A plasmid was a gift from Dr. Marci A. Scidmore (Rzomp et al., 2003). The plasmids expressing HA-GST-Rheb1, HA-GST-Rap2A, HA-GST-RagB(Q99L), ($RagB^{GTP}$), and RagC(S75L) ($RagC^{GDP}$) (Sancak et al., 2008) were acquired from AddGene. To generate Rab1A-GFP and Rab1A-HA, Rab1A cDNAs were subcloned into the EcoRV and NotI sites of pEGFP-C1 and the SalI and NotI sites of pRK5, respectively. Raptor and Rictor cDNAs were subcloned into the AscI and MluI sites of pCMV6-AN-Myc. $Rab1A^{GTP}$ (Q70L), $Rab1A^{GDP}$ (S22N), and $Rab1A^{C2S}$ (C202, 203S) were generated by PCR site-directed mutagenesis and confirmed by sequencing. The GM130-GFP plasmid was generated by cloning GM130 cDNA into pEGFP-C1.

### Immunological Reagents, Chemicals, Cell Extracts, Western Blot, and Immunoprecipitation

Immunological reagents were obtained from the following sources. The Tor1-specific antibody has been described previously (Li et al., 2006). Horseradish peroxidase-labeled secondary antibodies were from Santa-Cruz Biotechnology. Antibodies for mTOR (catalog no. 2983), Raptor (catalog no. 2280), Rheb (catalog no. 4935), RagA (catalog no. 4537), RagB (catalog no. 8150), RagC (catalog no. 5466), RagD (catalog no. 4470), Lamp1 (catalog no. 9091), Rictor, P-S6K1(T398), S6K1, P-AKT(S473), AKT, P-4E-BP1(T37/46), 4E-BP1, ERK, P-ERK(T202/Y204), α-tubulin, and the Myc-epitope were from Cell Signaling Technology. The antibody for the HA epitope was from Bethyl Laboratories. The antibodies for Rab1A and P-S6K1(T398) for IHC and the Lamp2 antibody (catalog no. ab25630) were from Abcam. The antibody for Rab1A (catalog no. 11671 for western blot and immunoprecipitation IP) and Rab1B were from Proteintech Group. The Rab1A antibody for immunofluorescence (IF) (catalog no. H00005861-M07A) was from Abnova. Protein G-Sepharose was from GE Healthcare. EDTA-free complete protease inhibitor cocktail and PhosSTOP were from Roche. Rapamycin and PD98059 were from Selleck Chemicals. Cell lysis and western blot (Drenan et al., 2004; Sancak et al., 2008) and immunoprecipitation (Sancak et al., 2008) were performed as described previously. For determining whether Ratpor or mTOR mediates the interaction with Rab1A, anti-Rab1A immunoprecipitates were washed with cell lysis buffer containing 0.25% or 0.50% TX-100.

### Xenograft Tumors and Drug Therapy in Athymic Nude Mice

To generate xenograft tumors, a total of $5 \times 10^6$ to $1 \times 10^7$ cells in 100 ml PBS were injected subcutaneously into 4-week-old female BALB/c nude mice. The tumor volume was measured using a Vernier caliper and calculated according to the formula tumor volume ($mm^3$) = (shorter diameter$^2$ × longer diameter) / 2. The tumor volume was measured every other day and is presented as means ± SD. At the endpoint, mice were sacrificed, and tumors were removed and photographed. For drug therapy experiments, DLD-1 and RKO cells were injected subcutaneously into nude mice to establish a xenograft model. After tumors were established, rapamycin was administered with 5 mg/kg rapamycin dissolved in DMSO and sterile saline by daily intraperitoneal injections for 10–12 days. Drug vehicle-treated mice received a daily injection of identical solution without rapamycin. At least six mice were included in each treatment group. Animal experiments were approved by Rutgers and Shanghai Jiaotong University School of Medicine (SJTUSM) Animal Care and Use committees.

### CRC Tissue Array and Immunohistochemistry

Ninety pairs of deidentified malignant infiltrating CRC tumors with paired noncancerous samples (cutting edge of surgical excision beyond 5 cm from the cancer areas) were randomly obtained from July 2006 to August 2007. The CRC tissue array was prepared by Shanghai OUTDO Biotech (Shanghai, China). The survival time was calculated from the day of operation to the end of the follow-up or the date of death because of the recurrence and metastasis. This study was carried out according to the provisions of the Helsinki Declaration of 1975 and was reviewed and approved by the SJTUSM

(E) Representative images of the anchorage-independent growth of NIH 3T3 or NIH 3T3/H-$Ras^{V12}$ cells with or without overexpression of Rab1A.
(F and G) NIH 3T3 cells stably expressing GFP or GFP-Rab1A were injected subcutaneously into the flanks of nude mice, and the tumor volume was measured every 3 days. Representative images of dissected tumors at the end of the experiment (F) and quantification of tumor growth (G) are shown. The numbers at the bottom Figure 7F show the number and percentage of xenograft tumors formed. Data represent means ± SD.
(H) Representative xenograft NIH 3T3/GFP-Rab1A tumor tissue sections with indicated staining. The mitotic index was obtained by morphological evaluation of 10 random HPFs) (400 × ) for each tumor. Data represent the mitotic index ($p < 0.001$ by Student's t test). Scale bar, 50 μm. Inset scale bar, 10 μm.
(I) RKO cells stably expressing GFP or GFP-Rab1A were analyzed for mTORC1 signaling.
(J) The growth of RKO cells overexpressing GFP or GFP-Rab1A was determined by SRB assay. Data represent means ± SD.
(K) RKO cells overexpressing GFP or GFP-Rab1A were determined for colony formation. Shown are representative images.
(L and M) RKO cells stably expressing GFP or GFP-Rab1A were injected subcutaneously into the flanks of nude mice, and the tumor volume was measured every 3 days. Representative images of dissected tumors at the end of the experiment (L) and quantification of tumor growth (M) are shown. Data represent means ± SD.
(N) H&E staining of RKO xenograft tumor tissues and IHC staining for Rab1A and P-S6K1 levels. Scale bar, 50 μm.
See also Figure S6.

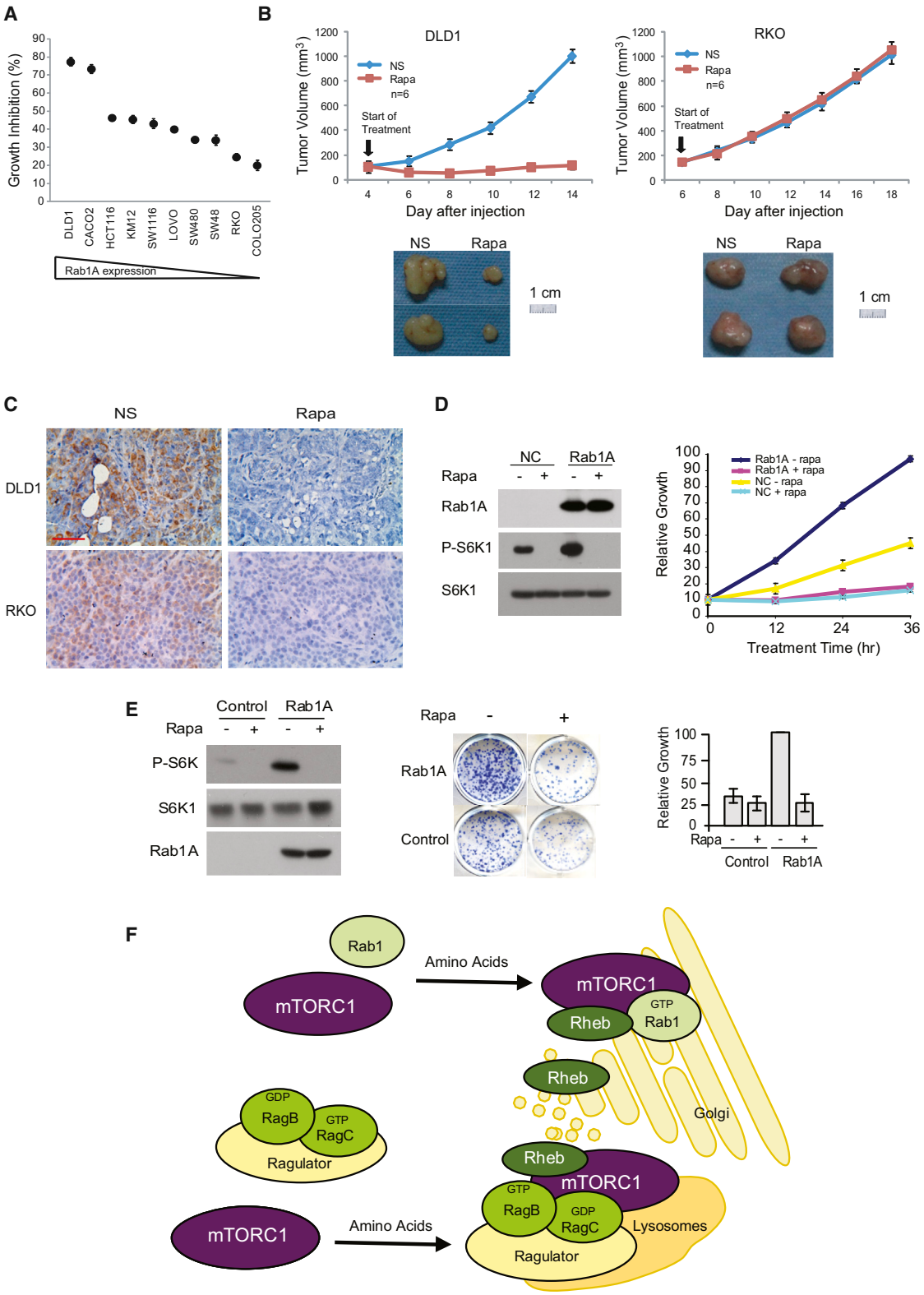


**Figure 8. Rab1A Overexpression Promotes mTORC1-Dependent Oncogenic Growth and Determines Rapamycin Sensitivity**

(A) Relative growth inhibition of CRC cells treated with 10 nM rapamycin for 48 hr.

(B) DLD-1 or RKO tumor-bearing animals were treated with rapamycin or a drug vehicle (NS) and measured for tumor growth. Shown are representative tumors dissected at the end of treatment. Data represent means ± SD.

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Ethics Committee. The streptavidin-biotin complex method was used in immunohistochemistry to detect Rab1A and P-S6K1(T389). Primary antibodies against Rab1A and P-S6K1(T389) were used at a concentration of 1:400 and 1:50, respectively. To score a tumor cell as positive, both cytoplasmic and nuclear staining were counted. For the quantitative analysis, a Histo score (H score) was calculated based on the staining intensity and percentage of stained cells using the Aperio ScanScope systems. The intensity score was defined as follows: 0, no appreciable staining in cells; 1, weak staining in cells comparable with stromal cells; 2, intermediate staining; 3, strong staining. The fraction of positive cells was scored as 0%–100%. The H score was calculated by multiplying the intensity score and the fraction score, producing a total range of 0–300. A cutoff of 30 was used for P-S6K1 positivity and 90 for Rab1A positivity. Tissue sections were examined and scored separately by two independent investigators blinded to the clinicopathologic data.

### Statistical Analysis

Statistical analyses were carried out using SAS 9.13 software. The statistical analysis of numeration data was done using Pearson's chi-square test or Fisher's exact chi-square test. The comparisons of continuous data between groups were performed using Student's t tests or Wilcoxon signed rank test. A nonparametric Spearman correlation test was performed to analyze the correlation between Rab1A and P-S6K1 expression levels. For survival analysis, data collection was locked on August 16, 2011. Kaplan-Meier plots and log rank tests were applied to determine the significance of differences in cumulative survival. All statistical tests were conducted at a two-sided significance level of 0.05. For additional methods, see Supplemental Information.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.ccell.2014.09.008.

## AUTHOR CONTRIBUTIONS

J.D.T., Y.J.Z., Y.H.W., J.H.C., and L.M. designed and performed the experiments and prepared the manuscript. H.Y.W. advised on experimental design and provided experimental expertise. X.F.Z. designed the experiments and prepared the manuscript.

## ACKNOWLEDGMENTS

We thank Drs. Cecilia Alvarez, Bert Vogelstein, Marci Scidmore, and Nava Segev for providing plasmids, cancer cell lines, and yeast strains; Drs. Peter Yurchenko and Guangye Du for advice on histological characterization of primary and xenograft tumors; and Brian Kain for assistance with confocal cell imaging. This work was supported by NIH R01 Grants CA123391 and CA173519 (to X.F.Z.), by New Jersey Commission on Cancer Research Postdoctoral Fellowship DFHS14PPC032 (to J.D.T.), and by Natural Science Foundation of China Grant 81270035 (to Y.J.Z.).

Received: May 21, 2014
Revised: July 16, 2014
Accepted: September 19, 2014
Published: October 23, 2014

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(C) DLD-1 and RKO tumors treated with rapamycin or a drug vehicle were analyzed for P-S6K1 by IHC. Scale bar, 50 μm.
(D) NIH 3T3 cells overexpressing GFP or GFP-Rab1A were treated with 10 nM rapamycin and assayed for mTORC1 signaling and cell growth. Data represent means ± SD of three independent experiments.
(E) RKO cells stably expressing GFP or GFP-Rab1A were treated with 10 nM rapamycin and assayed for mTORC1 signaling and colony formation. Data represent means ± SD of three independent experiments.
(F) Model comparing Rab1- and Rag-mediated mTORC1 activation by AA. Rab1 and Rag GTPases represent two distinct AA signaling branches anchoring on the Golgi and lysosomes, respectively, to activate mTORC1 through Rheb in response to AA sufficiency.

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