A Conserved Role of IQGAP1 in Regulating TOR Complex 1

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**Running title:** Role of IQGAP1 in S6K-Akt1 Feedback

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Summary

Defining the mechanisms that orchestrate cell growth and division is crucial to understanding cell homeostasis, which impacts human diseases like cancer and diabetes. IQGAP1, a widely conserved effector/regulator of the GTPase CDC42 is a putative oncogene that control cell proliferation, however, its mechanism in tumorigenesis is unknown. The mTOR pathway, the center of cell growth control, is commonly activated in human cancers, but proved ineffective as clinical target due to incomplete understanding of its mechanisms in cell growth inhibition. Using complementary studies in yeast and mammalian cells, here we report a potential role for IQGAP1 in regulating the negative feedback loop (NFL) of the target of rapamycin complex1 (mTORC1) that control cell growth. Two-hybrid screens identified yeast TORC1-specific subunit, Tco89p, as an Iqg1p-binding partner sharing roles in rapamycin-sensitive growth, axial bud-site selection and cytokinesis, thus coupling cell growth and division. Mammalian IQGAP1 binds mTORC1 and Akt1 and in response to epidermal growth factor (EGF), cells expressing the mTORC1/Akt1-binding region, IQGAP1IR-WW, contained attenuated pERK1/2 activity and inactive glycogen synthase kinase 3α/β (pGSK3α/β), which control apoptosis. Interestingly, these cells displayed high level of pAkt1S473, but attenuated level of the mTORC1-dependent kinase, pS6k1T389 and induced mTORC1/Akt1- and EGF-dependent transformed phenotypes. Moreover, IQGAP1 appears to influence cell abscission and its activity is elevated in carcinoma cell lines. These findings support the hypothesis that IQGAP1 acts upstream on the mTORC1/S6K1→Akt1 NFL and downstream of it to couple cell growth and division and thus like a rheostat regulates cell homeostasis, dysregulation of which leads to tumorigenesis or other diseases. These results could have implications on developing the next generation of anticancer therapeutics.
Introduction

The mechanisms that orchestrate cell proliferation continue to be central to cell biology research (Tapon et al., 2001; Sturgill, and Hall. 2007; Moseley et al., 2009) and to understanding prevalent human diseases like diabetes and cancer. The evolutionarily conserved serine/threonine kinase, mTOR the center of cell growth control interfaces nutrient and growth factor signals to regulate cell proliferation (Guertin and Sabatini 2007, Sengupta et al., 2010). It is believed that mTOR couples cell growth and division by integrating the nutrient and growth factor signals through the PI3K/Akt1-mTOR pathway to control cell size, a pre-requisite to entry into the cell cycle, but despite much progress, how the two activities are integrated remains unclear (Tapon et al., 2001; Fingar and Blenis, 2004; Sabatini 2006; Wullschleger et al., 2006; Polak and Hall. 2006; Sturgill, and Hall. 2007; Hall, 2008, Laplante and Sabatini, 2009; Huang and Manning 2009; Sengupta et al., 2010).

Yeast and mammalian TORs form two distinct complexes, TORC1 and TORC2, each containing shared and unique subunits (Fingar and Blenis, 2004; Sabatini 2006; Wullschleger et al., 2006, Hall, 2008, Laplante and Sabatini, 2009). In addition to other subunits, yeast TORC1 contains the shared subunits Tor1p or Tor2p, Lst8p and the unique subunits Tco89p and Kog1p (Loewith et al., 2002; Reinke et al., 2004). In mammals, where mTOR is a shared subunit, Raptor defines mTORC1 and Rictor defines mTORC2 (Sabatini 2006). The mTORC1 is a rapamycin-sensitive complex that regulates cell mass by activating mRNA translation by directly phosphorylating, thereby activating its effector S6K1 (ribosomal S6 kinase 1), therefore, S6K phosphorylation is a widely used marker of mTORC1 activation (Fingar and Blenis, 2004; Ruvinsky and Meyuhas, 2006; Guertin and Sabatini, 2007). In contrast, mTORC2, believed to control the actin cytoskeleton, exhibits rapamycin-insensitive properties, but responds to long-
term-rapamycin treatment and acts upstream of mTORC1 by directly activating Akt1, the effector of PI3K (Sabatini, 2006). Akt1 in turn activates mTORC1 by inhibiting the GAP (Tsc1/2 complex) thus activating the GTPase, Rheb, which activates mTOR and increases cell mass (Inoki et al., 2002; 2003; 2005 Manning, 2004, Laplante and Sabatini, 2009; Huang and Manning 2009).

This activation process is regulated by a negative feedback loop (NFL) whereby activated $pS6K1^{T389}$ suppresses $pAkt1^{S473}$ to regulate the cell size. How this regulatory inhibitory mechanism is controlled remains undefined (Laplante and Sabatini, 2009; Huang and Manning, 2009; Dibble et al., 2009; Julien et al., 2010; Sengupta et al., 2010). The importance of defining the mTORC1/S6K1 NFL regulation is that while aberrant activation of mTOR and Akt1 is a common oncogenic and diabetic signal, the mTOR-inhibitors has been ineffective in clinical trials or animal models due to their inhibition of the S6K NFL and activation of Akt (Manning, 2004; Guertin and Sabatini 2005; 2007; Huang and Manning 2009; Hsieh, et al., 2011). Therefore, understanding the regulations of the mTORC1/S6K1 NFL is crucial to developing the next generation of effective anticancer and antidiabetic therapeutics.

This study reports novel role for IQGAP1 in integrating mTORC1 and Akt1 signaling by modulating the mTORC1/S6K1 NFL to control cell proliferation. IQGAP1 is a modular protein and a widely conserved effector/regulator of the putative oncogene CDC42 GTPase and has been implicated in regulating cell polarity, migration, actin cytoskeleton dynamics and epithelial cell organization (Osman and Cerione, 1998, Osman et al., 2002; Mateer et al., 2003; Noritake et al., 2004; 2005; Bensenor et al., 2007; Le Clainche et al., 2007; Brandt and Grosse, 2007) and integrating signaling networks (reviewed in Mateer et al., 2003; white et al., 2009, Osman 2010). IQGAP1 has oncogenic activity, it induces transformed phenotypes in cell cultures and
tumorigenesis in mice and its aberrant expression or mislocalization associates with a wide range of human carcinomas (Wang et al., 2009; White et al., 2009; Johnson et al., 2009; Osman, 2010; Chen et al., 2010). Despite being heavily investigated, to date its molecular mechanism in oncogenesis remains unknown.

The yeast ortholog, Iqg1p, is similarly modular and promotes cytokinesis (Epp and Chant, 1997; Lippincott and Li, 1998; Osman and Cerione, 1998), cooperating with the mitotic exit network (Corbett et al., 2006). It regulates cytokinesis by serving as a positional marker for axial (haploid) bud-site selection, linking cytokinesis with bud-site selection and polarized growth (Osman and Cerione, 1998; 2006; Osman et al., 2002) thus fulfilling the tenet of the “cytokinesis tag” model, which predicts that proteins involved in bud-site selection early in the cell cycle, control cytokinesis at the end of the cycle (Madden and Snyder, 1998).

Together, these features support the concept that IQGAP1 essential role is to control cell homeostasis by coupling cell growth and division (Rittmeyer et al., 2008; Wang et al., 2009). It regulates insulin synthesis and secretion (Rittmeyer et al., 2008) and promotes cell size via its N-terminal domain, which binds mTOR (Wang et al., 2009) and it promotes cytokinesis and cell proliferation via its C-terminal domain, which binds and activates CDC42, however it required mTOR for this activity (Wang et al., 2009). The mechanism by which IQGAP1 regulates cell proliferation through the shared mTOR subunit remains to be defined. Because IQGAP1, CDC42 and mTORC2 are separately implicated in regulating the actin cytoskeleton it appeared that IQGAP1 would associate with mTORC2. Surprisingly, this appears to be not the case.

Utilizing the conserved roles of yeast and mammalian IQGAPs, here we report a novel role for IQGAP1 in modulating mTORC1/S6K1→Akt1 signaling to control cell proliferation. Screening for novel binding partners of Iqg1p by Two-hybrid identified the TORC1-specific
subunit Tco89p. Iqg1p and Tco89p bind through their N-terminal domains and share roles in coordinating rapamycin-sensitive cell growth and division, which we further tested in mammalian cells and demonstrated that human IQGAP1 regulates cell proliferation through mTORC1/Akt1. In both systems an association with TORC2 was undetected, supporting a conserved role of IQGAP1 through TORC1. Genetic and biochemical analyses of the effects of IQGAP1 on mTORC1/Akt1 and ERK1/2/GSK3α/β signaling, support the model that IQGAP1 serves as an upstream regulator of the mTORC1/S6K1-dependent inhibitory mechanism that regulates pAkt1S473 to modulate cell proliferation. Dysfunction of such mechanism can explain IQGAP1’s oncogenicity and its association with diverse carcinomas, presenting it as a potential therapeutic target.

Results

Yeast Iqg1p Associates with TORC1

Previously we demonstrated a conserved role for IQGAP1/Iqg1p in coupling cell growth and division and promoting cell proliferation (Osman et al., 2002; Rittmeyer et al., 2008; Wang et al., 2009). To investigate the molecular basis of this role, we used Iqg1p as bait to screen three yeast Two-hybrid libraries, and identified four positive library clones “LC” encoding the first 300 amino acids of the *S. cerevisiae* scYPL180w (Figs. 1A-D) that was identified as the TORC1 subunit Tco89p (Reinke et al., 2004). The specificity of the association was validated by testing the interaction with full-length Tco89p (Fig.1B) and by mapping the binding region on Iqg1p using several mutants and quantitative Two-hybrid assays, compared to empty vector as control (Fig. 1C). The results show that Iqg1-N directly or indirectly mediated Iqg1p-Tco89p binding (Fig. 1C, second bar) and that deletion of the 294 amino acids upstream of the CHD of Iqg1p, significantly reduced this binding (Fig. 1C, third bar). These findings demonstrate that the
interaction between Iqg1p and Tco89 is specific and mediated by the N-terminal region of each protein. Because Bud4p, another marker for axial bud-site and cytokinesis, was previously identified as an Iqg1p-binding partner from the same libraries (Osman et al., 2002), we examined whether it would associate with Tco89p by using Two-hybrid quantitative assays. The results in Fig. 1C, last bar demonstrate that the two proteins strongly interact.

Next, we verified the Two-hybrid results by in vivo biochemical assays. Tco89-Myc co-precipitated both ways with HA-tagged Iqg1p and Iqg1-N (Fig. 1D; and not shown), confirming the two-hybrid results. A mammalian counterpart of Tco89p is yet to be identified, but since it binds the TORC1-specific subunit Kog1p, the homologue of mammalian Raptor, we demonstrated that Iqg1-HA co-precipitates with Kog1-Myc (Fig. 1E) both ways (not shown), which affirms that Iqg1p interfaces TORC1. Furthermore, co-IP with Tor2 or TORC2-specific subunits was not detected (not shown). These results represent the first report of a link between TORC1 and Iqg1p, the effector of Cdc42p, a GTPase involved in bud-site-selection and polarized growth and support a potential role for Tco89p in bud-site selection and/or cytokinesis which we investigated next.

Iqg1p and Tco89p Confer Rapamycin-Sensitive Growth and Influence Axial Budding and Cytokinesis

Genetic and cell biological analyses indicate that Tco89p, like Iqg1p, has a role in coupling cell growth and division by linking bud-site selection and cytokinesis (Fig. 2). Analysis of 22 tetrads from iqg1Δtco89Δ heterozygous diploid cells did not yield double mutants (not shown), suggesting synthetic lethality and that the two genes affect the same essential function. Moreover isogenic strains harboring iqg1Δ or tco89Δ grew at 30ºC (Fig. 2A), but they were temperature-sensitive at 37ºC compared to their isogenic wild type (Fig. 2B), suggesting an
effect on cell growth (proliferation). Because \textit{tco89}\Delta causes rapamycin-sensitivity in a different genetic background (Reinke et al., 2004), we tested whether Tco89p and Iqg1p confer the same property in our strain background. We extended this analysis further by comparing the effects on diploid and haploid mutant strains growing on rapamycin-containing plates. Surprisingly, rapamycin caused cell cycle arrest in the haploid \textit{iqg1}\Delta and \textit{tco89}\Delta mutants (Fig. 2C, lower right) without affecting their isogenic homozygous diploids or the wild type strains (Fig. 2C, upper right). Interestingly, the \textit{iqg1}\Delta haploid strain was at least two-fold more sensitive to rapamycin than the \textit{tco89}\Delta strain (Fig. 2C, lower right). These findings demonstrate a previously unknown role for Iqg1p in rapamycin-sensitive growth and uncover a role for TORC1 in haploid yeast cell growth, which we investigated further by testing effects on bud-site selection, a well analyzed polarized event in yeast cell cycle.

Haploid yeast cells bud according to an axial budding program and the diploid cells follow a bipolar program. Each program is controlled by a well-defined signaling cascade sharing at the top the GTPases Bud1p and Cdc42p, which control general budding (Chant and Pringle, 1995; Madden and Snyder, 1998; Osman and Cerione, 2006). Components involved in the axial budding program directly control cytokinesis in the haploid cells and their dysfunction results in both cytokinesis and budding pattern defects (Madden and Snyder, 1998; Osman and Cerione, 2006). Comparing the budding patterns of the isogenic haploid and homozygous diploid mutant strains with their respective wild type revealed that Tco89p impacts the axial budding program (Fig. 2D). Haploid \textit{iqg1}\Delta and \textit{tco89}\Delta cells, but not their diploid counterpart, both exhibited defects in budding pattern that is predominantly (~70%) semi-random with fewer (~10-20%) bipolar (Fig. 2D), identical to the phenotype of \textit{iqg1}\Delta cells as reported previously (Osman et al., 2002). The random budding defect phenotype is a known marker of dysfunction...
of proteins serving upstream in the general budding control cascades such as the GTPases Bud1p and Cdc42p (Chant and Pringle, 1995; Osman and Cerione 2006). Thus this phenotype suggests that Tco89p serves upstream of the axial budding markers with Iqg1p-Cdc42p. The presence of a bipolar budding phenotype in haploid cells is a known marker of defects in the axial budding program (Chant and Pringle, 1995; Osman et al., 2002; Osman and Cerione 2006) and supports that Tco89p has a role upstream in the axial budding and growth cascade. Furthermore, the tco89Δ strains exhibited pronounced cell wall defects consistent with a previous report in a different strain background (Reinke et al., 2004). This defect in cell wall deposition was manifested as faint chitin staining (Fig. 2D, lower panels) and lyses/rupture in cell culture. These defects were rescued by re-expression of either Iqg1p or Tco89p in their respective mutant (Osman et al., 2002; and not shown).

Additionally, tco89 mutant strains displayed cytokinesis defects, exhibited by the presence of more than one bud per cell (Fig. 2D, lower panels), reminiscent of iqg1sec3 double mutants (Osman et al., 2002). The significance of these findings is that the axial bud-site selection program in yeast links cell growth to cytokinesis directly (Madden and Snyder, 1998; Osman et al., 2002; Osman and Cerione, 2006) and mimics directed cell division in mammals required for pattern formation and cell fate determination, dysfunction of which leads to cancer, diabetes and a myriad of developmental diseases (Gladfelter et al., 2001; Nelson, 2003). Accordingly, these data raised the possibility that mammalian IQGAP1 controls cell proliferation by coupling cell growth and division through the mTOR pathway, a hypothesis that we investigated next.
Mammalian IQGAP1 Interfaces mTORC1 and Modulates mTORC1→Akt Signaling

Previous evidence revealed that IQGAP1 regulates cell size, binds and requires mTOR for promoting cell proliferation (Wang et al., 2009). However, mTOR is a shared subunit of mTORC1 and mTORC2, and the results obtained above from yeast suggest that the conserved IQGAP1 interfaces mTORC1. Thus we examined IQGAP1 association with both complexes (Fig. 3). Both ways, V5-IQGAP1-F or V5-IQGAP1IR-ww but not V5-IQGAP1-C, co-precipitated with Raptor, the mTORC1-specific subunit, but not with Rictor, the mTORC2-specific subunit (Fig 3B, and not shown). This result supports that IQGAP1 serves through mTORC1 and explains previous reports of its roles in modulating protein synthesis (Rittmeyer et al., 2008) and cell size (Wang et al., 2009). Accordingly, we examined likely effects of IQGAP1 on mTORC1 signaling.

IQGAP1 and CDC42 both are activated downstream of the epidermal growth factor receptor, EGFR (Tu et al., 2003; Rittmeyer et al., 2008). EGF induces serine phosphorylation of IQGAP1 (pSer-IQGAP) leading to binding and activation of CDC42 (Rittmeyer et al., 2008; Wang et al., 2009). Binding of active CDC42-GTP to IQGAP1 inhibits insulin secretion (Rittmeyer et al., 2008), and reduces the cell size, but enhances pIQGAP1S1443-dependent cell proliferation (Wang et al., 2009). Thus we examined EGF-mediated effects of IQGAP1 on mTOR signaling. HeLa cells stably expressing wild type or mutant V5-IQGAP1 (Fig. 3A) as reported previously (Rittmeyer et al., 2008; Wang et al., 2009) showed fast maximal response to EGF at 5 min that remained constant after 15 or 30 min (not shown), therefore treatment for 5 min was selected for measuring signaling. Interestingly, phosphorylation of the MAPK extra cellular signal-regulated kinase (pERK1/2 p44/42) was attenuated in IQGAP1IR-ww cells (Fig. 3C, upper two panels) whereas phosphorylation of the serine/threonine protein kinase
pAkt1\textsuperscript{S473}/PKB (middle two panels), and its substrate the glycogen-synthase kinase-3 (pGSK3\textsubscript{α/β}\textsuperscript{S21/9}, bottom two panels) were augmented. These results demonstrate that expression of IQGAP1\textsuperscript{R-WW} down regulates ERK1/2 and GSK3\textsubscript{αβ} signaling and augments Akt1 signaling in response to EGF and they are consistent with the known pAkt1 inactivation of GSK3 whose activity alters glucose and fat metabolism and induces cell apoptosis (Jobe and Johnson, 2004, Manning, 2004).

Cells that were not treated with EGF for control had no detectable level of phosphorylation of any of the tested kinases except for pAkt1\textsuperscript{S473}, which was slightly increased in IQGAP1-F and -C cells (-EGF, Fig. 3D). This finding explains previous results why these cells induced transformed phenotypes that were sensitive to rapamycin (Wang et al., 2009) and to the PI3K/Akt1 inhibitor LY294002 (see Fig. 5) irrespective of EGF, supporting that IQGAP1-F or IQGAP1-C expression bypasses EGF stimulation and activates Akt1.

Because mTOR binds IQGAP1 (Wang et al., 2009) and Akt1 is an mTOR substrate (Sarbassov et al., 2005), these data raised the possibility of an IQGAP1-Akt1 association, which we detected by co-immunoprecipitation (Fig. 3E), indicating that they directly or indirectly form a complex in vivo. While these data explain that pIQGAP1 induces cell proliferation through Akt1, they do not explain how it augments pAkt1\textsuperscript{S473} (Fig. 3) and reduces the cell size (Wang et al., 2009). To investigate molecular basis of these observations, we examined mTOR downstream signaling.

**IQGAP1 Modulates S6K1 Activity**

As mentioned earlier, it is well established that pAkt1\textsuperscript{S473} activates mTORC1 leading to S6K1 activation by phosphorylation on Thr389. Subsequently, pS6K1\textsuperscript{T389} suppresses pAkt1\textsuperscript{S473} activity to control the cell size, thus the level of pS6K1\textsuperscript{T389} is widely used as a marker of mTORC1.
activity and cell growth. Evaluation of the $pS6K1^{T389}$ level in IQGAP1 mutant cell lines revealed that under nutrient conditions (full serum), $pS6K1^{T389}$ activity increased in IQGAP1$^{IR-WW}$ cells (Fig. 4A), and decreased (30-50%, $p > 0.001$) when EGF was applied (Fig. 4B, third lane; lower 4B, third bar). This attenuation of $pS6K1^{T389}$ was reproducible and specific to IQGAP1 because knockdown of endogenous IQGAP1 by RNAi in the IQGAP1$^{IR-WW}$ cells (Fig. 4C) restored the $pS6K1^{T389}$ level (Figs. 4D, first two lanes and 4E, first two bars). Notably, EGF increased ($p < 0.05$) the $pS6K1^{T389}$ level in cells expressing full-length, IQGAP-F compared to control cells expressing the vector (V) (Fig. 4B). These data explain our previous results that expression of IQGAP1$^{IR-WW}$ enhanced nutrient-induced protein synthesis (Rittmeyer et al., 2008) and cell size (Wang et al., 2009) by binding to mTORC1 (Fig. 3) and increasing $pS6K1^{T389}$. They also demonstrate that IQGAP1$^{IR-WW}$ mediates EGF-responsive augmentation of $pAkt1^{S473}$ by attenuating $pS6K1^{T389}$ thus suppressing its known inhibitory effects on $pAkt1^{S473}$. Collectively, our data support the concept that while IQGAP1-C serves as dominant active (DA) gain of function mutant in cell proliferation and IQGAP1$^{IR-WW}$ serves as dominant negative (DN) (Rittmeyer et al., 2008; Wang et al., 2009), it can serve as DA in response to EGF. This predicted that expression of IQGAP1$^{IR-WW}$, which arrests cytokinesis in presence of nutrients (Wang et al., 2009), would induce an EGF-mediated cell proliferation, a hypothesis that we tested next.

**IQGAP1 induces EGF-stimulated Akt-mediated Proliferation and Localizes to the Midbody During Cell Abscission**

We demonstrated capacity of IQGAP1$^{IR-WW}$ cells to induce EGF-stimulated and Akt1-mTORC1-dependent transformed phenotypes by RNAi, pharmacology and growth in low-serum (Fig. 5). The IQGAP1$^{IR-WW}$-mediated, EGF-stimulated transformed phenotype in fibroblast relied on
mTORC1-Akt1 because it was abolished by applying rapamycin or the PI3K/Akt1-inhibitor LY294002. It was specific to IQGAP1 because it was abolished by knockdown of \textit{IQGAP1}, indicating requirement of the endogenous protein (Fig. 5), consistent with the behavior of these dominant mutants (Rittmeyer et al., 2008; Wang et al., 2009). Similarly, the positive control transformed IQGAP1-C cells that are rapamycin-sensitive (Wang et al., 2009) and contain EGF-independent \( p\text{Akt1}^{S473} \) (Fig. 3D), are sensitive to LY294002, whereas IQGAP1\textsuperscript{IR-WW} cells that were left untreated with EGF as a negative control, whether they express IQGAP1-siRNA or not, failed to proliferate in low-serum (Fig. 5) as previously reported (Wang et al., 2009).

Collectively, these results demonstrate that IQGAP1 differentially responds to nutrient and mitogenic signals and accordingly modulates ERK1/2/GSK3\( \alpha/\beta \) and mTORC1/S6K1\( \rightarrow \text{Akt1} \) signaling to regulate cell proliferation by coupling cell growth and division thus raising the possibility that IQGAP1 differentially localizes during the cell cycle.

We tested this possibility by determining IQGAP1 localization during the cell cycle in synchronized HeLa and NIH3T3 cells, using \( \alpha \)-tubulin as a cytokinesis marker. IQGAP1 and \( \alpha \)-tubulin distributed diffusely in the cytoplasm throughout the cell cycle then co-localized on the midzone mitotic spindles (supplementary Fig. 1) during telophase, as revealed previously by proteomics (Skop et al., 2004; Morita et al., 2007). However, in late cytokinesis, during cell abscission, \( \alpha \)-tubulin remained on the mitotic spindles whereas IQGAP1 mostly concentrated in the middle of the midbody (Fig. 6, arrows and supplementary Fig. 2), resembling the centrosome localization (Gromley et al., 2005). This localization pattern was consistent in several cell lines that we screened with IQGAP1 antibodies, including pancreatic \( \beta \)-cells and renal cell carcinoma (RCC) (Fig. 6B). This novel finding implies that IQGAP1 has a role in midbody abscission, which is the distinct final step in animal cytokinesis (Gromley et al., 2005). This result explains
the previous finding that while IQGAP1-F and IQGAP1–C served as DA mutants and accelerated the cell cycle and induced transformed phenotypes, IQGAP1\textsuperscript{IR-WW} acted as DN and arrested cytokinesis (Wang et al., 2009). Together, these findings support an additional potential role for IQGAP1 in cell abscission downstream of the mTORC1/S6K/Akt1 pathway.

**IQGAP1 Activity is Deregulated in Carcinoma Cell Lines**

EGF or overexpression of IQGAP1 activates both IQGAP1 and CDC42, resulting in their binding and induction of cell proliferation (Rittmeyer et al., 2008; Wang et al., 2009). Thus we hypothesized that unregulated $p$IQGAP1 signaling resulting from overexpression (Rittmeyer et al., 2008) and not the mere overexpression, underlies tumorigenesis. To begin testing this hypothesis we evaluated the level of $p$Ser-IQGAP1 in a variety of carcinoma cell lines, including brain, breast RCC, and pancreatic carcinomas. We detected a significant increase in $p$Ser-IQGAP1 level in carcinomas compared to normal human mammary epithelia, HME1 (Fig. 7A), and normal kidney epithelia (Fig. 7B). Interestingly, when comparing pancreatic $\beta$-cell lines, we found higher $p$Ser-IQGAP1 level in carcinoma than in insulin-secreting insulinoma cell lines (compare dark bars). Furthermore, EGF treatment evoked higher levels of $p$Ser-IQGAP1 in the insulin-secreting cells but not in the pancreatic carcinoma cell lines (compare open bars), perhaps because the pancreatic carcinomas have maximal starting levels. These findings enforce our hypothesis and will be tested by further studies. The collective data discussed in this study support the model that IQGAP1, regulated by phosphorylation in response to nutrient or mitogenic signals, has a role in modulating mTORC1/S6K$\rightarrow$Akt1 NFL and serves downstream of it to control cell homeostasis. Dysfunction of this process can underlie IQGAP1’s tumorigenesis and explain its association with human carcinomas (Fig. 8).
Discussion

This study presents a number of novel findings relevant to the central question of how cells coordinate their growth and division to maintain homeostasis, which is crucial to understanding the mechanism of tumorigenesis as well as diabetes in human. It demonstrates for the first time, involvement of yeast TORC1 in bud-site selection and cytokinesis in baker’s yeast via the actin cytoskeleton regulators Iqg1p-Cdc42p. Thus despite the prevailing paradigm, which assigns cytoskeleton regulation to TORC2, our results support that TORC1 also impacts the actin cytoskeleton, an idea that requires further investigation. Moreover, this study reveals for the first time, a role for yeast and mammalian IQGAPs in rapamycin-sensitive growth. Interestingly, yeast Iqg1p was more sensitive to temperature and rapamycin-mediated growth inhibition than the TORC1 subunit Tco89p (Fig. 2B and C), which could potentially be utilized for predicting rapamycin-sensitivity in human cancers. Together these findings support the concept that IQGAP1 serves as a TORC1-scaffolding regulator. Furthermore, it reports a potentially conserved role for IQGAP1 in coupling cell growth and division through a potentially conserved novel CDC42/IQGAP1/TORC1/S6K1/Akt1 pathway. In absence of detecting an interaction with mTORC2, our findings raise the possibility that mTOR/IQGAP1 form a distinct mTOR sub-complex that impacts Akt1 signal-specificity towards mitosis and cell proliferation and experiments are underway to test this hypothesis.

Previously we reported that yeast Iqg1p specifies axial bud-site and links budding to cytokinesis (Osman et al., 2002; Osman and Cerione, 2006). In yeast, budding and cytokinesis both require directed deposition of new cell wall material to the growing bud-site as well as to the cytokinesis plate to bisect the daughter cells (Chuang and Schekman, 1999; Hales et al., 1999; Wolfe and Gould, 2005; Zhang et al., 2006; Osman and Cerione, 2006). Cell wall
synthesis is part of the cellular growth programs and is regulated with the cell cycle through spatial cues with the cell polarity machinery and the secretory pathway (Lesage and Bussey, 2006) and requires coordination with the Tor pathway (Fuchs and Mylonakis, 2009). However, the molecular basis of the proposed cross talk between Tor and the polarity machinery has not been determined (Fuchs and Mylonakis, 2009). Our finding that Iqg1p, the effector of Cdc42p GTPase implicated as positional markers in cell polarity, actin cytoskeleton and cytokinesis (Ziman et al., 1991; Osman and Cerione 1998; Johnson, 1999; Osman et al., 2002) interacts with TORC1 subunit Tco89p (Fig. 1) implicated in cell wall synthesis (Loewith et al., 2002; Reinke et al., 2004) represents the first molecular evidence for such cross talk. Moreover, our results implicates TORC1 for the first time, in budding yeast cytokinesis, which appears to be a conserved function, as a recent evidence has also implicated TORC1 in fission yeast cytokinesis (Hartmuth and Petersen, 2009). However a prevailing concept in the field is that TORC1 controls cell growth whereas TORC2 controls the actin cytoskeleton, our results now invite revisiting this paradigm with further investigation.

For relevance to human health, we dominant mutants of IQGAP1 and translated our findings into mammalian cells, as they permit study of growth factors signal. Biochemical and two-hybrid evidence suggested that IQGAP1-C- and N-termini dimerize with full-length and that the C-terminus can fold on itself and the N-terminus folds on the C-terminus (Grohmanova et al., 2004; Rittmeyer et al., 2008). These findings led to the adoption of the autoinhibition-folding as a mechanism, which has general support from structural evidence from like proteins (Agazadeh et al., 2000). On this basis, we demonstrated previously that IQGAP1IR-WW serves as DA in secretion and cell size and as DN in cell proliferation and migration with the converse being true for IQGAP1-C, which binds and activates CDC42 (Rittmeyer et al., 2008; Wang et al., 2009).
However, these two activities are interdependent because the phenotype of these mutants requires the endogenous protein as substantiated by RNAi (Rittmeyer et al., 2008; Wang et al., 2009). While this finding supports the autoinhibition-folding model where these mutants likely heterodimerize with the endogenous protein to cause dominant effects revealing cryptic functions of IQGAP1, it also support the concept that cell growth is prerequisite to cell division (Tapon et al., 2001); explaining why the endogenous protein is required. Overall, we propose that these features underlie the mechanism of actions of these mutants reported in this study.

On the basis of the above concept, we unveiled a potential mechanism by which IQGAP1 inhibits mTORC1 to augment Akt1 signaling and promote cell proliferation and survival while attenuating ERK1/2/GSK3α/β signaling (Figs. 3-5) implicated in cell differentiation and apoptosis. This finding is consistent with the opposing signals of ERK1/2/GSK3α/β and Akt1 (Ding et al., 2005). GSK3α/β regulates apoptosis, lipid and fat metabolism and is a critical PI3K/Akt1 substrate where active \( pAkt_{1S473} \) inhibits GSK3 by phosphorylating Ser21 on GSK3α and Ser9 on GSK3β (Jobe and Johnson, 2004, Manning, 2004). Furthermore, the finding that IQGAP1IR-WW attenuates ERK1/2 agrees with and further extends with new insights, previous reports that IQGAP1 modulates ERK2 (Roy et al., 2004) and serves as a scaffold for c-Raf-MEK1/2-ERK1/2 and Akt1 and controls stress-mediated cardiomyocyte apoptotic death (Sbroggiò et al., 2011). ERK1/2 are effectors of Ras/Raf/MEK1 and they regulate cell differentiation (Minden & Karin, 1997), therefore their attenuated activity is consistent with the increased \( pAkt_{1S473} \) signal, which promotes cell survival and mitosis eliciting transformed phenotypes (Figs. 3 and 5). Thus, our results demonstrate, for the first time, the capacity of IQGAP1 to interplay ERK1/2 and Akt1 signaling to promote cell proliferation and potentially suppress apoptosis, a concept that is currently under investigation.
Our data reveal, for the first time, a potential mechanism by which IQGAP1 augments \( pAkt1^{S473} \) and promotes cell proliferation by inhibiting mTORC1/S6K1 signaling. IQGAP1 mediated an EGF-stimulated attenuation of S6K1 activity thereby directly or indirectly activating Akt1\(^{S473} \) (Figs. 3 and 4), however binding to Akt1 (Fig. 3E) predicts a direct role to be further investigated. It has been demonstrated that mTORC2 (mTOR-Rictor) activates Akt1 by phosphorylation on Ser473, subsequently, \( pS6K1^{T389} \) phosphorylates Rictor on T1135 (Dibble et al., 2009; Julien et al., 2010) to suppresses mTORC2 and inhibit Akt1\(^{S473} \) activity via a negative feedback loop. Expression of the mutant Rictor\(^{T1135A} \) resistant to phosphorylation by \( pS6K1^{T389} \) increases \( pAkt1^{S473} \) activity and enhances mitosis by activating FoxO proteins in response to EGF (Julien et al., 2010). This finding agrees with our results that attenuated \( pS6K1^{T389} \) (Fig. 4) led to enhanced \( pAkt1^{S473} \) activity (Fig. 3C) and increased EGF-stimulated cell proliferation (Fig. 5). Moreover, several lines of evidence support that inhibition of mTORC1 advances mitosis (Hartmuth and Petersen, 2009; Gwinn et al., 2010; Julien et al., 2010) through Akt1 and its downstream effectors the forkhead (FKH-Tfs) FoxO transcription factors, however how this signaling axis is regulated remains incompletely defined (Alvarez et al., 2001; Shiota et al., 2006; Frias et al. 2006; Jacinto et al., 2006; Dibble et al., 2009; Julien et al., 2010). Our results present a novel and physiologically plausible mechanism whereby IQGAP1 inhibits mTORC1/S6K1 and abrogates its inhibitory feedback mechanism on \( pAkt1^{S473} \) thus advancing mitosis and cell proliferation, and IQGAP1’s role in cytokinesis lends additional credence to this hypothesis.

Like in yeast, animal cytokinesis requires membrane expansion and directed secretion in the cleavage furrow to bisect the daughter cells (Gromley et al., 2005). Our data supports a role for IQGAP1 downstream of mTORC1/Akt1 in promoting mitosis and executing cytokinesis,
which is consistent with our previous observation that IQGAP1-F and IQGAP1-C served as DA and accelerated the cell cycle while IQGAP1<sup>IR-ww</sup> served as DN and arrested cytokinesis (Wang et al., 2009). The spatial distribution of IQGAP1 as a ring in the midbody during cytokinesis (Fig. 6) implies a direct role in cell abscission (Fig. 8). In support of this concept, IQGAP1 was identified as partner of midbody proteins both by proteomics and two-hybrid (Skop et al., 2004; Morita et al., 2007). This localization pattern is identical to that of the centrosome and Exo70, an IQGAP1-binding partner in secretion (Rittmeyer et al., 2008), found to cooperate in midbody abscission by promoting asymmetric secretion (Gromley et al., 2005). Thus, IQGAP1 midbody localization, binding to the exocyst, regulation of exocytosis (Rittmeyer et al., 2008), acceleration of the cell cycle and promotion of cell proliferation (Fig. 5; Wang et al., 2009), predict key role in bisecting the daughter cells in mammals, which is currently being characterized. This finding represents the first report of localization of a member of IQGAP family to the cleavage furrow and may not apply to all three members of the family, which may regulate cytokinesis by different mechanisms, as yet, to be determined. Collectively, our data suggest that IQGAP1 has a conserved role in cell homeostasis by coupling cell growth and division, serving both upstream and downstream of mTORC1/S6k1/Akt1 NFL (Fig. 8).

This mechanism would also explain IQGAP1’s role in tumorigenesis. Several studies reported that IQGAP1 expression or mislocalization associates with a diversity of human carcinomas (reviewed in Johnson et al., 2009). However, our data support that it is not mere overexpression, but is unregulated IQGAP1 signaling that leads to tumorigenesis (Figs. 7 and 8), marked by increased pIQGAP1<sup>S1443</sup> and possibly aberrant sub-cellular redistribution (supplementary Fig. 1; Figs. 6-8), thus IQGAP1 would serve as a marker in tumors and as a potential therapeutic target. Because of IQGAP1 and mTOR roles in insulin secretion, it is
appealing to assume that the same mechanism applies to controlling insulin homeostasis and its deregulation underlies diabetes.

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Materials and Methods

Yeast Cell Culture, Genetic Manipulations and Two-hybrid analyses

Yeast strains, culture and two-hybrid screens and analyses were used as described previously (Osman et al., 2002). Briefly, IQG1 was cloned in frame into HA-pGBD-C2 vector, verified by sequencing and Western blotting of the right size HA-tagged protein with HA antibody and used as bait for screening the three genomic libraries YL2H-C1-3 in the yeast strain PJ694A (James et al., 1996) as described previously (Osman et al., 2002). The putative interacting library clones (LC) were identified by sequencing and the relevant gene was cloned by high fidelity PCR into the two hybrid vectors, verified by sequencing and the specificity of the interaction retested by qualitative (plate) and quantitative (liquid) Two-hybrid assays. The chromosomal copy of the genes was epitope-tagged and the interaction confirmed by IP. Gene cloning, C-terminal tagging or deletions were performed as described previously (Osman et al., 2002) using the pFA6a plasmid cassettes and procedures of Longtine et al., 1998 and Baudin et al., 1993. For
quantitative two-hybrid analyses, β-Galactosidase activity (Miller units) was calculated from at least five independent clones as previously described (Osman et al., 2002). Growth curves were determined by using a Bioscreen C Machine (Growth Curves USA). The cells were grown to OD₆₀₀ 0.2, serially diluted with YEPD in 96-well sterile plates in duplicates and incubated overnight (16 h) with shaking at 30°C or 37°C. The optical densities were automatically determined every 10 min and the average of two independent experiments was used to generate the growth curves with the algorithms in the Microsoft Excel software.

**Mammalian Cell Culture**

All cell lines were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% calf serum (NIH3T3) or 10% fetal bovine serum, FBS and 100 units/ml penicillin, 100 µg/ml streptomycin (Invitrogen) in a humidified 5% CO₂ incubator at 37°C. HeLa cells (ATCC) were cultured in MEM plus FBS under the same conditions. NIH3T3 and HeLa cell lines stably expressing the V5-IQGAP1 constructs were described previously (Rittmeyer et al., 2008; Wang et al., 2009). Glioblastoma U87, breast carcinomas, kidney RCC and normal HME1 and normal kidney cell lines as well as the human pancreatic carcinoma PANC-1, the insulin-secreting insulinomas βHC-9 and βTC-6 and the insulin-secreting RIN-5F and HIT-T15 were obtained from ATCC (Manassas, VA).

**Cell Synchronization**

Mammalian cells were cultured under the conditions described above and synchronized at G1/S with a double aphidicolin block (1.25 µg/ml) in growth media overnight followed by release for 9 h and treatment again overnight as described previously (Surka et al., 2002). Sample cells growing on cover slips were fixed every 15 min over 12 h period and used for immunofluorescence.
Fluorescence Microscopy

Calcofluor staining of chitin cell wall and bud-scars in yeast was done as described previously (Osman et al., 2002) following Pringle (1991). Briefly, cells at log phase were collected and stained with Calcofluor (Fluorescent Brightener, Sigma-Aldrich) and cells with more than three bud-scars were scored for budding pattern. The bud scars were visualized with a 100X oil emersion lens using an Olympus fluorescence microscope fitted with a Hamamatsu ORCAER monochrome CCD camera. Synchronized cells were fixed as described previously (Rittmeyer et al., 2008; Wang et al., 2009) and stained with IQGAPI primary antibodies (polyclonal was from Santa Cruz (H-109) or abcam (ab86064) and monoclonal was from Epitomocs (RabMAb Cat#3548-1) or Novus Biologicals (2C5) followed by secondary antibodies (Texas Red and AlexaFluor 488, Molecular Probes) and α-Tubulin-FITC monoclonal antibody (Sigma) for 1 h each at room temperature. The nuclei were stained with DAPI (Sigma), and the images were captured with a Leica confocal microscope.

Immunoprecipitation (IP) and Western Blotting

The stable cells were washed with ice-cold phosphate-buffered saline (PBS), scraped into 500 µl lysis buffer (25 mM HEPES, pH 7.4, 15 mM MgCl₂, 150 mM NaCl, 1% NP40, 10 µg/ml each of leupeptin and aprotinin and 0.2 mg/ml phenylmethylsulfonic chloride) and incubated on ice prior to centrifugation at top speed for 30 min. Yeast cells were disrupted with acid-washed glass beads and the lysate collected by centrifugation. Incubation of the lysates with beads alone or with an unrelated antibody was included as controls. Protein concentrations were determined with the Bio-Rad D₆ kit and equal amounts were precleared with beads (15 µl) for 1 hr at 4°C and used for IP. Briefly, the antibody was added to the precleared lysate and incubated on ice for 1 hr and 40 µl of PBS-equilibrated protein A or G beads were added and gently rocked for 3 hrs or
overnight at 4°C. The beads were washed 4X with 1 ml lysis buffer, boiled for 10 min in 40 µl 2X SDS sample buffer and resolved with SDS-PAGE. For IP with TOR, the detergent in the buffer was replaced by 3% CHAPS to preserve the TOR complexes (Sorbassov et al., 2005). Immunoblotting was performed with the antibodies indicated on the figures. IQGAP1 and yeast Tor1 and Tor2 antibodies were from Santa Cruz Biotech and the antibodies for mTOR, S6K, PCKC-substrate pan pSer and Akt1 were from Cell Signaling Technology. The antibodies were used in combination with Signal Enhancer Hikari (Nacalai, USA). Band signal was detected and quantified as below.

**Detection of Kinase Activity**

Confluent cells stably expressing the IQGAP1 constructs and vector control (neomycin-resistant cells) were serum-starved for 12 h and stimulated with epidermal growth factor, EGF (100 ng/mL) in serum-free media for 5 min or were left untreated for control. After washing with ice-cold PBS, the cells were lysed and ~ 80 µg of proteins were resolved in 7-20% gradient SDS-PAGE, transferred to PVD membranes and immunoblotted with total and phospho-specific (active) antibodies (Cell Signaling Technology) for ERK (p44/42), Akt1 (Ser473), GSK3αβ (Ser21/9) and S6K (Thr389). The signal was developed by Enhanced Chemiluminescence (Amersham) and band intensities detected and quantified from three experiments by ChemiDocXRS and Quantity One, 4.4.1 (Bio-Rad). Statistical analysis was performed by the algorithms in the Microsoft Excel software.

**Transformation Assays.**

Ability to proliferate in low serum is a phenotype acquired by transformed cells. Transformation assay in low serum was used to compare NIH3T3 stables with neomycin-resistant control cells expressing the empty vector as described previously (Wang et al 2009): Briefly, to measure
serum-independent growth of the transformed IQGAP1-C and the EGF-treated IQGAP1IR-WW cells, confluent cells were trypsinized and seeded in triplicates at 10x10⁴ in 12-well plates in DMEM plus 10% CS and incubated for five hours to allow cell adherence. Thereafter, the cells were washed with low-serum DMEM containing 1% CS and grown in the same medium that was changed every other day for 6 days. At 2, 4 and 6 days, the cells were washed thoroughly with PBS, trypsinized and counted with a haemocytometer. To measure EGF-induced transformation, 100 ng/mL EGF were added to the growth media. Experiments were performed in triplicates from three clones with similar expression levels of IQGAP1 constructs. Data are presented as the means of three independent experiments and statistical analyses was performed by the algorithms in the Microsoft Excel software.

**RNA interference**

RNAi was performed as described previously (Rittmeyer et al., 2008; Wang et al., 2009). Two human IQGAP1-siRNAs, scramble and the siCONTROL oligomers that do not target IQGAP1-C or other IQGAP-family members as demonstrated previously (Rittmeyer et al., 2008; Wang et al., 2009), were obtained from Dharmacon.

**Figure Legends**

**Fig. 1.** Identification of yeast TORC1 as an Iqg1p-partner. A. Schematic representation of Iqg1p and mutants used for two-Hybrid or immunoprecipitation (IP) analyses: F, full-length; N, N-terminus; NΔ294, lacking the N-terminus upstream sequences; CHD, Calponin Homology Domain that binds actin; C, the C-terminus containing the Ras-Gap Related Domain (GRD). B. Tco89-N and full-length association with Iqg1p by Two-hybrid plate assay. Yeast cells harboring β-Gal as reporter gene and expressing HA-pGBDC2-Iqg1p full-length together with
either the genomic library clone, LC, encoding the first 300 residues of Tco89p (dark, blue), the full-length pGADC2-Tco89p (dark, blue) or the empty vector pGADC2 as negative control (white), were patched on nutrient-dropout triple-selection plates containing β-Galactosidase. 

C. Mapping the domain of Iqg1p that interacts with Tco89p. Quantitative Two-hybrid analysis of Tco89p association with Iqg1p mutants and with Bud4p. The β-Galactosidase activity in Miller Units, was calculated from five different clones and plotted as the means± SEM for n=3.

*Significantly (p<0.005) > Iqg1-C or vector. 

D. Iqg1p and Tco89p interact in vivo. Lysates from cells co-expressing the tagged chromosomal copies Tco89-Myc and Iqg1-HA mutants were immunoprecipitated with HA antibodies and blotted with Myc antibodies, 5% of the whole cell lysate (WCL) was blotted with Myc antibodies as a loading control. 

E. Iqg1p co-precipitates with Kog1p, another TORC1-specific subunit. Cells co-expressing tagged chromosomal copies were processed as in D.

Fig. 2. Iqg1p and Tco89p Share Functions in Rapamycin-sensitive Growth, Axial Bud-site Selection and Cytokinesis. 

A-B. IQG1 or TCO89 Null Strains are Temperature-sensitive. Haploid WT and mutant cells were grown in YEPD to OD600 0.2, serially diluted into 96-well plates and the optical density was determined every 10 min overnight with a Bioscreen C plate reader A. at 30°C and B. at 37°C. 

C. Iqg1p Confers Rapamycin-sensitive Growth. Isogenic wild-type, iqg1Δ, tco89Δ, diploid (upper) and haploid (lower) strains were grown in YEPD to midlog phase (OD600=0.5), washed in sterile double-distilled water, serially diluted and plated on YEPD-agar for control (left) or on YEPD-agar containing 1.0 ng/mL rapamycin (right) and incubated at 30°C for three days then photographed with a BioRad XRS imager. 

D. Tco89p Specifies Axial Budding. Isogenic haploid wild type, iqg1Δ, and tco89Δ strains were grown on YEPD, washed and stained with Calcofluor (Sigma), which is a fluorescent dye specific for cell
wall and bud-scar chitin, and photographed using a 100X oil lens. The arrows denote additional buds from the same cell, indicating cytokinesis defects.

**Fig. 3.** IQGAP1 Associates with mTORC1 and Modulates mTOR-Akt1 Signaling. **A.** Schematic representation and expression level of the V5-IQGAP1 and mutants in stable HeLa or NIH3T3 cells (as described previously, Rittmeyer et al., 2008; Wang et al., 2009) used for signaling or IP. CHD: Calponin Homology Domain; IR: IQGAP- specific Repeats; WW: A Proline-rich domain resembling the SH3 protein interacting domains; IQ: 4 IQ motifs that bind calmodulin; GRD: rasGAP-Related Domain containing sequences that binds activated CDC42. The V5-IQGAP1 constructs: IQGAP1-F (F), IQGAP1IR-WW (IR-WW) and IQGAP1-C (C) and (V) denotes vector control. **B.** IQGAP1 Associates with Raptor (mTORC1) and *not* with Rictor (mTORC2). IQGAP1 or mutants were precipitated with V5 antibodies from HeLa cell lysate, separated on SDS-PAGE and blotted with antibodies for Raptor or Rictor. Rictor and Raptor in the whole cell lysate (WCL) was blotted as a loading control. **C.** IQGAP1 Modulates mTOR/Akt-signaling. 80 μg of total proteins from HeLa cells stably-expressing the indicated IQGAP1 constructs, serum-starved and treated with EGF, were blotted with total or phospho-specific antibodies for ERK1/2 (Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2), Akt1Ser473 or GSK3Ser21/9. **D.** Signal-dependent differential activation of Akt1 by IQGAP1. Cells were serum-starved and either left untreated or treated with EGF and blots obtained as in C were processed at SE, short (30 sec) and LE, long (2 min) exposure times of the same blot. The –EGF (control) and +EGF (from C) blots were processed on the same gel. **E.** Akt1 co-precipitates with IQGAP1. IQGAP1 and mutants were precipitated with V5 antibodies and blotted with Akt1 antibodies. Akt in the cell lysates (WCL) used for IP was blotted as equal loading control. The results are representative of different stable cell types NIH3T3, β-cell lines, MDCK or kidney.
**Fig. 4.** IQGAP1 Modulates S6K$^{T389}$ activity. **A.** IQGAP1 stimulates $pS6K^{T389}$ activity in high-serum (-EGF): **Upper panel:** A representative blot: lysates from HeLa cells expressing the V5-IQGAP1 constructs and growing in full serum were blotted with total and phospho-Thr389 S6K1 antibodies. **Lower panel:** bands intensities were quantified by densitometry, normalized against total S6K and presented as the means± SEM for n=3 independent experiments. *Significantly (p<0.001) lower than IQGAP1-F cells. **B.** IQGAP1 attenuates $pS6K^{T389}$ activity in response to EGF (+EGF): stable HeLa cell were serum-starved and treated with EGF and equal amount of lysates processed and the band intensities quantified as in A. **C.** Lysates from cells expressing two IQGAP1-siRNA, which down regulates IQGAP1 by >90%. **D.** Knockdown of endogenous IQGAP1 in cells expressing IQGAP1$^{IR-WW}$ and treated with EGF, restores $pS6K^{T389}$ level: control and stable IQGAP1$^{IR-WW}$ HeLa cells expressing the IQGAP1-siRNAs were serum-starved and treated with EGF and their lysate blotted with antibodies for total and phospho-Thr389 S6K1. **E.** Quantification by densitometry of bands represented in D and presented as the means± SEM for n=3 independent experiments. *Significantly (p<0.001) lower than the siRNA-treated cells. The results are representative of several cell lines including pancreatic β-cell and kidney cell lines.

**Fig. 5.** IQGAP1 induces EGF-stimulated transformed phenotype and plays a role in late cytokinesis. **A.** IQGAP1$^{IR-WW}$ induces EGF-stimulated and mTORC1/PI3K/Akt1-dependent cell proliferation. NIH3T3 cells stably expressing IQGAP1$^{IR-WW}$, IQGAP1-C (positive control), vector (V) as negative control or IQGAP1-siRNA, were serum-starved then left untreated or treated with EGF, DMSO (as drug-vehicle control), 10 $\mu$M PI3K/Akt1-inhibitor LY294002 or 100 nM mTOR-inhibitor rapamycin and evaluated by growth in low-serum media. Cell number was determined in triplicates every other day for 6 days and presented as the means± SEM for
n=3. EGF significantly ($p<0.001$) induced transformed phenotype and the drug inhibitors significantly ($p<0.001$) revered this phenotype in treated vs. untreated IQGAP1<sup>IR-ww</sup> cells.

**Fig 6.** IQGAP1 localizes in the midbody during cell abscission. **A.** Synchronously growing cells were fixed every 15 min for 16 h. and stained with the indicated rabbit polyclonal or rabbit monoclonal IQGAP1 antibodies (red, arrows) and FITC-α-Tubulin (green) and photographed under a confocal microscope. Enlarged midbody regions are shown, for full-view of representative cells and throughout the cell cycle see supplementary Figs. 1 and 2. **B.** Different cell lines were screened for midbody localization with antibodies for IQGAP1 and two examples: RCC (SW839) and β-cells (βTC-6 insulin-secreting insulinoma) cell lines are presented.

**Fig. 7.** Increased level of pSer-IQGAP1 in carcinoma cell lines. **A.** 80 µg of total proteins from glioblastoma (U87), breast carcinoma (MD-MB-231 and SKBR3) and renal cell carcinomas (RCC) and normal (as control) human mammary epithelia HME1, cell lines were immunoprecipitated with antibodies for IQGAP1 and blotted with PKC-substrate pSer-specific antibodies (upper lanes). Equal amount of cell lysate was blotted with antibodies for IQGAP1 (middle panel) or actin as loading control. **B.** The pSer-IQGAP1 level is elevated in several clear cell renal cell carcinoma (RCC) cell lines. Blots were obtained as described in B and the level of pSer-IQGAP1 bands was quantified by band densitometry, normalized against total IQGAP1 and expressed as the means± SEM for n=3. *pSerIQGAP1 in Normal epithelia is significantly ($P<0.05$) lower than any of the RCC cell lines. **C.** EGF effects on pSer-IQGAP1 levels in differentiated and undifferentiated carcinoma cell lines. EGF evoked higher pSer-IQGAP1 levels in insulin-secreting insulinoma cell lines compared to undifferentiated pancreatic carcinomas PANC-1, which already contain high levels in serum alone. Cells were starved for 12 hours and
either untreated (dark bars) or treated (open bars) with EGF and equal amount of lysates immunoprecipitated with IQGAP1 antibodies and processed as in panel A.

**Fig. 8. IQGAP1 Regulates Cell Homeostasis Through mTORC1:** A model depicting IQGAP1’s action in coupling cell growth and division through a novel CDC42-mTORC1/S6k1/Akt1 in response to nutrient or mitogenic signal. Black arrows denote previous findings of IQGAP1’s regulation of cell size and a known mTORC1/S6k1 negative feedback loop (NFL) and red arrows denote new findings in this study. In closed conformation, IQGAP1 enhances insulin secretion, protein synthesis and cell growth (Rittmeyer et al., 2008; Wang et al., 2009) through mTORC1/S6k1. In open conformation pIQGAP1 promotes cell division and proliferation (Wang et al., 2009) via pIQGAP1S1443↑/mTORC1/S6k1↓pAkt1S473↑ and suppresses differentiation and/or apoptosis by attenuating ERK1/2 and GSK3αβ. IQGAP1 attenuates pS6K1T389 activity to enhance pAkt1S473 activity, which in turn activates mitosis where IQGAP1 has a role in cell abscission. Thus IQGAP1 may act like a rheostat to modulate the NFL of pS6K1T389 → pAkt1S473 and maintain cell homeostasis, dysregulation of which could underlie tumorigenesis and/or aberrant insulin signaling in diabetes.

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A

B

C

D

E

Osman Fig. 1
Osman Fig. 4
Osman, Fig. 5