mTORC1 and mTORC2 Regulate EMT, Motility, and Metastasis of Colorectal Cancer via RhoA and Rac1 Signaling Pathways

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Abstract

Activation of phosphoinositide 3-kinase (PI3K)/Akt signaling is associated with growth and progression of colorectal cancer (CRC). We have previously shown that the mTOR kinase, a downstream effector of PI3K/Akt signaling, regulates tumorigenesis of CRC. However, the contribution of mTOR and its interaction partners toward regulating CRC progression and metastasis remains poorly understood. We found that increased expression of mTOR, Raptor, and Rictor mRNA was noted with advanced stages of CRC, suggesting that mTOR signaling may be associated with CRC progression and metastasis. mTOR, Raptor, and Rictor protein levels were also significantly elevated in primary CRCs (stage IV) and their matched distant metastases compared with normal colon. Inhibition of mTOR signaling, using rapamycin or stable inhibition of mTORC1 (Raptor) and mTORC2 (Rictor), attenuated migration and invasion of CRCs. Furthermore, knockdown of mTORC1 and mTORC2 induced a mesenchymal–epithelial transition (MET) and enhanced chemosensitivity of CRCs to oxaliplatin. We observed increased cell–cell contact and decreased actin cytoskeletal remodeling concomitant with decreased activation of the small GTPases, RhoA and Rac1, upon inhibition of both mTORC1 and mTORC2. Finally, establishment of CRC metastasis in vivo was completely abolished with targeted inhibition of mTORC1 and mTORC2 irrespective of the site of colonization. Our findings support a role for elevated mTORC1 and mTORC2 activity in regulating epithelial–mesenchymal transition (EMT), motility, and metastasis of CRCs via RhoA and Rac1 signaling. These findings provide the rationale for including mTOR kinase inhibitors, which inhibit both mTORC1 and mTORC2, as part of the therapeutic regimen for CRC patients. Cancer Res; 71(9); 3246–56. ©2011 AACR.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States (1). The prognosis for advanced CRCs remains dismal, mainly due to the propensity for metastatic progression and resistance to chemotherapy (2). The metastatic cascade entails an orderly sequence of steps enabling tumor cells to detach from the primary tumor, migrate and invade into surrounding tissue, intravasate into systemic circulation, extravasate to distant organs, and colonize at secondary sites (3). CRCs predominantly spread along the mesenteric circulation to the liver and less frequently to the lungs and bone.

It is believed that the initial step, acquisition of migratory and invasive capability, is the rate-limiting step in this cascade (4). Epithelial–mesenchymal transition (EMT) is proposed to be a crucial mechanism regulating the initial steps in metastatic progression (3). EMT is a molecular program whereby epithelial cells undergo reprogramming from a polarized, differentiated phenotype with numerous cell–cell junctions to acquire a mesenchymal phenotype, including lack of polarization, decreased cell–cell junctions, and increased motility and chemotherapeutic resistance (3). The plasticity of this event is highlighted by the fact that EMT is reversible—the reverse process is termed mesenchymal–epithelial transition (MET; ref. 3). Another important regulatory mechanism in the initial steps of the metastatic cascade involves activation of small GTPases, including RhoA and Rac1, which play a crucial role in actin cytoskeletal rearrangement and cell migration (5). Specifically, RhoA induces formation of actin stress fibers and cell–cell adhesions, whereas Rac1 stimulates formation of lamellipodia.

Activation of phosphoinositide 3-kinase (PI3K)/Akt signaling through activating mutations in PIK3CA (encoding the p110α catalytic subunit of PI3K) or loss of PTEN (encoding a lipid and protein phosphatase) is associated with the growth
and progression of CRC (6, 7). We have previously shown that PI3K and Akt2 can regulate the metastasis of CRCs (8, 9). However, identification of downstream proteins that are directly involved in regulating CRC metastasis would allow development of targeted therapies with fewer toxicities.

mTOR is a downstream serine/threonine kinase that exists in two complexes (10): mTORC1 (containing mTOR, Raptor, etc.) and mTORC2 (containing mTOR, Rictor, etc.). The bacterially derived drug rapamycin allosterically inhibits mTOR activity (10). mTORC1 is partially sensitive to rapamycin treatment, whereas mTORC2 is believed to be rapamycin-insensitive (10, 11). We have previously shown that mTORC1 and mTORC2 components, Raptor and Rictor, respectively, are overexpressed in CRCs and play an important role in CRC tumorigenesis (12). In this study, we investigated whether these proteins regulate motility, EMT, and metastasis of CRCs.

Materials and Methods

Reagents, cell lines, and lentiviral transduction

The human colon cancer cell lines HCT116, SW480, and KM20 were used as described previously (12). Identity of all cells was authenticated at the Johns Hopkins Genetic Resources Core Facility (Baltimore, MD) in October 2010 with short tandem repeat analysis using the Identifier Kit from Applied Biosystems. HCT116 and SW480 cells were obtained from American Type Culture Collection; KM20 cells were kindly provided by Dr. Isaiah J. Fidler (MD Anderson Cancer Center, Houston, TX). As described previously, stable knockdown HCT116, SW480, and KM20 cells were generated using short hairpin RNAs (shRNA) directed against human Raptor and Rictor genes constructed in pLKO.1-puro vector obtained from Addgene (12). A plasmid carrying nontargeting control (NTC) sequence was used to create control cells. Rapamycin and oxaliplatin were obtained from Sigma–Aldrich, TGFβ was obtained from PeproTech, Y27632 was obtained from Cayman Chemicals, and NSC23766 was obtained from Tocris Biosciences.

Immunohistochemistry

Paraffin-embedded tissue array sections of normal colon, primary CRC, and matched liver metastases were obtained from Accurate Chemical & Scientific Corporation. Each array category consisted of tissue derived from 18 patients: 18 normal cores, 36 primary tumor cores, and 36 liver metastasis cores. Staining was carried out as described previously with antibodies against mTOR (1:100), Raptor (1:100), and Rictor (1:100) obtained from Bethyl Laboratory (12). A modified Boyden chamber invasion assay with Matrigel-coated Transwell chambers was conducted with HCT116, KM20, and SW480 cells over 48 hours as described previously (13). The chemoattractant used was 10% FBS. Cells were counted in four different fields with an inverted microscope. All experiments were carried out in triplicate.

Transwell migration assay

A modified Boyden chamber invasion assay with Matrigel-coated Transwell chambers was conducted with HCT116, KM20, and SW480 cells over 48 hours as described previously (13). All experiments were carried out in triplicate.

Necrosis assay

Equal numbers of cells were serum starved overnight and then treated with 10 µmol/L oxaliplatin or dimethyl sulfoxide (DMSO; control) for 24 hours in serum-starved conditions. Necrosis was quantitated using the Cell Death Detection ELISAPlus (Roche) as detailed in the manufacturer’s instructions.

Western blotting and antibodies

Western blotting was carried out as described previously (12). The following antibodies were from Cell Signaling: pAktSer473, total Akt, Snail, Twist, E-cadherin, Vimentin, and β-actin. The following antibodies were from Bethyl Laboratory: mTOR, Raptor, and Rictor. Smooth muscle actin (SMA) antibody was obtained from Abcam.

Gelatin zymography

Equal numbers of cells were seeded and treated with 5 ng/mL TGFβ for 12 hours in serum-free conditions. Supernatants were collected, normalized for total protein concentration, mixed with sample buffer (Invitrogen), and analyzed by electrophoresis with a 10% zymogram gel (Invitrogen) for 90 minutes. The gel was developed according to the manufacturer’s instructions and stained with Coomassie Blue (Invitrogen).

Immunofluorescence

Immunofluorescence staining was carried out as described previously (13). Actin, Vimentin, and E-cadherin antibodies were obtained from BD Biosciences, whereas SMA and fibronectin antibodies were obtained from Abcam. Images were acquired using either a Nikon TE2000 inverted microscope or a Nikon Total Internal Reflection Fluorescence microscope (×200 objective) and NIS Elements AR3.10 software.

RhoA/Rac1 activity assays

RhoA and Rac1 activity was assessed using glutathione S-transferase (GST)-tagged Rho-binding domain of Rhotekin.
(TRBD) and GST-tagged p21 binding domain of PAK1 (GST–PBD) pull-down assays, respectively, as described previously (14, 15). Briefly, cells were grown to ~70% confluency in regular growth medium, serum starved overnight, and stimulated with 10% FBS for 5 minutes. For rapamycin treatment, cells were preincubated in 50 nmol/L rapamycin for 24 hours. RhoA monoclonal antibody was obtained from Santa Cruz Biotechnology, and Rac1 monoclonal antibody was obtained from Millipore.

**In vivo experimental metastasis**

A total of 1.5 × 10⁶ KM20 sh NTC, sh Raptor, or sh Rictor CRC cells were collected in 200 μL of PBS and inoculated i.v. into tail veins of 6-week-old male athymic Crl:NU–Foxn1–/- nude mice (Charles River Labs; n = 5 per group). All procedures were carried out in an animal facility by using protocols approved by the Institutional Animal Care and Use Committee. Six weeks after inoculation, mice were sacrificed by cervical dislocation and metastatic tissues were examined in paraffin-embedded sections stained with either hematoxylin and eosin or cytokeratin 7 and cytokeratin 20 antibodies from Abcam.

**Statistical analysis**

Analysis of variance was used to compare qRT-PCR and immunoreactivity scores across CRC stages. Contrasts were generated from the model to conduct specific hypothesis tests, such as pair-wise comparisons between cancer stages and linear trend tests across increasing stage. Paired t test or non-parametric analogue was used for comparisons of immunoreactivity scores between matched normal, primary, and metastatic tumor samples. A generalized linear mixed model was used to analyze migration, invasion, and apoptosis data. Cell line, treatment, dose, and location (bottom, middle, top) were included as independent variables in the model and contrasts for pair-wise comparisons between treatment versus control as well as linear trend test for increasing treatment doses were carried out. Comparisons between control versus knockdown Raptor and Rictor groups of proportion of mice exhibiting metastases were made using Fisher’s exact test. Tests for normality were carried out to assess validity of parametric models.

**Results**

**Expression of mTORC1 and mTORC2 components is elevated in CRC**

To delineate the extent of mTOR pathway alterations in CRC, we analyzed mTOR, Raptor, and Rictor expression by qRT-PCR in cDNA from 48 patients representing all stages of disease and normal colon. Comparison of mTOR, Raptor, and Rictor expression profiles showed elevated mRNA expression of all three genes in stage I–IV CRC compared with normal colon (Fig. 1A). Interestingly, increasing levels of mRNA for all genes were observed with increasing stage of disease as confirmed by a test for linear trend (P < 0.0001).

To further determine specific expression changes associated with CRC progression, we analyzed tissues from 18 stage IV patients for mTOR, Raptor, and Rictor expression in normal colon, primary tumor, and matched liver metastases. Each sample was assigned an immunoreactivity score ranging from 0 to 6. Representative samples for each protein are shown (Fig. 1B) along with analysis (Fig. 1C). Normal colon showed either negative or focal mild cytoplasmic staining for mTOR, Raptor, and Rictor. Meanwhile, primary tumor and matched liver metastases exhibited diffuse cytoplasmic staining for all three proteins. Paired comparisons of immunoreactivity scores between normal versus primary tumors and normal versus metastatic tumors were significant (P < 0.01) for all three proteins. No significant differences were observed between primary tumors versus liver metastases. Taken together, these findings show that mTOR, Raptor, and Rictor are overexpressed at both mRNA and protein levels in primary CRCs and this increased expression is maintained in the associated distant metastases.

**mTORC1 and mTORC2 regulate CRC migration and invasion**

Migration and invasion are critical steps in initial progression of cancer that facilitate metastasis. We used HCT116, SW480, and KM20 cells to determine the effects of rapamycin on CRC migration and invasion. First, cells were treated with increasing doses of rapamycin, and migration was assessed using the short-term Transwell migration assay. Treatment with rapamycin significantly decreased migration of HCT116, SW480, and KM20 cells in a dose-dependent manner (Fig. 2A). We further confirmed these findings by using a wound-healing assay. Consistent with results obtained from Transwell migration assay, rapamycin significantly decreased migration of HCT116 and SW480 cells (Fig. 2B). Finally, the effect of rapamycin on invasion was assessed using a modified Boyden chamber assay. Rapamycin potently decreased invasion of both HCT116 and SW480 CRC cells in a dose-dependent manner (Fig. 2C). In summary, pharmacologic blockade of mTOR signaling by using rapamycin significantly inhibits migration and invasion of CRC cells in a dose-dependent manner.

To decipher the contribution of mTORC1 and mTORC2 in regulating migration and invasion, we selectively silenced Raptor and Rictor, essential components of mTORC1 and mTORC2, respectively. HCT116, SW480, and KM20 cells with stable shRNA-mediated knockdown of Raptor or Rictor were generated and showed more than 90% reduction of the targeted proteins (Fig. 3A). All cells expressing Rictor shRNA had significantly reduced levels of pAkt Ser473, whereas pAkt Ser473 levels in cells expressing Raptor shRNA were increased compared with control cells.

Migration of stably silenced cells was assessed using the short-term Transwell migration assay. Stable knockdown of both Raptor and Rictor significantly decreased migration of HCT116, SW480, and KM20 cells (Fig. 3B). Consistent with results obtained from Transwell migration assay, knockdown of both Raptor and Rictor significantly decreased migration of HCT116 and SW480 cells by using wound-healing assay (Fig. 3C). Finally, invasion of stably silenced cells was assessed using a modified Boyden chamber assay. Targeted inhibition of both Raptor and Rictor significantly decreased invasion of
HCT116 and SW480 cells (Fig. 3D). Taken together, our findings suggest that both mTORC1 and mTORC2 regulate migration and invasion, critical steps in the initial progression of CRC.

mTORC1 and mTORC2 regulate EMT in CRC

Several biochemical markers are used to characterize EMT: Epithelial cells express E-cadherin predominantly, whereas mesenchymal cells express Vimentin, SMA and fibronectin (3). Because targeted inhibition of mTORC1 and mTORC2 attenuated migration and invasion, we determined whether their inhibition is sufficient to induce MET by examining expression of the aforementioned markers. Stable knockdown of Raptor and Rictor in SW480 cells increased E-cadherin levels, whereas it decreased Vimentin and SMA levels (Fig. 4A). Immunofluorescence microscopy confirmed increased levels of E-cadherin and decreased levels of Vimentin, SMA, and fibronectin in Raptor and Rictor knockdown cells compared with control cells (Fig. 4B).

The transcription factors, Snail and Twist, are implicated in transcriptional repression of E-cadherin expression and orchestrating the molecular EMT program (4). Because knockdown of Raptor and Rictor increased E-cadherin expression and induced MET, we determined whether these proteins regulate expression of Snail and Twist (Fig. 4C). Consistent with the increase in E-cadherin levels, we noted that Snail expression was decreased with knockdown of both Raptor and Rictor, whereas Twist expression decreased predominantly with knockdown of Raptor in HCT116 and SW480 cells.

EMT is further associated with increased production of matrix metalloproteases (MMP), which serve to degrade extracellular matrix proteins and facilitate cell invasion. Because mTORC1 and mTORC2 can regulate the invasion of CRCs, we determined whether silencing mTORC1 or mTORC2 could decrease MMP production. As shown in Fig. 4D, knockdown of Raptor and Rictor significantly decreased levels of MMP-9 secreted by SW480 cells.

Finally, EMT is associated with acquisition of chemotherapeutic resistance (3). To address this issue, we determined whether silencing mTORC1 or mTORC2 could enhance sensitivity to oxaliplatin-induced apoptosis. As shown in Fig. 4E, HCT116 and SW480 cells expressing shRNA targeting Raptor or Rictor were sensitized to oxaliplatin-induced apoptosis compared with control cells. Taken together, these results suggest that inhibition of mTORC1 and mTORC2 induces morphologic, biochemical, and functional changes reminiscent of MET.

mTORC1 and mTORC2 regulate actin cytoskeleton rearrangements involved in CRC motility via RhoA and Rac1 signaling

Polymerization and depolymerization of filamentous (F-) actin control cytoskeletal reorganization, leading to morphologic changes (such as lamellipodia formation) associated with motility. We investigated whether mTORC1 and mTORC2 regulate changes in the actin cytoskeleton by staining for F-actin in SW480 cells with stable Raptor or Rictor knockdown (Fig. 5A). Control cells showed a mesenchymal morphology, spreading out evenly over the substratum with reduced cell–cell contacts (left) and showed abundant lamellipodia and stress fibers (right). Conversely, both Raptor and Rictor knockdown

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cells showed epithelial morphology, packed together closely with numerous cell–cell contacts (left), and displayed a dramatic decrease in lamellipodia and stress fibers (right). RhoA and Rac1 belong to a family of GTPases that regulate F-actin assembly and disassembly while controlling cell migration. Because Raptor and Rictor knockdown cells showed decreased lamellipodia, stress fibers, and migration compared with control cells, we determined whether inhibiting mTOR signaling pharmacologically by using rapamycin and shRNA-mediated inhibition of Raptor and Rictor can alter activation of RhoA and Rac1 by using GST–TRBD and GST–PBD pull-down assays, respectively. As shown in Fig. 5B and C, RhoA and Rac1 activity decreased basally following rapamycin treatment in both HCT116 and SW480 cells. Serum stimulation increased activation of RhoA and Rac1, whereas treatment with rapamycin attenuated this activation. Next, we evaluated activation of RhoA and Rac1 in Raptor or Rictor knockdown HCT116 and SW480 cells. Both a 30% to 40% decrease in activated RhoA and a 50% to 85% decrease in Rac1

Figure 2. Rapamycin attenuates migration and invasion of CRCs. A, Transwell migration assay carried out with HCT116, SW480, and KM20. Cells in the control group were treated with DMSO; hpf, high-power field; *, $P < 0.05$ versus control. B, wound-healing assay carried out with HCT116 and SW480 cells over 48 hours. Cells in the control group were treated with DMSO; *, $P < 0.05$ versus control. C, Transwell invasion assay was conducted with HCT116 and SW480 cells over 48 hours. Cells in the control group were treated with DMSO; *, $P < 0.05$ versus control.

activity were observed on Raptor or Rictor knockdown compared with control cells (Fig. 5D and E). Finally, to implicate these downstream pathways in regulating migration of CRC cells, we treated HCT116 and SW480 cells with small molecule inhibitors of Rho-associated kinase (Y27632) and Rac1 (NSC23766) and assessed their effect on migration by using Transwell migration assay. We found that both compounds significantly reduced migration of HCT116 and SW480 cells (Fig. 5F). Taken together, these findings suggest that mTORC1 and mTORC2 are required for activation of RhoA and Rac1. The decrease in RhoA and Rac1 activity may prevent rearrangement of the actin cytoskeleton, thus accounting for the attenuated migratory capability noted on mTORC1 and mTORC2 inhibition.
mTORC1 and mTORC2 are critical for establishment of CRC metastases

To further extend our findings in vivo, we examined the effect of mTORC1 and mTORC2 inhibition in an experimental metastasis model. Green fluorescent protein (GFP)-labeled KM20 cells with stable knockdown of Raptor or Rictor (Fig. 3A) were injected i.v. into athymic nude mice and formation of systemic metastases was assessed; findings are summarized in Fig. 6A. Macroscopic lung nodules were not observed on gross examination of lungs from animals inoculated with control, Raptor, or Rictor knockdown cells. However, histologic examination showed the presence of micrometastases in 20% of mice from the control group (Fig. 6B). In contrast, knockdown of Raptor or Rictor completely abolished formation of pulmonary micrometastases in all mice. Surprisingly, we found that 100% of mice inoculated with control cells exhibited metastatic nodules on the upper back, bone (knee joint and ribcage), and mesenteric lymph nodes (Fig. 6C and D). In contrast, knockdown of Raptor and Rictor cells completely abolished establishment of these metastases. To further confirm that the cellular origin of metastases was indeed an epithelial CRC cell line (KM20), metastatic tissues from the aforementioned locations were stained with cytokeratin 7 (CK7) and cytokeratin 20 (CK20). As expected for metastatic CRC, the tumor showed diffusely strong immunoreaction for CK20, whereas immunoreaction for CK7 was only focally noted in this poorly differentiated (high-grade) CRC (Fig. 6E; ref.16). Taken together, our findings suggest that mTORC1 and mTORC2 are critical for establishment of CRC metastases irrespective of their site of colonization.
Discussion

In this study, we determined the role of mTORC1 and mTORC2 in regulating motility, EMT, and metastasis of CRC. First, we found increased expression of mTOR, Raptor, and Rictor mRNA with more advanced stages of CRC. In addition, mTOR, Raptor, and Rictor protein levels were significantly elevated in stage IV CRCs and this overexpression profile was maintained in their matched distant metastases. Second, we show that inhibition of mTORC1 and mTORC2 attenuated migration and invasion of CRCs concomitant with altered cytoskeletal rearrangement and decreased activation of RhoA and Rac1. Third, we show that inhibition of mTORC1 and mTORC2 induces changes reminiscent of MET. Finally, we show that establishment of metastasis in vivo was completely abolished upon targeted inhibition of mTORC1 and mTORC2. We propose that mTORC1 and mTORC2 regulate motility of CRCs via RhoA and Rac1 signaling.

Although intensive studies have focused on the role of mTOR signaling in regulating growth and survival, its role in EMT, motility, and metastasis of cancers is not well understood. We found that pharmacologic (using rapamycin) and genetic (using RNA interference) inhibition of mTORC1 (Raptor) and mTORC2 (Rictor) significantly decreased migration, invasion, and establishment of metastasis in vivo. Our findings are consistent with several studies showing inhibition of migration and invasion by rapamycin in various types of cancers (17). A recent study used knockdown of Raptor and Rictor to show that both mTORC1 and mTORC2 regulate insulin-like growth factor 1 (IGF-1) stimulated migration of...
Figure 6. Inhibition of mTORC1 and mTORC2 abolishes establishment of CRC metastases in vivo. GFP-labeled KM20 sh NTC, sh Raptor, and sh Rictor cells were inoculated i.v. into athymic CrI:NU-Foxn1
nude mice and assessed after 6 weeks. A, summary of metastases in each location; *, P < 0.05 versus NTC shRNA. B, representative images from histologic assessment of lungs. C, representative images from assessment of systemic metastases using GFP Illumatool. D, representative subcutaneous, bone, and lymph node metastases along with histologic analysis (×200). E, expression of CK20 and CK7 in metastatic tissues (×200).
The cyclin-dependent kinase inhibitor p27Kip1 and, in turn, and colleagues (22) showed that rapamycin increases levels of migration. Consistent with these findings, Jacinto and colleagues (5) observed that mTORC1 and mTORC2 decreases the formation of lamellipodia. Consistent with our findings, a recent study showed that both mTORC1 and mTORC2 are involved in regulating F-actin reorganization and formation of lamellipodia in a panel of cancer cells (18). Moreover, previous studies have also shown that silencing of mTORC2 prevents cell spreading and F-actin polymerization via PKCα in fibroblasts (20).

The Rho family of GTPases, including RhoA and Rac1, participate in regulating actin cytoskeleton reorganization and cell migration. Our findings in this study implicate signaling through RhoA and Rac1 pathways as a critical downstream mechanism by which mTORC1 and mTORC2 may regulate changes in the actin cytoskeleton and cell migration. Consistent with these findings, Jacinto and colleagues (21) observed that mTORC2 signals through Rac1 and found that mTOR also signals through RhoA. Similarly, Moss and colleagues (22) showed that rapamycin increases levels of the cyclin-dependent kinase inhibitor p27Kip1 and, in turn, inhibits RhoA activation, thereby blocking cytoskeletal reorganization and cell migration. Finally, a recent study showed that disruption of mTORC1 and mTORC2 by inhibiting Raptor and Rictor, respectively, inhibits the activity of RhoA and Rac1 (23). In addition, mTORC1 was also shown to control the translation of RhoA and Rac1 mRNAs via 4E-BP1 and p70S6K.

EMT is a key reversible step that facilitates tumor migration, invasion, and metastasis. Owing to the clinical importance of this process, inhibition of EMT is an attractive therapeutic approach that can significantly alter disease outcome. However, it remains unknown which pathways should be inhibited to reverse EMT. Our findings implicate mTORC1 and mTORC2 as key regulators of EMT in CRCs. This conclusion is based on the observation that silencing mTORC1 and mTORC2 induces a repertoire of biochemical (increased E-cadherin and decreased vimentin, SMA, and fibronectin), morphologic (increased cell–cell contact, decreased formation of lamellipodia), and functional (decreased MMP-9 production and enhanced sensitivity to oxaliplatin-induced apoptosis) changes characteristic of MET. Consistent with our findings, a recent study found that the cytokine TGFβ, which is known to play a major role in promoting EMT, induces activation of mTOR signaling and phosphorylation of p70S6K and 4E-BP1, which subsequently increased protein synthesis and cell size (24). Inhibition of mTOR signaling by using rapamycin inhibited the increase in protein synthesis and cell size, whereas it inhibited cell migration and invasion associated with TGFβ-induced EMT. These studies suggest that mesenchymal–epithelial reprogramming may be an important mechanism underlying the attenuated metastasis of CRC noted on inhibition of mTORC1 and mTORC2.

Several findings support the targeting of mTOR signaling as an anti-metastatic therapy. First, we show that mTOR, Raptor, and Rictor are overexpressed at both the mRNA and protein levels in primary CRCs and this increased expression is maintained in the associated distant metastases. The positive correlation noted between expression and stage suggests that mTOR signaling may contribute toward CRC progression. Second, multiple upstream pathways ultimately converge on mTORC1 and mTORC2 to facilitate cancer cell migration and invasion. Recent studies have shown that IGF-I, EGF, and TGFβ, all critical mediators of migration, EMT, and metastasis, activate mTOR signaling; inhibition of mTOR, using rapamycin, potently inhibited cell motility induced by IGF-I, EGF, and TGFβ (17, 19, 24). These findings suggest that mTOR signaling may be a critical node in regulating progression and metastasis of cancers. Targeting mTORC1 and mTORC2 could be a more beneficial strategy than targeting individual, upstream, redundant signaling pathways. Third, advanced cancers are difficult to treat because they are chemoresistant. We show that knockdown of mTORC1 and mTORC2 sensitizes CRCs to undergo apoptosis on treatment with oxaliplatin.

Despite the incontrovertible rationale for targeting cancers addicted to PI3K/Akt/mTOR signaling, such as CRC, with rapamycin, clinical results have been disappointing. One proposed mechanism of resistance to rapamycin involves its inability to inhibit mTORC2 (10, 12). Our findings support a role for elevated mTORC1 and mTORC2 activity in regulating EMT and metastasis of CRC. Taken together with our previous results showing that both mTORC1 and mTORC2 contribute to CRC tumorigenesis, we hypothesize that the inherent redundancy in functions of both complexes may allow mTORC2 to compensate for loss of mTORC1 activity upon rapamycin treatment, thereby leading to rapamycin resistance. Our findings provide the rationale for including mTOR kinase inhibitors targeting the ATP binding pocket, which inhibit both mTORC1 and mTORC2 more completely, as part of the therapeutic regimen for treating CRC patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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