AKT Activity Determines Sensitivity to Mammalian Target of Rapamycin (mTOR) Inhibitors by Regulating Cyclin D1 and c-myc Expression

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Prior work demonstrates that AKT activity regulates sensitivity of cells to G1 arrest induced by mammalian target of rapamycin (mTOR) inhibitors such as rapamycin and CCI-779. To investigate this, a novel high-throughput microarray polysome analysis was performed to identify genes whose mRNA translational efficiency was differentially affected following mTOR inhibition. The analysis also allowed the assessment of steady-state transcript levels. We identified two transcripts, cyclin D1 and c-myc, which exhibited differential expression in an AKT-dependent manner: High levels of activated AKT resulted in rapamycin-induced down-regulation of expression, whereas low levels resulted in up-regulation of expression. To ectopically express these proteins we exploited the finding that the p27kip1 mRNA was efficiently translated in the face of mTOR inhibition irrespective of AKT activity. Thus, the p27kip1 5'-untranslated region was fused to the cyclin D1 and c-myc coding regions and these constructs were expressed in cells. In transfected cells, expression of cyclin D1 or c-myc was not decreased by rapamycin. Most importantly, this completely converted sensitive cells to a phenotype resistant to G1 arrest. Furthermore, the AKT-dependent differential expression patterns of these two genes was also observed in a mouse xenograft model following in vivo treatment with CCI-779. These results identify two critical downstream molecular targets whose expression is regulated by AKT activity and whose down-regulation is required for rapamycin/CCI-779 sensitivity.

Drugs that specifically inhibit the mammalian target of rapamycin (mTOR) are currently being developed as potential anti-tumor agents. mTOR is a critical protein that integrates signals that link the ability of cells to undergo cell cycle transit to the availability of nutrients in their immediate environment (1–4). By inhibiting mTOR, these drugs essentially trick the cell into believing that conditions are not appropriate for cell cycle progression to ensue and induce G1 arrest. Rapamycin is the prototype mTOR inhibitor and CCI-779, a recently developed analog of rapamycin, is currently in clinical trials for cancer patients.

mTOR activation is mediated by upstream signals from the phosphatidylinositol-3 kinase (PI3-K)/3-phosphoinositide dependent protein kinase 1 (PKD-1)/AKT cascade. mTOR activity, in turn, results in phosphorylation of the p70S6 kinase (p70) and 4E-BP1 translational repressor (5–8). Phosphorylation of p70 is critical for ribosome biogenesis, whereas phosphorylation of 4E-BP1 disrupts its interaction with the eIF-4E translation initiation factor, allowing eIF-4E to participate in assembly of a translation initiation complex (eIF-4F). In this complex, eIF-4E binds to the cap structure at the 5'-end of mRNAs, which promotes ribosome recruitment to mRNAs and the initiation of translation. By up-regulating the components of the protein synthetic machinery and cap-dependent translation, both of these mTOR-dependent phosphorylation events lead to translation of proteins required for cell cycle transit.

Because malignant clones frequently demonstrate up-regulation of the PI3-K/PDK-1/AKT/mTOR pathway (9–12), mTOR inhibitors may be particularly effective as they target this pathway. In keeping with this notion, high levels of AKT activity, whether due to PTEN mutation or introduction of activated AKT alleles, result in hypersensitivity to CCI-779-induced G1 arrest (13). At first glance, this correlation is intuitive because AKT activity is an indicator of activation through the PI3-K/PDK-1/AKT pathway, and this pathway is an upstream stimulator of mTOR function. However, mTOR inhibition should result in inhibition of ribosome biogenesis and cap-dependent translation in all cells, irrespective of AKT activity, and, in fact, cells with lower levels of basal activity (i.e. those with less stimulation through AKT to mTOR) might be more susceptible to rapamycin-induced decreases in mTOR activity below a specific threshold.

The critical cytostatic effect of mTOR inhibitors is theoretically a reduction in cap-dependent translation of cell cycle...
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...proteins. Thus, a second possible explanation for the regulatory role of Akt might be that it can regulate the ability of cells to maintain levels of these proteins by controlling their translation through cap-independent mechanisms. To test this hypothesis, we screened the translational state of >5,000 mRNAs, using the methodology of Zong et al. (14). Our results identified two potentially critical mRNAs for cell cycle transit, the cyclin D1 (clone 9E11, Upstate Biotechnology) and c-myc, whose translational response to mTOR inhibitors both in vitro and in vivo were remarkably regulated by the degree of Akt activation. Further studies confirmed that the differential effects on the expression of these proteins determined sensitivity to mTOR inhibitors.

EXPERIMENTAL PROCEDURES

Cell Culture and Plasmids—U87 and LAPC-4 parental cell lines were originally obtained from ATCC and maintained as described previously (13). They were subsequently stably transfected using retroviral constructs with a wild-type PTEN gene to generate U87PTEN and a constitutively active Akt allele to generate LAPC-4Akt, as described previously (13). Plasmids expressing p27-IRES-EGFP, p27-IRES-cyclin D1 and p27-IRES-myc mRNAs were constructed using pcDNA3 as the backbone vector. The 5' UTR of p27(IRES-EGFP containing the 365 nucleotide IRES was PCR amplified from IMAGE clone 4298538 and inserted immediately upstream of the cyclin D1 open reading frame in pcDNA3. The c-myc open reading frame was amplified from pBABEpuro (a gift from D. Felsher, Stanford University) and subcloned into pcDNA3. Subsequently the p27(IRES-EGFP 5' UTR was inserted immediately upstream of c-myc. Lastly, the EGFP open reading frame was subcloned into pcDNA3 and the p27(IRES-EGFP 5' UTR ligated immediately upstream. Plasmids were sequenced at all junction points to ensure that they were properly generated.

Polysome and Microarray Analysis—Extraction and display of polysomes was performed as previously described (15). Briefly, cells were lysed in buffer supplemented with 100 μg/ml cycloheximide at 4 °C. Following removal of nuclei and mitochondria, supernatants were layered onto 15–40% sucrose gradients and spun at 38,000 rpm for 2 h at 4 °C in a SW 40 rotor (Beckman Instruments). Centrifuged gradients were fractionated into eleven 1-ml fractions using an ISCO Density Gradient Fractionator at a flow rate of 3 ml/min. The polysome profile of the gradient was monitored via UV absorbance at 260 nm. RNA from individual fractions was extracted using phenol/chloroform following incubation with proteinase K and precipitated. RNA was then either reverse transcribed (SuperScript RTI, Invitrogen) in the presence of Cy3-DCtP or Cy5-DCtP, respectively. Custom spotted arrays containing >60,000 mRNAs were hybridized with the labeled p27(IRES-EGFP, 5.5 μg of plasmid containing p27(IRES-EGFP, 5.5 μg of plasmid containing p27(IRES-cyclin D1, or 5.5 μg of plasmid containing p27(IRES-myc. In groups where both IRES-containing cyclin D1 and c-myc plasmids were transfected only 2.75 μg of each plasmid were used. To measure GFP expression and DNA content, cells were first fixed in 2% buffered formaldehyde (Polysciences) and then permeabilized with 70% ethanol at -20 °C followed by propidium iodide staining. Texas Red-conjugated c-myc antibody and Alexa 488-conjugated c-myc antibody were used for staining (Molecular Probes). Data were acquired and analyzed on a FACScalibur flow cytometer using CellQuest software (BD Biosciences).

RESULTS

Akt Activity Regulates Sensitivity to mTOR Inhibitors both in Vitro and in Vivo—To investigate the mechanism by which Akt activity regulates sensitivity to mTOR inhibitors, we studied two separate cell lines whose Akt activity was altered by transfection. The LAPC-4 line containing quiescent Akt was transfected with a constitutively active Akt allele and the U87MG glioblastoma cell line (termed U87 in this work), containing a mutated PTEN gene and resulting in heightened Akt activity, was transfected with a wild-type PTEN gene. LAPC-4 cells transfected with myristoylated Akt (termed LAPC-4akt) had greatly increased expression of phosphorylated (activated) Akt compared with empty vector-transfected cells (LAPC-4pure), and U87 cells transfected with wild-type PTEN (U87PTEN) had significantly decreased expression compared with control U87 cells (Fig. 1A). In contrast, levels of phosphorylated ERK were unaffected by transfection in both cell lines, attesting to the specificity of the altered Akt activity.

The transfectants were then tested for sensitivity to the mTOR inhibitor CCI-779 by cell cycle analysis (Fig. 1B). As shown, a clear differential sensitivity was demonstrated between the “high Akt” cell lines (U87 and LAPC-4akt) versus their “low Akt” counterparts (U87PTEN and LAPC-4pure). The ID50 for decrease in S-phase distribution was 7.5–10 nm of CCI-779 for the sensitive U87 and LAPC-4akt cell lines, whereas the U87PTEN and LAPC-4pure cell lines were completely resistant with no effect on S-phase distribution at any dose. There was no induction of apoptosis as detected by sub-G1 peak or annexin V staining (data not shown).

To determine whether Akt activity could regulate sensitivity to CCI-779 in vivo, we examined the growth of the four cell lines in SCID mice. Tumor cells were injected subcutaneously, and mice were randomly assigned to treatment with CCI-779 at different doses when tumors reached 200 mm3 in size. Mice...
FIG. 1. AKT-dependent sensitivity to rapamycin. A, immunoblot analysis of PTEN, phospho-AKT, total AKT, phospho-ERK, total ERK, and actin levels in the four cell lines. B, S-phase cell cycle analysis on U87, U87PTEN, LAPC-4puro, and LAPC-4AKT cell lines treated with CCI-779 for 48 h. Data represent % cells in S-phase, mean ± S.D., n = 3. C, inhibition of tumor growth by CCI-779 in xenografts of U87, U87PTEN, LAPC-4AKT, and LAPC-4puro, in SCID mice. Mice were treated with CCI-779 for 5 days after randomization (see “Experimental Procedures”) with the indicated doses of drug and tumor growth assessed at day 8 (D8) or 12 (D12) after initiation of treatment. Horizontal bars indicate 50% reduction in control tumor growth (vehicle-treated). Data represent tumor volume, mean ± S.D. of three experiments (8 mice/experiment).
were treated with CCI-779 each day for five consecutive days, and at 8 and 12 days after initiation of treatment tumor growth was assessed. As shown in Fig. 1C, CCI-779 inhibited growth of all the cell lines in a dose-dependent fashion. However, the low AKT cell lines were relatively more resistant (IC_{50} values for U87\_PTEN and LAPC-4\_puro were 1 and 3 mg/kg, respectively, when assessed at day 12) then their high AKT counterparts (IC_{50} values for U87 and LAPC-4\_AKT were 0.07 and 0.9 mg/kg, respectively). These results are consistent with previous work (13) demonstrating the increased sensitivity of tumors containing high AKT activity to CCI-779 both in vitro and in vivo.

Prior work with PTEN\_−/− and PTEN\_+/− mouse embryonic fibroblasts demonstrated that AKT-dependent differential sensitivity to CCI-779 was not due to differences in the ability of the drug to inhibit the mTOR pathway (13). This was also true in our paired prostate and glioblastoma cell lines. p70 and 4E-BP1 phosphorylation were completely abrogated at 10 nM of CCI-779 in all four cell lines and the ED_{50} values did not differ between the high AKT and low AKT lines.

**AKT Activity Regulates the Translational State and Transcriptional Response of Specific mRNAs to mTOR Inhibition**—By inhibiting p70 and 4E-BP1 phosphorylation, mTOR inhibitors prevent translation. Thus, another possible downstream determinant of sensitivity is differential effects on the translation of specific critical mRNAs. To investigate this hypothesis we utilized a high-throughput methodology whereby microarray analysis of mRNA translational state can be assessed. This technique is based on the observation that well-translated transcripts are typically associated with polysomes, whereas poorly translated mRNAs are monosomes. Thus, the two pairs of LAPC-4 and U87 cell lines were treated with or without the mTOR inhibitor, rapamycin, for 6, 24, or 72 h and extracts prepared for polysomal analysis. Polysomes were then separated from monosomal material on sucrose gradients and the associated RNAs were extracted from gradient fractions. Gradient fractions corresponding to monosomal (fractions 1–4) and polysomal material (fractions 5–11) were pooled, and the RNA from these two groups was reverse transcribed separately to generate fluorescently labeled probes and hybridized to microarrays. The mRNA translation state for a given transcript—untranslated, polypeptide initiated, or elongating—was determined by measuring the signal intensity of the transcript in the polysomal fraction. Transcripts were deemed to represent full-length mRNA if their signal intensity in polysomes was at least 2.5-fold greater than their signal intensity in monosomes.

In addition to translational state, a measure of total steady-state level of any mRNA could be obtained from these microarrays by summing the signal intensities of the polysomal and monosomal fractions. By this analysis, rapamycin had significant effects on mRNA expression in a small subset (63 genes) of the detectable genes (greater than 2.5-fold change), inhibiting transcription in 34 genes (54%) and enhancing transcription in 29 genes (31%). Again, our analysis confirmed the published finding (20, 21) that the transcripts of many ribosomal components are inhibited by rapamycin. In general, these effects on transcription were comparable between high AKT and low AKT paired cell lines. However, in similar fashion to translation, rapamycin differentially regulated cyclin D1 and c-myc transcription, inhibiting it in high AKT lines and enhancing it in low AKT lines. These major alterations in transcription and translation of cyclin D1 and c-myc mRNAs are summarized in Fig. 2C.

To confirm the results of the translational state microarray analysis, we performed Northern blot analysis on mRNAs associated with polysomes (well translated) versus monosomes (poorly translated) as separated by sucrose gradients. At the top of each series of Northern blots in Fig. 3 is shown the individual polysome profile obtained from each cell line during sucrose gradient fractionation. Northern blot analysis for cyclin D1, c-myc, and actin mRNA is shown below the profiles on total RNA isolated from the corresponding fractions of the sucrose gradient. Densitometric analysis of the signals obtained from the monosomal fractions (fractions 1–4) versus the polysomal fractions (fractions 5–11) allowed a calculation of percent mRNA found in polysome fractions (well translated) as shown to the right of the Northern blots. The differential regulation is very clear; in rapamycin-sensitive, high AKT cell
**Fig. 2. Global and specific changes in translation of mRNAs following treatment with rapamycin.**

A, false-color images of the 181 genes whose translational state was significantly altered in the four cell lines over the indicated time points were clustered together using the GeneSpring software package (K-means cluster analysis). Translational state change is indicated by color value according to the color bar on the left side of the panel (red, increase in translation state; yellow, no change; green, decrease in translation state). Fluorescence signal strength from the microarrays is indicated by color brightness, and gray bars indicate missing data points. B, plots of mRNA translational state changes showing specific expression patterns of cyclin D1 and c-myc in the four cell lines following exposure to rapamycin for 48 h. The translation state of a particular mRNA was expressed as the ratio of the normalized signal intensities between the polysomal and monosomal fractions (described under “Experimental Procedures” and “Results”). C, summary of transcriptional and translational changes for cyclin D1 and c-myc in LAPC-4AKT, U87 high AKT rapamycin-sensitive cells versus LAPC-4puro, U87PTEN low AKT rapamycin-resistant cells. Arrows indicate relative increase or decrease in transcription or translation with rapamycin and values are average transcriptional and translational state fold changes for the indicated cell lines over the course of the 48 h rapamycin treatment.
lines (LAPC_{AKT} or U87), rapamycin produces a marked shift in cyclin D1 and c-myc mRNA from polysome fractions (fractions 5–11) to monosome fractions (fractions 1–4), indicating a significant decrease in translational efficiency. In rapamycin-resistant, low AKT cell lines (LAPC_{4puro} or U87_{PTEN}), rapamycin induces an opposite response, increasing the percentage of cyclin D1 and c-myc mRNA found in polysome fractions versus monosome fractions. For example, the percent of cyclin D1 message associated with polysomes in U87 cells decreases from 45% to 8% after treatment with rapamycin. Likewise, the percent of c-myc message in polysomal fractions decreases from 39% to 4%. In contrast, rapamycin increases the percent of cyclin D1 mRNA associated with polysomes in U87_{PTEN} cells from 27% to 67% and the percent of c-myc mRNA from 34% to 78%. As expected, in all cell lines regardless of AKT activity, rapamycin inhibits translation of actin (i.e., shift of actin mRNA from well translated polysomes to poorly translated monosomes). These data confirm the results of microarray analysis, namely that the level of AKT activity regulates the translational state response to rapamycin of cyclin D1 and c-myc.

The Northern blots further confirm the assessment of total steady-state mRNA transcription from the microarrays. The relative amounts were determined by densitometry on equally exposed autoradiographs and summing the signals from monosome and polysome fractions (data not shown). Although the total amount of c-myc and cyclin D1 mRNA in all fractions (monosome + polysome fractions) is significantly decreased by rapamycin in LAPC_{4AKT} and U87 cell lines (decreased by 35.3% for c-myc and 27.2% for cyclin D1 in LAPC_{4AKT}; decreased by 25.7% for c-myc and 31.3% for cyclin D1 in U87) the
total c-myc and cyclin D1 mRNA is increased by rapamycin in resistant LAPC-4puro and U87PTEN cell lines (increased by 27.4% for c-myc and 32.8% for cyclin D1 in LAPC-4puro; increased by 31.7% for c-myc and 27.6% for cyclin D1 in U87PTEN).

Western analysis of protein extracts obtained from the high AKT and low AKT cell lines prior to and following rapamycin exposure also demonstrated differential expression (Fig. 4A). Both cyclin D1 and c-myc protein levels decreased or became undetectable in the rapamycin-sensitive cell lines following exposure, whereas, in the resistant cell lines, the relative levels of both proteins increased upon rapamycin treatment. By densitometric analysis, cyclin D1 levels increased 3.5-fold in LAPC-4puro cells (3.5 ± 0.7, mean ± S.D. of three experiments) and 5.1-fold in U87PTEN cells. C-myc levels increased 6.2-fold in LAPC-4puro cells (mean ± S.D. of three experiments) and 5.2-fold in U87PTEN cells.

To examine whether in vivo treatment with CCI-779 induced AKT-dependent differential effects on cyclin D1 or c-myc protein expression, we performed Western analysis on tumor material removed from mice following treatment with the drug for 5 days. As shown, in vivo administration of CCI-779 resulted in a dose dependent decrease in both cyclin D1 and c-myc protein levels in “sensitive” U87 cells, (Fig. 4B, left panel). In “resistant” U87PTEN cells, cyclin D1 and c-myc protein levels increased and accumulated in a dose dependent manner (by densitometry, cyclin D1 levels increased 3.5-fold and c-myc levels increased 2.5-fold following treatment with 4 mg/kg; Fig. 4B, right panel). Similarly, cyclin D1 and c-myc expression decreased in sensitive LAPC-4AKT cells (Fig. 4C, left panel), whereas accumulating in resistant LAPC-4puro cells (Fig. 4C, right panel).

Aberrant Levels of Cyclin D1 and c-myc Are Critical Determinants of Rapamycin Sensitivity—To determine if the downregulated expression of cyclin D1 or c-myc was specifically responsible for sensitivity to rapamycin-induced G1 arrest, we attempted to revert the rapamycin-sensitive phenotype in the U87 cell line model by restoring expression of these genes. However a potential obstacle to this strategy was the ability of rapamycin to inhibit the translation of any transfected genes in rapamycin-sensitive cells. However, we observed from our array analysis that rapamycin did not significantly alter the translation of the p27kip1 mRNA in either the sensitive high AKT or resistant low AKT cell lines. This suggested to us that the p27kip1 mRNA contained sequences capable of directing translation in the face of mTOR inhibition. Indeed, recent work has demonstrated that a 365 nucleotide sequence in the human p27kip1 mRNA leader is capable of directing cap-independent translation (22). Furthermore, the highly conserved IRES within the murine p27kip1 mRNA (overall 78% sequence identity) has also been shown to mediate cap-independent translation that is resistant to rapamycin (23). Thus, we generated a series of constructs in which we replaced the native 5′-UTR of cyclin D1 and c-myc with the leader from the p27kip1 mRNA containing the IRES sequences. The transcription of cyclin D1 and c-myc IRES-containing genes was driven by a cytomegalovirus promoter. One of these constructs also expressed the EGFP gene downstream of these 5′-UTR sequences, which allowed us to specifically examine successfully transfected
cells. Rapamycin-sensitive high AKT U87 cells were transiently transfected. To ensure that the p27\textsuperscript{kip1}-IRES-containing cyclin D1 and c-myc mRNAs were being faithfully transcribed (termed p27-IRES-cyclin D1 and p27-IRES-myc), we also performed Northern analysis on EGFP sorted cells using probes specific for these transcripts and were able to confirm mRNA expression (data not shown). To test effects of rapamycin on translation of the transfected genes, we next performed flow cytometry for cyclin D1 or c-myc expression of EGFP-gated, transiently transfected cells treated with or without rapamycin. Shown in Fig. 5A are the percent cells positively stained for cyclin D1 or c-myc expression (above a threshold fluorescence obtained from an isotype matched control antibody) and the respective mean fluorescence intensities (in parentheses). As expected, rapamycin-sensitive U87 cells, when either untransfected or transfected with an empty vector, demonstrated a marked decrease in endogenous cyclin D1 and c-myc expression (percent positive cells and fluorescence intensity) when exposed to rapamycin (compare lines 1–5 and 2–6 in Fig. 5A). However, U87 cells expressing the p27-IRES-cyclin D1 mRNA (lines 3 and 7) or the p27-IRES-myc mRNA (lines 4 and 8) demonstrated little effect on cyclin D1 or c-myc expression, respectively, following rapamycin treatment. The specificity of this effect is demonstrated by the fact that in these same transiently transfected cells, rapamycin was still capable of down-regulating expression of the other protein (i.e. c-myc expression in p27-IRES-cyclin D1-transfected cells and cyclin D1 expression in p27-IRES-myc-transfected cells). These data demonstrated that the p27\textsuperscript{kip1} leader was capable of directing translation of either cyclin D1 or c-myc in sensitive U87 cells, which was resistant rapamycin.

With no inhibitory effect of rapamycin on ectopic expression of transfected cyclin D1 or c-myc, we were able to test the role of these genes in G1 arrest. For these experiments, rapamycin-sensitive U87 and PTEN-resistant U87 cells were similarly transfected with the indicated constructs and cell cycle distribution determined on EGFP-gated cells. Data presented are means ± S.D. of three separate experiments.

![Fig. 5. Ectopic expression of cyclin D1 and c-myc in rapamycin-sensitive U87 cells confers resistance. A, summary of cyclin D1 and c-myc expression in transfected U87 cells with or without 100 nM rapamycin for 48 h. Expression presented as percent positively stained cells and their relative mean fluorescence intensity (in parentheses). B, S-phase cell cycle distribution of transiently transfected U87 and U87PTEN cells. Cells were transfected with the indicated constructs and cell cycle distribution determined on EGFP-gated cells. Data presented are means ± S.D. of three separate experiments.](image-url)
**DISCUSSION**

mTOR inhibitors have demonstrated considerable potential as anti-cancer agents. Thus, our efforts to identify, in an unbiased analysis, how sensitivity to these drugs is regulated by AKT may have future clinical significance. Our study is particularly significant as it utilized isogenic cell lines and the differences in sensitivity to \\G_1 arrest were not artificially induced by in vitro selection with increasing concentration of drug. The study clearly indicates that AKT activity determines the ability of mTOR inhibitors to specifically down-regulate both transcription and translation of cyclin D1 and c-myc. Such down-regulation was necessary for cell sensitivity.

The importance of cyclin D1 and c-myc on the effects of mTOR inhibitors has been demonstrated previously in other systems (24–26) and more recently by Nelsen et al. (27). Of particular importance, Barbet et al. (26), also found that the yeast homolog of cyclin D1, CLN3 under control of the rapamycin-insensitive UBI4 5′-UTR suppressed rapamycin-induced \\G_1 arrest. Our study utilized a similar strategy to translate rapamycin-sensitive mRNAs following exposure to the drug.

Rapamycin and CCI-779 classically block protein translation via their inhibitory effects on ribosome biogenesis, initiation complex formation and cap-dependent translation. However, our data clearly demonstrate important effects on transcription as well. This is especially true for cyclin D1 and c-myc, two critical genes that determine sensitivity. These results are consistent with prior work (18–20) that also documented effects of mTOR inhibitors on transcription. We cannot discern from our experiments whether the inhibitory effects on transcription are mechanistically linked to inhibitory effects on translation.

Although it is unclear how AKT activity might regulate the transcriptional response to mTOR inhibitors, prior literature allows us to speculate on the regulation of translation. Inhibition of mTOR prevents phosphorylation of the p70S6 kinase and 4E-BP1 translational repressor. The dephosphorylation of these mTOR substrates results in the inhibition of translation. Our data clearly demonstrate that the AKT-dependent differential alterations in cyclin D1 and c-myc translation are not due to different effects on p70 or 4E-BP1. Both proteins were dephosphorylated equally and with the same sensitivity to rapamycin in high AKT versus low AKT cell lines. Furthermore, the mRNA translational state array analysis indicates that the global downstream effects were also comparably present irrespective of AKT activity. How then can one explain differential effects on cyclin D1/c-myc translation? One possibility is that, in the face of mTOR inhibition, 4E-BP1 dephosphorylation, and diminished cap-dependent translation, some transcripts can be translated via internal ribosome entry sites (IRESes), and IRES-dependent translation might be regulated by AKT activity for particular mRNAs. The c-myc mRNA is well known to contain an IRES within its 5′-UTR (28–30). The human cyclin-D1 leader is relatively long, has high CG-content, and is predicted to be highly structured in addition to having limited rRNA-complementarity (31). These properties are common to other mRNAs known to contain IRES sequences (32, 33). It is possible that the cyclin D1 leader also contains se-
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...mTOR inhibitors like rapamycin or CCI-779 may have clinical implications. First, sampling tumor tissue soon after initiation of therapy for cyclin D1/c-myc expression may provide prognostic information concerning the likelihood of an ensuing response. This may be more informative than pre-therapy assessment of phosphorylated/activated AKT in tumor tissue as the latter would be difficult to evaluate as a relative marker of sensitivity. Second, it might be more difficult to treat tumors that contain unregulated expression of cyclin D1 or c-myc such as some non-Hodgkin’s lymphomas. The excessive transcription of these genes in some tumors may prevent significant G1 arrest induced by the mTOR inhibitors.

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