Cyclin D1 and c-myc Internal Ribosome Entry Site (IRES)-dependent Translation Is Regulated by AKT Activity and Enhanced by Rapamycin through a p38 MAPK- and ERK-dependent Pathway

YiJiang Shi‡, Anushree Sharma‡, Hong Wu§, Alan Lichtenstein¶¶¶, and Joseph Gera‡‡‡

From the ‡Department of Research and Development, Veterans Affairs Greater Los Angeles Healthcare System and the Departments of **Medicine and §Molecular and Medical Pharmacology, ¶Jonsson Comprehensive Cancer Center, and †Howard Hughes Medical Institute, David Geffen School of Medicine, University of California, Los Angeles, California, 91343

The macrolide antibiotic rapamycin inhibits the mammalian target of rapamycin protein (mTOR) kinase resulting in the global inhibition of cap-dependent protein synthesis, a blockade in ribosome component biosynthesis, and G1 cell cycle arrest. G1 arrest may occur by inhibiting the protein synthesis of critical factors required for cell cycle progression. Hypersensitivity to mTOR inhibitors has been demonstrated in cells having elevated levels of AKT kinase activity, whereas cells containing quiescent AKT activity are relatively resistant. Our previous data suggest that low AKT activity induces resistance by allowing continued cap-independent protein synthesis of cyclin D1 and c-Myc proteins. In support of this notion, the current study demonstrates that the human cyclin D1 mRNA 5′ untranslated region contains an internal ribosome entry site (IRES) and that both this IRES and the c-myc IRES are negatively regulated by AKT activity. Furthermore, we show that cyclin D1 and c-myc IRES function is enhanced following exposure to rapamycin and requires both p38 MAPK and RAF/MEK/ERK signaling, as specific inhibitors of these pathways reduce IRES-mediated translation and protein levels under conditions of quiescent AKT activity. Thus, continued IRES-mediated translation initiation may permit cell cycle progression upon mTOR inactivation in cells in which AKT kinase activity is relatively low.

Received for publication, July 13, 2004, and in revised form, December 9, 2004
Published, JBC Papers in Press, January 4, 2005, DOI 10.1074/jbc.M407874200

© The American Society for Biochemistry and Molecular Biology, Inc., 2005.

This paper is available on line at http://www.jbc.org/
differential AKT-dependent cyclin D1 and c-myc IRES activity is dependent on p38 MAPK and RAF/MEK/ERK signaling.

RESULTS

The Cyclin D1 and c-myc Leaders Function as IRESes in Transfected Cells in an AKT-dependent Manner—The glioblastoma U87MG cell line has a PTEN-null mutation with resulting heightened AKT activity. It was stably transfected with a wild-type PTEN construct, which markedly down-regulated AKT activity (20). Similarly, the LAPC-4pare tumor cell line with relatively quiescent AKT activity was stably transfected with a myristoylated AKT allele (or empty vector control). The differential AKT/mTOR cascade activation of these paired isogenic lines has already been described (19, 20). In addition, for this study, we also utilized MEFs in which the PTEN gene had been disrupted (21). The AKT activity in these MEFs is markedly higher as compared with PTEN+/−/− MEFs (21, 27).

To determine whether the human cyclin D1 leader could internally initiate translation, it was cloned into the intercistronic region of a dicistronic reporter mRNA and tested in transiently transfected cells. The c-myc leader, containing a known IRES (28), was also cloned into this region. The dicistronic mRNAs used in these studies contain the Renilla and firefly luciferases as the first and second cistrons, respectively (pRF). Because we had previously observed a differential pattern of cyclin D1 and c-myc polysome association in quiescent as compared with activated AKT-containing cells (19), we investigated the ability of the cyclin D1 and c-myc leaders to initiate translation internally in transiently transfected cell lines containing differential AKT activities. We transiently transfected these cell lines with the indicated dicistronic constructs shown in Fig. 1A. Expression of the downstream firefly luciferase in the constructs containing the cyclin D1 5′-UTR or the c-myc IRES as compared with pRF (empty vector) was enhanced dramatically in those cell lines containing quiescent AKT activity (U87PTEN, LAPC-4puro, and PTEN+/−/− MEF in Fig. 1, B, C, and D, respectively). For example, in the U87MGPTEN, LAPC-4pare, and PTEN+/−/− MEF cell lines the presence of the 5′-UTR of cyclin D1 or the c-myc IRES resulted in an ~3–4-fold increase in firefly luciferase activity. In contrast, firefly luciferase activity was minimally affected in the relatively “active AKT” member cell lines of these isogenic pairs, indicating that AKT activity prevented cyclin D1 and c-myc IRES function. Only a modest ~1.5–2-fold increase was seen in myc-IRES activity, and there was no increase in cyclin D1 IRES activity. The presence of the human p27Kip1 IRES-containing sequences (24, 29) in the dicistronic reporter construct resulted in an ~10-fold increase in firefly luciferase activity in all of the cell lines tested irrespective of AKT activity. This was consistent with our earlier observations that the p27Kip1 mRNA was well translated in the face of mTOR inhibition regardless of the AKT status of the cell (10) and indicates that p27Kip1 IRES function is independent of AKT activity. It also provides a control confirming that reporter expression will occur in the “high-AKT” cell lines if an IRES is functional.

To evaluate whether the AKT-dependent enhanced translation of the downstream cistron in the cell lines tested was the result of initiation from shorter monocistronic transcripts or possibly from cryptic promoter activity, we analyzed the dicistronic mRNAs via Northern blot and luciferase activities in constructs where the SV40 promoter was absent (pRF−p), pRCDF1−p, and pMyecF−p (30). Fig. 2A shows a schematic diagram of the promoterless dicistronic constructs transfected into PTEN+/− and PTEN+/−/− MEFs. As shown in Fig. 2B, introduction of the promoterless constructs resulted in minimal luciferase activities in both the PTEN+/− and PTEN+/−/− MEFs, indicating that the AKT-dependent firefly luciferase expression from these constructs was not the result of internal promoter activities. Further...
However, Northern analysis of mRNAs from transfected PTEN−/− or PTEN+/+ MEFs detected only the presence of the full-length dicistronic transcripts when probed for sequences within the downstream firefly luciferase open reading frame (Fig. 2C). The 5'-UTR of cyclin D1 is 208 nucleotides in length, whereas the c-myc IRES sequences are 396 nucleotides in length.

Fig. 1. AKT-dependent cyclin D1 and c-myc IRES activity in vivo. A, schematic diagrams of the dicistronic constructs used in this study. Constructs used are pRF, pRCD1F, which contains the 5'-UTR of human cyclin D1, pRmycF, containing the human c-myc IRES, and pRp27F, containing the human p27 Kip1 IRES. Luc, luciferase. B, normalized (to values obtained for pRF alone) Renilla (white bars) and firefly (black bars) luciferase activities of U87 and U87PTEN cells transfected with the indicated constructs. C and D are identical to B except for the cell line used, as indicated. Transfection was performed in quadruplicate with each construct in each indicated cell line.
These data further supported the notion that the cyclin D1 5′-UTR was capable of internal initiation and that both the IRES activities of the 5′-UTR of cyclin and the c-myc IRES were regulated by AKT activity.

The Cyclin D1 5′-UTR and c-myc IRES Mediate Internal Initiation in an AKT-dependent Fashion in Cell-free Extracts—To rule out that firefly luciferase reporter expression could be due to unusual cryptic splicing events, we analyzed the AKT-dependent IRES activities of the cyclin D1 and c-myc mRNAs in cell-free extracts. These extracts were prepared according to Carroll and Lucas-Lenard (26) and demonstrated to have high efficiencies in initiating protein synthesis. Capped dicistronic mRNAs that either lacked (pRF) or contained the IRES (pRCD1F) were transcribed and subse- quently used to program translation in lysates from U87MG, U87MGPTEN, LAPC-4puro, LAPC-4myrAKT, and pRCD1F constructs. pRF and pRCD1F are identical to the constructs in Fig. 1A except the SV40 promoter sequences have been removed (51). Luc, luciferase. B, relative luciferase activities of the indicated promoterless construct transfected into PTEN−/− MEF or PTEN+/− MEF. The positions of the 28 S and 18 S ribosomal RNAs are shown.

This again was consistent with the results from the dicistronic mRNAs in cell-free extracts. These extracts were prepared according to Carroll and Lucas-Lenard (26) and demonstrated to have high efficiencies in initiating protein synthesis. Capped dicistronic mRNAs that either lacked (pRF) or contained the IRES (pRCD1F) were transcribed and subse-

quently used to program translation in lysates from U87MG, U87MGPTEN, LAPC-4puro, LAPC-4myrAKT, PTEN−/− MEFs, or PTEN+/− MEFs. Translation of the parent pRF mRNA yielded firefly luciferase activities, which were indistinguishable from the background obtained from control reaction mixtures that lacked the firefly luciferase reporter mRNAs. In contrast, as shown in Fig. 3, an equivalent amount of either pRCD1F or pRmycF mRNAs generated firefly luciferase activities, which were ~2–4-fold higher in extracts prepared from cell lines with relatively quiescent AKT levels. The firefly luciferase activities generated from the pRF27F mRNA were consistently ~6-fold higher in all cell extracts tested as compared with pRF mRNA. This again was consistent with the results from the dicistronic in vivo experiments, demonstrating that the IRES-dependent translation of p27Kip1 was independent of AKT activity.

To evaluate whether the translation of the cyclin 5′-UTR and the c-myc IRES in these reporter mRNAs was indeed cap-independent within these cell extracts, in vitro transcribed and capped mRNAs from pRCD1F and pRmycF were translated in the presence of increasing concentrations of the cap analog m’GTP. This analog blocks cap-dependent translation by binding to the initiation factor eIF-4E (31). Using mRNA in vitro transcribed from pRF and capped, translation of the Renilla luciferase cistron was blocked by ~80–90% at 150 μM of m’GpppG but was not affected by comparable concentrations of the non-methylated form of the analog GTP, and the translation of the firefly cistron was unaffected by m’GpppG (data not shown). As shown in Fig. 4, with both the in vitro transcribed and capped mRNAs from pRCD1F and pRmycF, the translation of the Renilla cistron was inhibited by ~80–90% at concentrations of 100 μM m’GpppG or higher in all cell extracts tested. However, the translation of the firefly cistron remained consistent in the extracts with relatively high levels of active AKT and increased ~1.5–2.5-fold in extracts from the cell lines with relatively quiescent AKT levels.

 AKT-dependent Cyclin D1 5′-UTR and c-myc IRES Activity Is Enhanced by Rapamycin—Because our previous studies (19) demonstrated a stimulation of cyclin D1 and c-myc polysome association and protein levels by rapamycin under conditions of quiescent AKT activity, we assessed whether cyclin D1 or c-myc IRES activity would also be enhanced following rapamycin exposure in cells with quiescent AKT activity. To address the AKT-dependent affects of rapamycin on cyclin D1 or c-myc IRES function, we transfected our dicistronic constructs into...
the cell lines shown in Fig. 5 and determined Renilla and firefly luciferase activities prior to and following rapamycin exposure. Renilla luciferase activity was reduced by rapamycin \(70\% - 90\%\) as compared with values obtained in the absence of the drug for each construct tested (data not shown). However, the results show that in the cell lines with relatively quiescent AKT (U87<sub>PTEN</sub>, LAPC-4<sub>myrAKT</sub>, and PTEN<sup>-/-</sup> MEFs), rapamycin treatment resulted in a 3–6-fold stimulation of firefly luciferase activity as compared with values obtained for lines containing active AKT. Interestingly, rapamycin exposure also stimulated p27<sub>Kip1</sub> IRES activity \(4–6\)-fold in all cell lines irrespective of AKT activity, again consistent with our previous data and results from others demonstrating the resistance of p27 IRES<sup>Kip1</sup> activity following exposure to the phosphatidylinositol 3-kinase inhibitor LY294002 (24). Northern blot analysis further demonstrated that rapamycin had no effect on pRF steady-state mRNA levels prior to and following rapamycin treatment (data not shown).

**Fig. 3.** **AKT-dependent cyclin D1 and c-myc IRES activity in cell-free extracts.** In vitro transcribed dicistronic RNAs from the indicated constructs were used to program translation in cell-free extracts in the three cell line pairs shown. Renilla and firefly luciferase activities of U87 versus U87<sub>PTEN</sub> (A), LAPC-4<sub>myrAKT</sub> versus LAPC-4<sub>puro</sub> (B), and PTEN<sup>-/-</sup> MEF versus PTEN<sup>-/-</sup> MEF (C) translation extracts. Values were normalized to those obtained for pRF alone, and the data are representative of three independent experiments for each cell line.

**Differential AKT-dependent Cyclin D1 and c-myc IRES Activity Is Dependent on Both p38 Mitogen-activated Protein Kinase and RAF/MEK/ERK Signaling**—Because it has been demonstrated previously that c-myc IRES function is dependent on p38 MAPK activity during apoptosis (28) and both p38 MAPK and ERK signaling following genotoxic stress (32), we investigated whether these cascades also contributed to the differential AKT-dependent cyclin D1 and c-myc IRES activity we had observed. An additional rationale was the known ability of AKT to down-regulate p38 (33) and ERK (34) activity. To determine whether differential p38 or ERK signaling could be correlated with AKT-dependent cyclin D1 or c-myc IRES activi-
ity, we initially examined the activities of these kinases in the PTEN−/− and PTEN+/+ MEFs prior to and following rapamycin exposure. As shown in Fig. 6A, the basal p38 and ERK kinase activities as determined by in vitro kinase assays were ∼3–4-fold higher in the PTEN−/− MEFs as compared with the PTEN−/− MEFs. Additionally, treatment with rapamycin resulted in an 8-fold induction of p38 and 10-fold induction of ERK activity in PTEN−/− MEFs while only modestly increasing basal p38 and ERK activities (∼1–2-fold) in the PTEN−/− MEFs. Total p38 and ERK content in the samples was similar, demonstrating that equivalent amounts of material were immunoprecipitated (Fig. 6A). These data suggest that p38 and ERK signaling is activated by rapamycin exposure in an AKT-dependent manner and is consistent with the known negative regulatory effects of AKT on p38 and on ERK (33, 34).

To investigate whether AKT-dependent cyclin D1 and c-myc IRES activity was regulated by p38 or ERK signaling we planned to transfet PTEN−/− and PTEN−/− MEFs with the
indicated dicistronic constructs in Fig. 6B and subsequently to treat these cells with the p38 inhibitor SB203580 or the ERK inhibitor PD98059. Our preliminary experiments demonstrated almost complete inhibition of kinase activity using these inhibitors (data not shown). Treatment of either PTEN+/− or PTEN−/− MEFs with SB203580 inhibited basal p38 kinase activity by more than 95% within 2 h of treatment at a concentration of 25 μM. Similarly, treatment of the MEFs with PD98059 inhibited ERK activity by more than 92% within 2 h of treatment at 25 μM.

These inhibiting concentrations of SB203580 or PD98059 were found to significantly affect AKT-dependent IRES function. The experiments shown in Fig. 6, B and C, are assays performed in the absence or presence of rapamycin, respectively. As shown in Fig. 6B, PTEN−/− MEFs expressed relatively little firefly luciferase activity when transfected with the dicistronic constructs containing the cyclin D1 5′-UTR or the c-myc IRES within the intercistronic regions. However, as previously observed, firefly luciferase activity was markedly increased (~3–4-fold) in the PTEN+/- MEFs transfected with pRCND1F or pRmycF. Treatment of these cells with either SB203580 or PD98059 resulted in more than 75% inhibition of firefly luciferase activity. The p38 inhibitor resulted in a modest decrease in Renilla luciferase expression (~5–10% inhibition), whereas the ERK inhibitor reduced Renilla luciferase expression by 60–65% in all of the constructs tested (data not shown). Interestingly, the p27Kip1 IRES was unaffected by treatment with either of the inhibitors and did not demonstrate differential AKT-dependent activity as shown before (Fig. 1).

To address whether these signaling cascades contributed to the rapamycin-induced differential cyclin D1 and c-myc IRES activity we had previously observed, we performed the same assays in MEFs pretreated with SB203580 or PD98059, which had been transiently transfected with the indicated dicistronic constructs shown in Fig. 6C. As shown in the relatively quiescent AKT-containing PTEN+/− MEFs, rapamycin induced firefly luciferase expression by ~4-fold relative to control experiments without the drug. This enhancement of IRES activity was markedly inhibited by pretreatment with either SB203580 or PD98059. 1 h of preincubation with either of these inhibitors resulted in greater than 80% inhibition of the rapamycin-induced firefly luciferase expression in PTEN−/− MEFs. Renilla luciferase expression was reduced by rapamycin (~65% inhibition) in all of the tests performed, and pretreatment with SB203580 or PD98059 in combination with rapamycin did not significantly reduce Renilla luciferase expression further (data not shown). As before, p27Kip1 IRES activity was enhanced by rapamycin irrespective of AKT activity but was not affected by inhibition of p38 or ERK signaling.

Differential cyclin D1 and c-Myc protein levels were also assessed in the PTEN+/− and PTEN−/− MEFs treated with rapamycin alone and in combination with either the p38 or ERK inhibitors. As shown in Fig. 7, treatment of PTEN−/− MEFs with rapamycin resulted in down-regulation of cyclin D1 and c-myc expression; however, PTEN+/− MEFs maintain or modestly increase expression in response to the drug. This differential response in cyclin D1 and c-myc expression is ablated by pretreatment of the cells with either the p38 or ERK inhibitors prior to exposure to rapamycin. As determined by densitometry, pretreatment with SB203580 inhibited cyclin D1 protein levels in rapamycin-treated PTEN−/− MEFs by 75-fold (Fig. 7, compare lane 7 with 8), whereas pretreatment with the ERK inhibitor PD98059 reduced cyclin D1 expression by 9-fold in these cells (lanes 11 and 12). Pretreatment with either SB203580 or PD98059 reduced c-Myc protein expression to below detectable levels in quiescent AKT-containing PTEN+/− MEFs upon rapamycin exposure (Fig. 7, lanes 7 and 8, lanes 11 and 12).

Sequence Analysis and Secondary Structure Prediction of the Cyclin D1 5′-UTR—The 5′-UTRs of the major human cyclin D1 and c-myc mRNAs consist of 209 and 400 nucleotides, respectively. Both leader sequences contain the hallmark 5′-UTRs from other mRNAs demonstrated to exhibit IRES activity. Both leaders are relatively long, highly structured, and contain upstream AUG or CUG initiation codons (22, 35). Although a model of the c-myc IRES structure has been described (36), the leader of the human cyclin D1 mRNA has not been characterized in this regard.

Some mRNAs capable of internal translation initiation have been shown to contain sequence complementarity to 18 S ribosomal RNAs (37, 38). It has been proposed that these regions of
complementarity may serve as cis-acting elements involved in the direct recruitment of ribosomal 40 S subunits to mRNAs and possibly regulate cap-independent translation (39). To address whether the cyclin D1 leader contained regions of sequence complementarity to 18 S ribosomal RNAs, we performed sequence comparisons. Comparisons of 18 S ribosomal RNAs and the cyclin D1 5'UTR identified several complementary sequence matches (see supplemental data). Seven of these regions ranged from 80 to 94% similarity to 18 S rRNA over 11–22 nucleotides for the cyclin D1 leader.

A secondary structural model of the cyclin D1 5'UTR was derived by free energy calculations using the MFOLD algorithm (40). 13 structures were calculated, which ranged in initial free energies (dG) from −71.5 to −88.9 kcal/mol. The most stable predicted structure is shown as supplemental data. The structure is highly complex with several long stems, junctions, and higher order bifurcations. Taken together, these data support the notion that the cyclin D1 5'UTR contains a bona fide IRES element.

DISCUSSION

Our previous studies (19) suggested that the cyclin D1 and c-myc mRNAs are transcripts that could be effectively translated under conditions of reduced cap-dependent initiation. In this report we have demonstrated that under specific signaling conditions, the leader of the human cyclin D1 mRNA can ini-

FIG. 6. AKT-dependent cyclin D1 and c-myc IRES activity requires p38 MAPK and ERK signaling. A, p38 MAPK and ERK activities in PTEN−/− and PTEN+/+ MEFs prior to and following rapamycin exposure. In vitro kinase reactions were immunoblotted for the indicated phosphorylated substrate and total p38 or ERK protein levels. B, PTEN−/− and PTEN+/+ MEFs were transfected with the indicated dicistronic constructs and treated with either SB203580 or PD98059. Changes in firefly luciferase (cap-independent) expression are shown. Values were normalized to pRF without treatment and were performed in triplicate. C, PTEN−/− and PTEN+/+ MEFs were transfected with the indicated dicistronic constructs, pretreated with either SB203580 or PD98059 for 1 h, and subsequently exposed to rapamycin (rapa) as shown. Relative -fold changes in firefly (cap-independent) luciferase activities are shown as compared with activities obtained in the absence of rapamycin. Data are representative of three independent experiments.
tiate protein synthesis via an IRES. Although the cyclin encoded by the Kaposi sarcoma-associated herpes virus has also been reported to contain an IRES (41); to our knowledge, this is the first report of the ability of this mRNA to initiate translation internally. We have also shown that AKT activity regulates cyclin D1 and c-myc IRES function and demonstrated that rapamycin increases cyclin D1 and c-myc IRES activity in an AKT-dependent manner. These results are consistent with our previously observed AKT-dependent effects on cyclin D1 and c-Myc protein synthesis following rapamycin exposure. Furthermore, we have extended our studies by implicating the p38 MAPK and RAF/MEK/ERK signaling cascades in the regulation of AKT-dependent cyclin D1 and c-myc IRES activity. Our results support a working model in which the AKT-dependent control of cyclin D1 and c-myc IRES function in response to rapamycin may regulate the expression of these critical determinants resulting in either G1 arrest or tumor cell survival. When AKT activity is relatively low, rapamycin treatment results in the inhibition of cap-dependent translation but stimulates the selective translation of cyclin D1 and c-myc via their IRESes mediated via p38 MAPK and RAF/MEK/ERK signaling, thus maintaining expression. However, when AKT is elevated, the rapamycin-induced inhibition of cap-dependent translation is not associated with enhanced IRES function, most likely because of the negative regulatory effects of AKT activity on the p38 MAPK and RAF/MEK/ERK pathways; thus, cap-independent translation is prevented, and protein levels fall. This differential regulation of cap-independent translation and overall cyclin D1/c-myc expression accounts for the differential sensitivity of "high versus low" AKT activity cell targets.

An interesting question arises; under what circumstances might there be a requirement for IRES-mediated translation initiation of cyclin D1 mRNA, particularly when cyclin D1 expression has been shown to be dependent on eIF-4E (42, 43)? Recent data suggest that cyclin D1 also normally accumulates during the G2 phase of the cell cycle and that synthesis during this phase may contribute to the rapid achievement of the levels of cyclin D1 required for the ensuing G1 transit in actively proliferating cells (44). It has also been recently appreciated that there is a reduction in cap-dependent protein synthesis during the G2/M cell cycle transition (45, 46), and interestingly, it is known that both AKT activity and protein levels transiently drop during the G2/M transition (47). Although it has been demonstrated that post-translational mechanisms contribute to the accumulation of cyclin D1 during G3 (48, 49), it is also possible that the IRES-mediated synthesis of cyclin D1 normally occurs during this phase of the cell cycle and supplements expression.

Our data imply that the factor(s) responsible for AKT-dependent cyclin D1 and c-myc IRES function are downstream of p38 and ERK. This is consistent with the results of others who have demonstrated roles for these effectors in regulating the IRES-mediated synthesis of c-myc during apoptosis or in response to genotoxic agents (28, 32). It is possible that these effectors regulate cyclin D1 and c-myc IRES activity directly or indirectly via phosphomodulation of an IRES trans-acting factor(s). Recently, three members of the poly(rC)-RNA binding family, PCBP1, PCBP2, and hnRNPK, have been shown to be required for c-myc IRES activity and stimulate IRES-mediated translation when overexpressed and bound to the mRNA (50). Moreover, it is known that the activity of these proteins is regulated by phosphorylation (51–53). Alternatively, p38 or ERK activity may lead to changes in IRES trans-acting factor expression thereby affecting IRES function. Along these lines it has been demonstrated that the expression of PCBP1 under hypoxic conditions is dependent on p38 activity in cortical neurons (54). Experiments designed to address these questions are currently in progress.

The observation that p27kip1 IRES activity was not AKT-dependent following rapamycin exposure is interesting and suggests that the regulation of this IRES is similar to but distinct from the cyclin D1 and c-myc IRESes in this setting. P27kip1 IRES function may be regulated by a specific IRES trans-acting factor(s) that enhances its function following rapamycin exposure but is nonresponsive to changes in p38, ERK, or AKT activities. The p27kip1 IRES has been demonstrated to be active under conditions of elevated cyclic AMP (29) and repressed by the neuronal ELAV HuD (24), whereas enhancement of c-myc IRES activity has been shown to be dependent on PCBP1, PCBP2, and hnRNPK (50). It is certainly possible that the factors mediating cap-independent translational control of p27kip1 are distinct from those regulating other cellular IRESes.

Our data also suggest that the ability of tumor cells to respond to mTOR inhibitors by stimulating cap-independent mechanisms of initiation of critical cell cycle proteins may constitute a mechanism of cellular resistance to these drugs. In particular, tumors that have relatively little dependence on the phosphatidylinositol 3-kinase/AKT/mTOR signaling cascade (i.e. low AKT activity) appear to markedly increase the cap-independent synthesis of cyclin D1/c-myc following mTOR inhibitor exposure. Further understanding the mechanisms regulating the expression of these determinants may assist in the development of compounds that function in a synthetically lethal manner with mTOR inhibitors.

Acknowledgments—We thank I. Mellinghoff, C. Sawyers, A. Willis, and J.-T. Zhang for providing cell lines and plasmids. We also thank M. Rettig for helpful discussions and critical reading of the manuscript.

REFERENCES

1. Raught, B., Gingras, A. C., and Sonenberg, N. (2000) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 245–293, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. Bjornsti, M. A., and Houghton, P. J. (2004) Nat. Rev. Cancer 4, 335–348
3. Rhouds, R. E. (1999) J. Biol. Chem. 274, 30337–30340
4. Raught, B., and Gingras, A. C. (1999) Int. J. Biochem. Cell Biol. 31, 43–57
5. Sekulic, A., Hudson, C. C., Homme, J. L., Yin, P., Otterness, D. M., Karnitz, L. M., and Abraham, R. T. (2000) Cancer Res. 60, 3594–3513
6. Cigan, A. M., and Denahue, T. F. (1987) Gene (Amst.) 59, 1–18
7. Hellen, C. U., and Sarnow, P. (2001) Genes Dev. 15, 1593–1612
8. Stoneley, M., and Willis, A. E. (2004) Oncogene 23, 3200–3207
9. Vagner, S., Galy, B., and Pyronnet, S. (2001) EMBO Rep. 2, 893–898
16. Zhang, H., Stalock, J. P., Ng, J. C., Reinhard, C., and Neufeld, T. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13118–13123
17. Oldham, S., Montagne, J., Radimerski, T., Thomas, G., and Hafen, E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 13571–13576
18. Figueroa, C., and Vojtek, A. B. (2003) Proc. Natl. Acad. Sci. U. S. A. 99, 42396–42397
19. Gera, J. F., Mellinghoff, I. K., Shi, Y., Rettig, M. B., Tran, C., Hsu, J. H., Neshat, M. S., Mellinghoff, I. K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Hu, M. C., Tranque, P., Edelman, G. M., and Mauro, V. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1339–1344
20. Neshat, M. S., Mellinghoff, I. K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Frost, P., Gibbons, J. J., Wu, H., and Sawyers, C. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 10314–10319
21. Wang, S., Gao, J., Lei, Q., Renzengurt, N., Pritchard, C., Jiao, J., Thomas, G. V., Li, G., Roy-Burman, P., Nelson, P. S., Liu, X., and Wu, H. (2003) Cancer Cell 4, 209–221
22. Xiong, Y., Connolly, T., Futcher, B., and Beach, D. (1991) Cell 65, 691–6999
23. Stoner, M., Paulin, F. E., Le Queene, J. P., Chappell, S. A., and Willis, A. E. (1998) Oncogene 16, 423–428
24. Kullmann, M., Gopfert, U., Sieve, B., and Hengst, L. (2002) Genes Dev. 16, 3087–3099
25. Zuker M. (2003) Nucleic Acids Res. 31, 3406–3415
26. Carroll, R., and Lucas-Lenard, J. (1993) Anal. Biochem. 212, 17–23
27. Stiles, B., Gilmore, V., Kanzler, N., Lash, R., Li, A., Quo, R., Liu, X., and Wu, H. (2002) Mol. Cell. Biol. 22, 3842–3851
28. Stoner, M., Chappell, S. A., Jaglom, C. L., Dickens, M., MacFarlane, M., and Willis, A. E. (2000) Mol. Cell. Biol. 20, 1162–1169
29. Miskimins, W. K., Wang, G., Hawkinson, M., and Miskimins, R. (2001) Mol. Cell. Biol. 21, 4960–4967
30. Han, B., and Zhang, J. T. (2002) Mol. Cell. Biol. 22, 7372–7384
31. Cai, A., Jarovetz, S., and Sarnow P. (2002) Biochemistry 38, 8538–8547
32. Sobhankulova, T., Mitchell, S. A., and Willis, A. E. (2001) Biochem. J. 359, 183–192
33. Gratton, J. P., Morales-Ruiz, M., Kureishi, Y., Fulton, D., Walsh, K., and Sessa, W. C. (2001) J. Biol. Chem. 276, 30359–30365
34. Zimmermann, S., and Moelling, K. (1999) Science 286, 1741–1744
35. Sessa, W. C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12238–12243
36. Le Queene, J. P., Stoner, M., Fraser, G. A., and Willis, A. E. (1999) J. Mol. Biol. 310, 111–126
37. Tranque, P., Hu, M. C., Edelman, G. M., and Mauro, V. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1162–1169
38. Hu, M. C., Tranque, P., Edelman, G. M., and Mauro, V. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1339–1344
39. Mauro, V. P., and Edelman, G. M. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 12031–12036
40. Jacobson, A. B., and Zuker, M. (1995) J. Mol. Biol. 233, 261–269
41. Bieleski, L, and Talbot, S. J. (2001) Mol. Cell. Biol. 21, 1162–1169
42. Mamane, Y., Petroulakis, E., Rong, L., Yoshida, K., Ler, L. W., and Sonenberg, N. (2004) Oncogene 23, 3172–3179
43. Rousseau, D., Kaspar, R., Rosenwald, I., Gehrke, L., and Sonenberg, N. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1065–1070
44. Sherr, C. J. (2002) Cell Cycle 1, 56–58
45. Pyronnet, S., and Sonenberg, N. (2001) Curr. Opin. Genet. Dev. 11, 13–18
46. Pyronnet, S., Dostie, J., and Sonenberg, N. (2001) Genes Dev. 15, 2083–2093
47. Roberts, E. C., Shapiro, P. S., Nahreini, T. S., Pages, G., Pouyssegur, J., and Ahn, N. G. (2002) Oncogene 21, 7545–7556
48. Evans, J. R., Mitchell, S. A., Spriggs, K. A., Ostrowski, J., Bomsztlyk, K., Ostarek, D., and Willis, A. E. (2003) Oncogene 22, 699–704
49. Abrass, C. K., and Bomsztyk, K. (2001) Mol. Cell. Biol. 21, 5619–5624
50. Ostrowski, J., Schullery, D. S., Denisenko, O. N., Higaki, Y., Watts, J., Aebersold, R., Stempka, L., Ghoshdini, M., and Bomsztlyk, K. (2000) J. Biol. Chem. 275, 3619–3624
51. Ostrowski, J., Kawata, Y., Schullery, D. S., Denisenko, O. N., Higaki, Y., Abrass, C. K., and Bomsztlyk, K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9044–9049
52. Zhu, Y., Sun, Y., Mao, O., Jin, K. L., and Greenberg, D. A. (2002) Neuroscience 110, 191–198
Cyclin D1 and c-myc Internal Ribosome Entry Site (IRES)-dependent Translation Is Regulated by AKT Activity and Enhanced by Rapamycin through a p38 MAPK- and ERK-dependent Pathway

YiJiang Shi, Anushree Sharma, Hong Wu, Alan Lichtenstein and Joseph Gera

J. Biol. Chem. 2005, 280:10964-10973.
doi: 10.1074/jbc.M407874200 originally published online January 4, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M407874200

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2005/01/05/M407874200.DC1

This article cites 53 references, 29 of which can be accessed free at
http://www.jbc.org/content/280/12/10964.full.html#ref-list-1