Mnk mediates integrin (α6β4)-dependent eIF4E phosphorylation and translation of VEGF mRNA

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Running title: Integrin-dependent translational control via Mnk

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Abstract

It was previously shown that integrin α6β4 contributes to translation of cancer-related mRNAs such as VEGF via initiation factor eIF4E. In this study, we found that integrin α6β4 regulates the activity of eIF4E through the Ser/Thr kinase Mnk. While a role for Mnk in various aspects of cancer progression has been established, a link between integrin and Mnk activity has not. Here we show that Mnk1 is a downstream effector of integrin α6β4 and mediates the α6β4 signaling important for translational control. Integrin α6β4 signals through MEK and p38 MAPK to increase phosphorylation of Mnk1 and eIF4E. Inhibition of Mnk1 activity by CGP57380 or downregulation by shRNA blocks α6β4-dependent translation of VEGF mRNA. Our studies suggest that Mnk1 could be a therapeutic target in cancers where the integrin α6β4 level is high.

Introduction

Integrin α6β4 is widely expressed on most epithelial cells and serves as a receptor for members of the laminin family of extracellular matrix proteins (1, 2). While its primary function is known to be the maintenance of normal tissue integrity, a number of recent findings strongly support a role in cancer cell functions associated with progression (1, 2). In aggressive cancer cells, the tumor microenvironment is thought to play a role in locating α6β4 to actin filament-rich structures such as lamellipodia and filopodia (3, 4). Localization of α6β4 in these structures enhances its signaling function through functional and physical interactions with other signaling receptors (3, 4, 5). Signaling-competent α6β4 can initiate a signal transduction cascade that stimulates the
ability of cancer cells to invade as well as survive under hostile conditions (6, 7, 8). For example, previous studies have shown that α6β4 protects breast carcinoma cells from apoptosis under stress conditions such as deprivation of serum and matrix (7, 8), a phenomenon linked to the ability of this integrin to activate the PI3K/Akt pathway. This survival pathway in α6β4-expressing breast carcinomas depends on expression of vascular endothelial growth factor (VEGF) (7, 8). The Ser/Thr kinase mTOR is activated by α6β4 via the PI3K/Akt pathway and mediates α6β4-dependent VEGF translation (8).

mTOR phosphorylates and inactivates 4E-BP1, which binds the translation initiation factor eIF4E and prevents its interaction with eIF4G (9). eIF4E complexed with eIF4G and eIF4A, which is called eIF4F, regulates recruitment of capped mRNA to 48S initiation complexes (10). The rate of translation initiation depends on both structural features of individual mRNA and the level and activity of eIF4F complex. Translation of mRNAs containing highly structured 5′-untranslated regions (UTRs) requires high levels of eIF4E (reviewed in 11). However, the PI3K/Akt/mTOR pathways may not be the only pathway for α6β4-dependent translational control since rapamycin, an mTOR-specific inhibitor, only partially blocks α6β4-dependent translation of VEGF mRNA (8). Yoon et al. demonstrated that α6β4 also promotes activation of Ras and phosphorylation of eIF4E in breast carcinomas under serum-depleted conditions (12), suggesting the involvement of a Ras-dependent mechanism in α6β4-dependent translational control. The prime candidate for mediation of Ras-dependent eIF4E phosphorylation is the Ser/Thr kinase Mnk, since Ras, MEK and p38 pathways have been shown to regulate Mnk kinase activity (13, 14).
Mnk is the only known physiological kinase that phosphorylates eIF4E (S209 in human eIF4E) and does so in response to extracellular stimuli that include stress, growth factors, cytokines, and hormones (reviewed in 15). Both Mnk and eIF4E bind to eIF4G. It was suggested that eIF4G provides a docking site for Mnk to phosphorylate eIF4E (16). There are four Mnk isoforms derived from two genes (MNK1 and MNK2) by alternative splicing. In the cytoplasm, activation of Mnk1a is responsible for the inducible phosphorylation of eIF4E, whereas Mnk2a mainly contributes to basal constitutive phosphorylation. Mnk1b and Mnk2b are localized primarily in the nucleus (reviewed in 17). There are several studies showing a positive correlation between the protein levels or activity of Mnk1 and protein synthesis, cell proliferation, transformation, or apoptosis (18, 19, 20, 21, 22, 23, 24, 25). Additional studies utilizing knock-out mice (26), siRNA (27, 28), or the Mnk inhibitor CGP57380 (23, 28, 29, 30) have confirmed a role of Mnk in apoptosis (26, 27), differentiation (29), cytokine production (28, 30), and cancer phenotype (23). The activation status of Mnk has been correlated not only with changes in cell fate but also with changes in the spectrum of mRNAs translated (24, 25, 28).

In this study, we assessed the role of Mnk in αβ4-dependent translational control. We found that Mnk is a downstream effector of αβ4 that mediates proliferation of MDA-MB-435 cancer cells. Studies with the Mnk inhibitor CGP57380 and shRNA against Mnk demonstrated that Mnk is required for αβ4-dependent phosphorylation of eIF4E and translation of the cancer-related VEGF mRNA.

Materials and Methods

Cells, Antibodies and Reagents
MDA-MB-435 cells were obtained from the Lombardi Breast Cancer Repository at Georgetown University. The generation of MDA-MD-435 sub-clones expressing β4 integrin (β4) (clone 3A7) as well as an empty vector (mock) (clone 6D2) were performed as previously described (31). All cells were grown in DMEM/Low Glucose (HyClone Lab Inc., Logan, Utah) supplemented with penicillin-streptomycin and 10% fetal bovine serum at 37°C in 5% CO₂. Anti-eIF4G-1 (peptide 7) antibodies were described previously (32). Anti-eIF4E, anti-phospho-eIF4E (S209), anti-Mnk1, anti-phospho-Mnk1 (Thr197/202), anti-MEK1/2, anti-phospho-MEK1/2 (S217/221), and anti-phospho-p38 (Thr180/185) antibodies were purchased from Cell Signaling Technology. Anti-p38 antibody was obtained from Santa Cruz Biotechnology. Anti-actin antibody was purchased from Sigma. CGP57380 was provided by Novartis Pharma AG, Switzerland, UO126 was purchased from Cell Signaling, and SB253508 was purchased from Calbiochem. Lentiviral particles expressing shRNA against Mnk1 (TRCN0000195343) were purchased from Sigma.

**Pharmacologic Inhibition and shRNA Expression**

For inhibition of Mnk1/2, mock and β4 cells were grown in 10% FBS media in 150 mm plates until they reached 50-60% confluency, at which point the cells were transferred to low serum media (0.5% FBS) and treated with 0.1% DMSO or 10 μM CGP57380 overnight at 37°C. Cells were counted and plated into 6-well culture dishes. For inhibition of MEK1/2 or p38 MAP kinases, β4 cells (at 50-60% confluency) were treated with various concentrations of the MEK1/2 inhibitor UO126 or the p38 MAP kinase inhibitor SB253508 under conditions of serum starvation overnight at 37°C.
Kinase inhibitors were stored as a 10 mM stock solutions in 100% DMSO; inhibitor-treated cells were always compared with cells treated with the same concentration of DMSO. For shRNA experiments, β4 cells were stably infected with lentiviral particles that express shRNA targeted against either GFP or Mnk1.

**Immunoblotting**

To analyze the expression and phosphorylation of proteins, cells were lysed in cold RIPA-EDTA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and 5 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride, 1 mM Na$_3$VO$_4$, and protease inhibitor (Pierce). Protein concentrations were determined using the BCA protein assay kit (Pierce). Proteins were separated by SDS-PAGE on 4% to 20% gradient gels and transferred to polyvinylidene fluoride membranes (Millipore). The blots were incubated with primary antibodies in TBST or TBST with 5% w/v nonfat dry milk, then with the appropriate secondary antibodies conjugated to horseradish peroxidase. Proteins were detected using the ECL Western blotting substrate (Pierce).

**Proliferation Assay**

Mock and β4 cells were plated in 6-well plates at 1 x 10$^4$ cells per well and allowed to adhere overnight. They were then grown in low serum media and treated with either 0.1% DMSO or 10 μM CGP57380 for up to 5 days. At various times, cells were trypsinized and counted with a hemocytometer.
Fractionation of Polysomes

Polysomes were analyzed in mock and β4 cells grown in low serum medium as described previously (33). Briefly, cycloheximide (VWR) was added directly to the plates to a final concentration of 50 μg/ml, and the cells were incubated for 5-10 min at 37°C. After briefly washing with PBS containing 100 μg/ml cycloheximide, cells were lysed in 0.5 ml lysis buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM MgCl2, 0.5% NP-40, 2 mM DTT, 100 μg/ml cycloheximide, 50 μg/ml heparin, 0.5 U/μl RNasin (Applied Biosystems), and Complete™ EDTA-free protease inhibitor cocktail (Roche)). Lysates were kept on ice for 10 min and centrifuged for 5 min at 28,000 x g at 4°C. The supernatants were kept at -80°C. An equal volume of lysate (generally 800 μl) was layered onto a 15-45% (w/v) sucrose gradient containing 100 μg/ml cycloheximide and centrifuged in a Beckman SW41Ti rotor at 38,000 rpm at 4°C for 3 h. Gradients were collected in 1-ml fractions with continuous monitoring of absorbance at 260 nm using an ISCO syringe pump with UV-6 detector.

RNA Isolation and Real-Time PCR

RNA was isolated from pools of light polysomes (L, fractions 1-7), and heavy polysomes (H, fractions 8-12) using an E.Z.N.A. Total RNA Kit (Omega Bio-Tek) according to the manufacturer’s instructions. RNA was measured by OD_{260}. The relative amount of RNA in the L and H pools is expressed as a percentage of the total amount of RNA in all fractions. Aliquots of 5 μl were then used for quantitative reverse transcription real-time PCR analysis (qRT-PCR). GFP mRNA (100 pg) was added to each fraction before RNA isolation as an internal standard to control for variation in RNA
yield. Reverse transcription was performed with random primers the TaqMan® Reverse Transcription Reagents kit (Applied Biosystems) by following the manufacturer’s protocol. qRT-PCR was performed with specific primers designed for each mRNA: GAPDH forward primer, 5’-gggtgtaaccatgagaagt 3’; GAPDH reverse primer, 5’-gacttgtgatcagtcct-3’; VEGF forward primer, 5’-ttgccaatcacctcct-3’; VEGF reverse primer, 5’-tttctggtgtcgctg-3’; and GFP reverse primer, 5’-accatgtggtctcttttcg- 3’ (MWG-Biotech AG). Amplification and detection were performed with the iCycler IQ Real-time PCR detection system and IQ™ SYBRgreen Supermix (Bio-Rad). The relative amount of a specific mRNA in the L and H pools is expressed as a percentage of the total amount of that mRNA in all fractions.

Results

α6β4 Integrin Increases the Phosphorylation of Mnk and eIF4E under Serum-deprived Conditions

A previous study demonstrated that α6β4 integrin affects the translation of VEGF mRNA by the PI3K/mTOR/eIF4E pathway (8). The mechanism involves mTOR-mediated phosphorylation of 4E-BP1 and subsequent release of eIF4E from 4E-BP1. Recently, this integrin was shown to be involved in translational activation of ErbB2 mRNA in an eIF4E-dependent manner in breast carcinoma cells under serum deprivation (12). Interestingly, this event coincides with activation of Ras and phosphorylation of eIF4E, which suggests that α6β4 could stimulate translation in an mTOR-independent manner (12). Considering that Mnk is a downstream effector of Ras and mediates eIF4E phosphorylation (25), we hypothesized that α6β4-dependent Ras activation leads to the
stimulation of Mnk1 activity and, as a consequence, to changes in cancer cell behavior via eIF4E.

We examined the relationship between α6β4 expression and Mnk1 activity. MDA-MB-435 cell lines were stably transfected with either a vector expressing β4-integrin (β4 cells) or an empty vector (mock cells). MDA-MB-435 cells do not express endogenous β4-integrin but do express the α6-integrin subunit that can pair with β4 (31). We examined the level of both Mnk1 and eIF4E phosphorylation in mock and β4 cells grown in media containing 0.5% FBS in the presence of 0.1% DMSO or 10 μM Mnk inhibitor CGP57380 (Fig. 1A). The expression of β4 integrin increases the steady state phosphorylation of Mnk1 at Thr-197/202 by 9-fold, which is an indicator of Mnk1 kinase activity (34). In agreement with a previous report (12), phosphorylation of eIF4E at S209 is also increased as a function of α6β4 expression by 2-fold. Moreover, the increased level of P-eIF4E in β4 cells correlates with high levels of P-Mnk1 in these cells (Fig. 1A). Treatment with the Mnk inhibitor CGP57380 effectively abolishes the phosphorylation of eIF4E in both β4 and mock cells (Fig. 1A). CGP57380 occupies an ATP-binding pocket of the Mnk1 kinase domain and thus affects kinase activity without affecting of Mnk1 phosphorylation. This explains why we do not observe a significant change in P-Mnk1 levels upon addition of CGP57380 to either cell line (Fig. 1A). The data suggest that under low-serum conditions, α6β4 stimulates Mnk1 activity and promotes the phosphorylation of eIF4E.

To further test the role of Mnk1 in α6β4-dependent eIF4E phosphorylation, we knocked down the levels of Mnk1 by stably expressing shRNA in β4 cells. As shown in Fig. 1B, shRNA against Mnk1 effectively reduces the levels of both phosphorylated and
MEK and p38 MAPK Mediate α6β4-dependent Mnk1 and eIF4E Phosphorylation

To define the signaling mechanism by which α6β4 stimulates phosphorylation of Mnk1 and eIF4E, we screened a number of pharmacologic inhibitors. This provided evidence that the MEK/p38MAPK pathway mediates α6β4-dependent Mnk1 and eIF4E phosphorylation. Inhibition of MEK by UO126 and p38 MAPK by SB253508 (Fig. 2A) effectively blocks phosphorylation of both Mnk1 and eIF4E. The result is consistent with previous report that MAP kinases activate Mnk1 (14). MEK inhibition also reduces p38 MAPK phosphorylation (Fig. 2A), indicating that p38 MAPK is downstream of MEK. Inhibition of MEK and p38 MAPK has no effect on the total protein levels of Mnk1 and eIF4E (Fig. 2A). We confirmed the role of MAP kinases in α6β4 signaling leading to Mnk1 phosphorylation by monitoring the phosphorylation levels of p38 MAPK at T197/202 and ERK 1/2 at T202/Y204 in mock and β4 cells (Fig. 2B). Expression of β4 integrin increases the phospho-levels of p38 MAPK but not Erk1/2 (Fig. 2B), suggesting that p38 MAPK is the major downstream effector of α6β4–mediated Mnk1 phosphorylation.

Inhibition of Mnk by CGP57380 Blocks α6β4-induced Proliferation

Based on previous reports that expression of β4 integrin in MDA-MB-435 cells enhances their malignant behaviors (8, 35, 36), we assessed the impact of Mnk inhibition by CGP57380 on the proliferation of MDA-MB-435 mock and β4 cells (Fig. 3A).
expression of β4 integrin increases cell proliferation roughly 1.8-fold over mock cells (Fig. 3A). This α6β4-dependent proliferation is effectively blocked by CGP57380, bringing the proliferation rate to the level of mock cells (Fig. 3A). To confirm the role of Mnk1 in α6β4-dependent proliferation, we compared β4 cells that stably express shRNA against either GFP (control) or Mnk1. As expected, depletion of Mnk1 by shRNA also blocks α6β4–dependent proliferation by day 5 (Fig. 3B).

**Inhibition of Mnk Blocks α6β4-dependent Translation Initiation of VEGF mRNA**

To investigate whether Mnk1 regulates α6β4–dependent translation initiation, we performed ultracentrifugal analysis of polysomes from mock and β4 cells grown under low serum conditions in the presence of either 0.1% DMSO or 10 μM CGP57380. Efficiently translated mRNAs are found in heavy polysomes that sediment faster (Fig. 4A, area H) compared to inefficiently translated mRNA, which are found in light polysomes and sediment slower (Fig. 4A, area L) (37). In agreement with previous data (8), expression of β4 integrin increases the pool of heavy polysomes (H) (Fig. 4A vs 4C). Incubation of cells with CGP57380 reduces the amount of heavy polysomes more dramatically in β4 cells (Fig. 4D vs 4C) than in mock cells (Fig. 4B vs 4A), suggesting that Mnk inhibition selectively affects α6β4-dependent translation. We also analyzed the polysomal profiles of β4 cells that stably express shRNA against either GFP or Mnk1. The profile for of β4 cells expressing the GFP shRNA (Fig. 4E) is similar to that of uninfected β4 cells (Fig. 4C), indicating that shRNA against GFP expression does not alter translation. By contrast, expression of shRNA against Mnk1 in β4 cells reduces the
pool of polysomal RNA (Fig. 4F vs 4E), confirming the role of Mnk1 in α6β4-dependent translation.

Finally, we assessed the effect of Mnk1 inhibition on α6β4-dependent translation initiation of VEGF mRNA (Fig. 5). We used GAPDH mRNA as a control in this assay. We extracted RNAs from the combined sucrose gradient fractions 1 through 7 (L) and 8 through 12 (H), and then analyzed them by qRT-PCR. The polysomal distribution of GAPDH mRNA is not altered by β4 integrin expression or by inhibition of Mnk1 or p38 MAPK (H is 60-65% for all conditions in Fig. 5A). This result suggests that the p38 MAPK/Mnk1 pathway does not affect general translation of housekeeping mRNAs. By contrast, VEGF mRNA shifts from L to H as a function of β4 integrin expression (Fig. 5B; H is 39% in mock cells and 54% in β4 cells), which is consistent with a previous report (8). This polysomal shift of VEGF mRNA is blocked when Mnk1 is inhibited by GCP57380 (H is 31%) or shRNA (H is 40%), or when p38 MAPK is inhibited by SB253508 (H is 42%). Importantly, Mnk1 inhibition has no impact on the polysomal distribution of VEGF mRNA in mock cells, indicating that Mnk1 inhibition selectively affects only α6β4-dependent translation initiation of VEGF mRNA.

Discussion

Translational control has emerged as an important aspect of cancer progression, but the mechanism by which aggressive cancer cells maintain efficient translation of cancer-related mRNAs is still unclear. A previous study demonstrated that integrin α6β4 increases the activity of eIF4E and the consequent translation of survival-factor mRNAs in carcinoma cells (8). Inhibition of the Akt/mTOR pathway only partially blocked α6β4-
dependent eIF4E activity and translation initiation, suggesting additional pathways by which this integrin regulates eIF4E activity (8). In the current study, we present a different mechanism by which α6β4 may regulate eIF4E activity, the regulation of Mnk activity. We provide evidence that Mnk inhibition by CGP57380 or shRNA blocks α6β4-dependent proliferation of MDA-MB-435 cells. We further demonstrate that Mnk mediates α6β4-dependent eIF4E phosphorylation and translation initiation of a cancer-related mRNA. This is the first demonstration, to our knowledge, of a functional connection between integrin and Mnk.

While it is thought that Mnk regulates eIF4E activity via its phosphorylation at S209, the role of phosphorylation of eIF4E in translational control remains to be determined. Translational upregulation of ErbB2 mRNA by α6β4 is correlated not only with the level of eIF4E but also with its phosphorylation status (12). Other studies indicate that translation of Mcl-1 mRNA (24), HIF1α mRNA (25), and cyclin D1 mRNA (38, 39) require either a high level of eIF4E or activation of Mnk. Since all these mRNAs have long structured 5'-UTRs and require high levels of eIF4E for translation, it was suggested that activation of Mnk leading to eIF4E phosphorylation promotes cap-dependent translation. It is now clear that mTOR and Mnk are two major kinases that mediate α6β4-dependent eIF4E regulation and subsequent translation of cancer-related mRNAs. While the PI3K/Akt pathway has been shown to mediate α6β4-dependent mTOR activation (40), the mechanism by which α6β4 activates Mnk seems to involve the Ras pathway, based on its requirement for eIF4E phosphorylation (12). We have identified the MEK/p38 MAPK pathway as a mediator that links α6β4-dependent Mnk and eIF4E phosphorylation (Fig. 2). The relative contribution of mTOR and Mnk
pathways in α6β4-dependent translational control is currently unknown. We speculate that actions of these two pathways are independent, since mTOR increases the availability of functional eIF4E via phosphorylation and inactivation of 4E-BP1 whereas Mnk directly phosphorylates eIF4E. Therefore, a dual approach of targeting both mTOR and Mnk might be more effective therapy for attacking cancer cells that overexpress α6β4.

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Figure Legends

FIGURE 1. β4 integrin expression in MDA-MB-435 cells increases phosphorylation of Mnk and eIF4E. (A) The lysates from mock and β4 cells grown under low serum conditions and 0.1% DMSO (−) or 10 μM CGP57380 (+) overnight were analyzed by immunoblotting with the indicated antibodies. Results are representative of three independent experiments. Densitometric analysis was performed to measure the relative intensity of the bands from immunoblotting analysis and is presented in arbitrary units (right). Statistical analysis was done using the Student’s t-test. P<0.01(∗) comparing to the control (mock cells) (B) β4 cells were infected with a lentivirus that stably expresses either GFP or Mnk1 shRNA. The lysates from β4 cells expressing shRNA against GFP or
Mnk1 grown under low serum conditions were analyzed as in (A). Results are representative of three independent experiments. Densitometric analysis was performed as in (A). Statistical analysis was done using the Student’s t-test. P<0.01(*) comparing to the control (GFP shRNA cells).

**FIGURE 2.** MEK and p38 MAPK mediate α6β4-dependent phosphorylation of Mnk and eIF4E. (A) β4 cells were grown under low serum conditions and incubated with 0.1% DMSO (0) or the indicated concentrations of either UO126 or SB253508 overnight. The lysates from these cells were analyzed as in Fig. 1. Results are representative of three independent experiments. (B) The lysates from mock and β4 cells grown under low serum conditions were analyzed as in Fig. 1. Results are representative of three independent experiments. Densitometric analysis was performed as in Fig. 1. Statistical analysis was done using the Student’s t-test. P<0.01(*) comparing to the control (mock cells)

**FIGURE 3.** CGP57380 blocks α6β4-dependent MDA-MB-435 cell proliferation. (A) Serum-starved mock and β4 cells were grown in the presence of 0.1% DMSO or 10 μM CGP57380 for the indicated periods of time. The total number of cells was counted on days 2, 3, and 5 (mean ± SEM, three experiments). The statistical analysis was done using the Student’s t-test. P<0.01(*) compared with the results of the control (DMSO Mock cells). (B) β4 cells expressing shRNA against either GFP or Mnk1 were monitored for proliferation as described above. The statistical analysis was done using the Student’s t-test. P<0.01(*) compared with the results of the control (day1).
FIGURE 4. Inhibition of Mnk1 affects α6β4 integrin-dependent translation. Mock (A, B) and β4 (C, D) cells were grown in low serum conditions overnight in the presence of 0.1% DMSO (A, C) or 10 μM CGP57380 (B, D). (E) and (F), β4 cells expressing the shRNA against GFP or Mnk1, respectively. The percent of RNA in untranslated complexes, monosomes and light polysomes (L, fractions 1-7) and in heavy polysomes (H, fractions 8-12) is shown. Numbers under (A) are fractions numbers. Results are representative of four independent experiments for (A) through (D) and two independent experiments for (E) and (F).

FIGURE 5. Mnk inhibition affects the initiation step of translation of VEGF but not GAPDH mRNAs in β4 cells. The relative amount of mRNA purified from pools L and H from sucrose density gradients in Fig. 4 was analyzed using qRT-PCR with specific primers for GAPDH (A) or VEGF (B). Solid bars, percentage of specific mRNA in L fractions compared to the total amount of this mRNA. Open bars, percentage of specific mRNA in heavy polysomes. Mean ± SEM, three experiments for mock and β4 cells grown in the presence of DMSO or CGP57380 and two independent experiments for β4 cells grown in the presence of SB253508 or expressing GFP or Mnk1 shRNA. The statistical analysis was done using the Student’s t-test. P<0.01(*). The effect of CGP57380 and SB253508 was compared to the effect of DMSO in β4 cells. The effect of expression of shRNA against Mnk1 was compared to that of shRNA against GFP in β4 cells. CGP, CGP57380. SB, SB253508.
References


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ERK and involves phosphorylation of tuberous sclerosis complex 2 (TSC2).


Figure 1

A.

- P-eIF4E (S209)
- CGP
- Mock
- β4

- P-Mnk1 (T197/202)
- CGP
- Mock
- β4
Figure 1

B.

- shRNA:
  - Actin
  - Mnk1
  - P-Mnk1 (T197/202)
  - elf4E
  - P-elf4E (S209)

- Expression level (10^-3):
  - 0
  - 20
  - 40
  - 60
  - 80

- GFP
- Mnk1

- β4
Figure 2

A.

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β4
Figure 3

A.

Cell numbers (10^5)

- DMSO
- CGP
- DMSO (Mock)
- CGP (β4)

B.

Cell numbers (10^5)

- GFP shRNA
- Mnk-1 shRNA

- day 1
- day 3
- day 5

β4
Figure 5

A.

GAPDH mRNA (%)

DMSO  CGP  Mock

DMSO  CGP  SB  β4

GFP  Mnk1  β4+shRNA
Figure 5

VEGF mRNA (%) vs. Treatment Conditions

- Mock
- CGP
- DMSO
- β4
- SB
- β4+shRNA
- Mnk1

* indicates statistical significance.
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