Extracellular Signal-regulated Protein Kinase/Jun Kinase Cross-talk Underlies Vascular Endothelial Cell Growth Factor-induced Endothelial Cell Proliferation

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Ligand binding to vascular endothelial cell growth factor (VEGF) receptors activates the mitogen-activated protein kinases extracellular signal-regulated kinase (ERK) and c-Jun N-terminal protein kinase (JNK). Possible cross-communication of ERK and JNK effecting endothelial cell (EC) actions of VEGF is poorly understood. Incubation of EC with PD 98059, a specific mitogen-activated protein kinase kinase inhibitor, or transfection with Y185F, a dominant negative ERK2, strongly inhibited VEGF-activated JNK. JNK was also activated by ERK2 expression in the absence of VEGF, inhibited 82% by co-transfection with dominant negative SEK1, indicating upstream activation of JNK by ERK. VEGF-stimulated JNK activity was also reversed by dominant negative SEK1. Other EC growth factors exhibited similar cross-activation of JNK through ERK. VEGF stimulated the nuclear incorporation of thymidine, reversed 89% by PD 98059 and 72% by Y185F. Dominant negative SEK1 or JNK-1 also significantly reduced VEGF-stimulated thymidine incorporation. Expression of wild type Jip-1, which prevents JNK nuclear translocation, inhibited VEGF-induced EC proliferation by 75%. VEGF stimulated both cyclin D1 synthesis and Cdk4 kinase activity, inhibited by PD 98059 and dominant negative JNK-1. Important events for VEGF-induced G1/S progression and cell proliferation are enhanced through a novel ERK to JNK cross-activation and subsequent JNK action.

VEGF is an important vascular permeability and EC proliferation/survival factor, stimulating many of the steps leading to angiogenesis (1, 2). VEGF binds and signals through several transmembrane tyrosine kinase receptors, including Flk-1 (KDR) and Flt-1 (3). Flk-1 is probably necessary for VEGF-induced EC proliferation, but signaling through Flt-1 also contributes to biological actions of VEGF on both EC and non-endothelial cells (4). The importance of VEGF-induced signaling is demonstrated in that genetic inactivation of either receptor leads to a complete lack of development of blood vessels in the embryo, and inactivation of Flk-1 function dramatically impairs the growth of cancer cells in vivo (5–7).

The precise mechanisms of VEGF action are incompletely understood, but both receptors phosphorylate and activate membrane-associated kinases, such as Src and phosphatidylinositol 3-kinase (8, 9). KDR activation assembles Shc-Grb2-Nck complexes, transmitting activating signals to the MAP kinase ERK through incompletely defined pathways (10). Recently, it has been shown that VEGF can stimulate ERK-dependent proliferation in cells transfected to express KDR (10). VEGF signaling to the c-Jun mitogen-activated protein kinase (JNK) has also been demonstrated (11). However, cell biologic functions of VEGF or other growth factors, resulting from interactions between members of the MAP kinase family remain largely undefined.

To delineate the role of the MAP kinases in VEGF function, we assessed the activation of ERK and JNK in primary cultures of bovine aortic EC. We found that VEGF-induced ERK was necessary and sufficient for rapid JNK activation and that both MAP kinases mediated the cell proliferation effects of VEGF, although JNK was the more important final effector. We addressed the mechanism of this communication and determined novel targets for MAP kinase cross-talk, essential to the angiogenesis-promoting actions of VEGF.

EXPERIMENTAL PROCEDURES

Materials—Antibodies and substrate for kinase activity or antibodies to cyclin D1 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). PD 98059 was a generous gift from Dr. Alan Saltiel (Parke-Davis). Growth factors were from Calbiochem. or Sigma. LipofectAMINE was from Life Technologies, Inc.

Cell Preparation—Primary cultures of bovine aortic EC were prepared as described previously and were used in passages 4 and 5, since passaging greatly increased the transfection efficiency of these cells (12, 13).

Kinase Activity Assays—For ERK and JNK activity assays, the cells were synchronized in serum and growth factor-free medium for 24 h and then incubated with 10 ng/ml VEGF for up to 30 min or preincubated with the MEK inhibitor, PD 98059 (14) (10 μM) for 10 or 15 min, respectively. In other studies, Ang II (100 nM), thrombin (0.5 unit/ml), bFGF (1 ng/ml), or platelet-derived growth factor (5 ng/ml) was added to the cell incubation medium for 10 min. The cells were lysed, and lysates was immunoprecipitated with protein A-Sepharose conjugated to anti-serum for ERK or JNK. Immunoprecipitated kinases were washed and then added to the protein myelin basic protein (MBP) for ERK or GST-c-Jun (1–79) (for JNK) for in vitro kinase assays, as described previously (12, 15). This was followed by SDS-PAGE separation and autoradiography/densitometry.

For CdK activity, EC were cultured with VEGFs for 8 h as described previously (16). Cell lysate was added to protein A-Sepharose-conjugated CdK4 antibody and then added to in vitro kinase activity tubes containing GST-pRb as substrate. Samples from each condition were...
assayed for Cdk4 protein by immunoblot. For JNK kinase activity, EC were incubated with VEGF for 10 min, and then the cell lysate was immunoprecipitated with ERK or SEK-1 antibodies conjugated to agarose beads. The precipitate was incubated in kinase buffer containing the fusion protein, mouse-specific antibody-JNK (New England Biolabs, Beverly, MA), as substrate. All experiments were performed two or three times.

Transient Transfections—Bovine aortic endothelial cell (passage 4–5) were grown to 40–50% confluence and then transiently transfected with 1.5–10 μg of fusion plasmids depending on the plate size and amount of cells. Plasmids included dominant negative SEK-1 (pcDNA3-3HA-SEK1-AL-HA) (17), wild type JNK-1 (pcDNA3Flag-JNK-1), or dominant negative JNK-1 (pcDNA3 Flag-JNK-1 APF) (18), dominant negative ERK2 (Y185F-Erk2) or wild type ERK2 (pCMV5Erf2) (19), wild type Jip-1 (Flag-JBD-Jip-1) (20) or control plasmids pCMV5 and pcDNA3, and transfection was carried out using LipofectAMINE. Cells were incubated with liposome-DNA complexes at 37 °C for 5 h, followed by overnight recovery in DMEM containing 10% fetal bovine serum. Then, prior to experimental treatment, cells were synchronized for 24 h in serum-free DMEM and then treated with VEGF.

Cell Proliferation—Nuclear thymidine incorporation was carried out as described previously (15). Subconfluent, nontransfected EC were synchronized in serum-free medium for 24 h and then incubated for 20 h in the absence or presence of VEGF 15 ng/ml, sometimes with 10 μM MEK, with or without the addition of 0.5 μCi of [3H]thymidine for 4 h, cells were washed, incubated with 10% trichloroacetic acid to precipitate nuclear thymidine, and lysed with 0.2 N NaOH and neutralized with 0.2 N HCl; the lysates were counted in a β-counter. Transfected cells were recovered and then synchronized in the absence of serum for 24 h, followed by treatment as indicated above. Data were analyzed by ANOVA plus Schefé's test.

RESULTS

VEGF Activates ERK and JNK Activity—We first examined the time course of VEGF stimulation of ERK and JNK activity. In cultured bovine aortic endothelial cell, VEGF-induced ERK activity was stimulated earlier (by 5 min) and more strongly (4–versus 2.5-fold), compared with VEGF-induced JNK activity (Fig. 1). The moderate, yet consistent and significant, activation of JNK by VEGF is comparable with that seen with some other growth factors (22), and we used a relatively low concentration of VEGF in these studies. Stress factors or cytokines produce strong increases of JNK activity and stimulate cell apoptosis (23–25), whereas growth factors are generally anti-apoptotic. The earlier activation of ERK that we observed in these studies suggested that this proline-directed serine/threonine kinase may activate JNK in this setting.

Relationship of ERK to JNK Activation by VEGF—We then examined the role of ERK in VEGF-activated JNK by several approaches. VEGF activation of JNK activity was almost completely reversed by preincubating the cells with PD 98059, a soluble inhibitor of MEK-1, which is a consensus direct activator of ERK (14) (Fig. 2A). To confirm this, we transfected the bovine aortic endothelial cell with a dominant negative ERK2, Y185F, and this also prevented the activation of ERK and JNK in response to VEGF, by 72 and 71%, respectively (Fig. 2B). We have previously shown that in primary cultures of vascular cells, expression of Y185F and co-transfected pGreen Lantern (Life Technologies, Inc.) was approximately 78% efficient (15). These results indicate that ERK activation by VEGF was importantly linked to the activation of JNK in EC.

It has been previously shown that MEK cannot directly activate JNK (26). Therefore, to further support ERK stimulation of JNK activity, EC were transfected with an ERK2 expression vector, pCMV5Erf2 (19, 27). In the absence of VEGF, ERK2 expression increased both ERK and JNK activity (Fig. 2B), further supporting ERK as the kinase that underlies VEGF-stimulated JNK.

ERK Activation of JNK Is Indirect—We then determined how ERK might activate JNK in this setting. One possibility is that ERK could directly phosphorylate and thus activate JNK, but the consensus ERK core target sequence, PXS/TP (28), is not present in JNK-1. Alternatively, ERK might enact an upstream signal mechanism leading to JNK activation. To test these ideas, ERK was immunoprecipitated from VEGF-incubated cells and was found to be active against the substrate MBP (Fig. 3A, lane 1 versus lane 2) but was not capable of directly phosphorylating the exogenous fusion protein, maltose-binding JNK protein (lane 3). In contrast, SEK-1, which was immunoprecipitated from VEGF-incubated EC, could phosphorylate JNK (lane 4 versus lane 5). This suggested that VEGF-stimulated ERK might activate SEK or another JNK kinase, since ERK was necessary for JNK activation in this setting.

To support this idea, EC were transfected with a dominant negative SEK-1, (pcDNA3-3HA-SEK1-AL-HA), and the ability of VEGF to stimulate JNK activity in this setting was reversed by 64% (Fig. 3B, lanes 1, 2, and 5). When dominant negative SEK-1 was co-transfected with pCMV5Erf2 in the absence of VEGF, ERK activation of JNK activity was inhibited by 82% (Fig. 3B, lanes 6 and 7). In parallel, PD 98059 prevented the VEGF-stimulation of JNK in the control vector-transfected cells by 73%. These results confirm that ERK activity is required for VEGF-induced JNK activation. This occurs when ERK activates JNK kinase(s), particularly SEK-1, through upstream signaling to this JNK kinase.

Other EC Growth Factor MAP Kinase Cross-activation—Although VEGF is the most specific EC growth factor, other growth factors such as bFGF have been implicated in angiogenesis (29). Also, tyrosine kinase as well as G protein-linked

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**Fig. 1. Temporal activation of ERK (●) and JNK activity (○) in response to VEGF.** EC were synchronized for 24 h in growth factor-deficient medium and then incubated with VEGF10 ng/ml over 30 min. Each kinase activity was immunoprecipitated, and equal aliquots of protein from each condition were used for an in vitro kinase tube assay, using MBP (ERK) or GST-c-Jun(1–79) (JNK) as substrates. Both kinase studies were repeated three times. Control EC levels of the MAP kinases did not change over the 30 min (data not shown).
vascular growth factor receptors stimulate ERK and JNK (29, 30). To determine whether similar cross-talk might underlie the activation of JNK for these proteins, EC were incubated with angiotensin II or thrombin (G protein-coupled receptors), platelet-derived growth factor, or bFGF (tyrosine kinase receptors). Each growth factor significantly stimulated both ERK (data not shown) and JNK activities (Fig. 4A), in part confirming previous studies (30, 31). Preincubation with PD 98059 inhibited the activation of JNK by each growth factor, ranging from 72 to 98% (Fig. 4A). In contrast, Y185F did not inhibit UV activation of JNK (Fig. 4B), and UV-activated JNK was also not affected by PD 98059 (data not shown). Thus, ERK participates in JNK activation in response to many EC growth factors that utilize both tyrosine kinase and G protein-linked
signal transduction pathways. However, ERK does not appear to participate in stress-activated JNK.

**Role of ERK in VEGF-induced Cell Proliferation**—We then determined if the activation of ERK was necessary for VEGF-induced EC proliferation. When nontransfected EC were preincubated with the soluble MEK inhibitor, PD 98059, VEGF-stimulated EC nuclear thymidine incorporation was reduced 89% (Table I). In EC transfected with the dominant negative ERK2 construct (Y185F), VEGF-induced thymidine incorporation was reduced 72%. Our results are consistent with the greater inhibition of ERK activity by the soluble MEK inhibitor compared with Y185F in this setting and indicate that ERK action is necessary and sufficient for VEGF-induced EC proliferation.

**Role of JNK in VEGF-induced Cell Proliferation**—Since VEGF-stimulated ERK leads to increased JNK activity, we postulated that JNK mediates some of the effects of ERK for EC proliferation. To determine this, EC were transfected with dominant negative SEK-1 or dominant negative JNK-1 constructs and then incubated with VEGF (Table II). In this setting, VEGF-stimulated thymidine incorporation was reduced 54 and 71%, respectively. SEK-1 is one of several immediate upstream kinases that activate JNK, and hence signaling from unaffected molecules, such as JNK kinase-2 (32), might account for the lesser reversal of VEGF-induced proliferation by the dominant negative SEK-1, compared with JNK-1 APF. Also, SEK-1 activates p38 MAP kinase (33), and alteration of p38 signaling by dominant negative SEK-1 might affect ERK-JNK cross-talk.

We also transfected the cells with Flag-JBD(Jip-1), an interacting protein that causes cytoplasmic retention of JNK and inhibits JNK-mediated transcription (20). Expression of Jip-1 reduced VEGF-induced EC proliferation by 75%. Thus, ERK activation is necessary for VEGF-induced EC proliferation, but activation of JNK by ERK and subsequent action of nuclear translocated JNK substantially accounts for ERK effects.

To further support this interactive role, we transfected EC with wild-type ERK2 or wild type JNK-1 in the absence of VEGF, each caused a 52% increase in thymidine incorporation (Table II). Thus, either MAP kinase can stimulate EC DNA synthesis in the absence of growth factor. We previously showed that ERK2 expression in the absence of growth factor is transcriptionally active (27). When ERK-transfected EC were co-transfected with dominant negatives SEK-1 or JNK-1, the proliferative effects were reduced 71 and 94%, respectively.

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**TABLE I**

The role of ERK in VEGF-induced EC proliferation

Nontransfected or transfected cells were synchronized for 24 h in serum-free media. EC were then incubated for 20 h in the absence or presence of VEGF. In some conditions, PD 98059 was added to the incubation mixture of nontransfected cells 1 h prior to the mitogen. VEGF incubation was followed by the addition of 0.5 μCi of [3H]thymidine for 4 h. Cells were then washed, incubated for 10 min with 10% trichloroacetic acid at 4 °C to precipitate the nuclear incorporated thymidine, washed two additional times, and lysed with 0.2 N NaOH overnight, and the lysates were counted in a β-counter. Data are combined from two or three experiments, each condition in duplicate for each experiment.

| Nuclear thymidine incorporation | cpm × 10^3 |
|--------------------------------|------------|
| Control                        | 55 ± 1.2   |
| PD 98059 (10 μM)               | 56 ± 1.8   |
| VEGF (15 ng/ml)                | 119 ± 3.3* |
| VEGF + PD                      | 62 ± 2.6*  |
| pCMV5 (control)                | 29 ± 1     |
| Y185F                          | 28.1 ± 1.2 |
| pCMV5 + VEGF                   | 58 ± 2.1*  |
| Y185F + VEGF                   | 37 ± 1.3*  |

* p < 0.05 for control versus VEGF by ANOVA plus Schefe’s test.

**FIG. 4.** A, activation of JNK by bFGF, angiotensin II (Ang), platelet-derived growth factor (PDGF), and thrombin depends on ERK activation. EC were incubated with each growth factor for 15 min in the absence or presence of PD 98059. JNK activation by each was prevented by the MEK inhibitor. B, UV-activated JNK is not dependent on ERK activation. Nontransfected and Y185F-transfected EC were subjected to 1 min of UV irradiation (20 J/s), followed by a 15-min incubation, and then JNK activity was determined.
or VEGF, inhibited 75% by the soluble MEK inhibitor or 68% by the dominant negative JNK-1 (Fig. 6). ERK2 expression in the absence of growth factor stimulated cyclin D1 synthesis, and this was almost completely inhibited by dominant negative JNK-1. A representative experiment is shown, while the bar graph represents three experiments combined.

**TABLE II**

| Target | Cyclin D1 (pixels) |
|--------|-------------------|
| Cont   | 3.0 ± 0.2         |
| VEGF+  | 2.5 ± 0.1         |
| PD     | 2.0 ± 0.1         |
| pcDNA3 | 1.5 ± 0.1         |
| +VEGF  | 1.0 ± 0.1         |
| +pcDNA3 | 0.5 ± 0.1     |

**FIG. 5.** VEGF-stimulated cyclin D1 synthesis. Transfected or non-transfected EC were synchronized for 24 h and then incubated with 250 μCi of [3H]thymidine in the presence or absence of VEGF 10 ng/ml for 16 h. Cell lysate protein was immunoprecipitated for cyclin D1 (antibody from Santa Cruz Biotechnology), solubilized, and denatured in SDS-reducing buffer and electrophoretically resolved by PAGE. VEGF-stimulated cyclin D1 synthesis (lanes 2 and 6) was prevented by PD 98059 (lane 3) or by dominant negative JNK-1 (lane 7). ERK-stimulated synthesis (lane 9) was almost completely inhibited by dominant negative JNK-1 (lane 10). A representative experiment is shown, while the bar graph represents three experiments combined.

**Discussion**

We have examined the signal transduction enacted by VEGF that may contribute to its angiogenic role (1, 2), specifically to the stimulation of EC proliferation. This is a relatively early and important step in the process of new blood vessel formation, and the essential contributions of the VEGF receptors Flk (KDR) and Flt-1 to this multistep process have been demonstrated through embryonic gene inactivation studies (5–7). We report here that VEGF stimulates a potent and rapid increase in ERK activation that precedes the still rapid onset of increased JNK activity. We further showed that VEGF-induced ERK is required for JNK activation and that this occurs through ERK activating JNK kinases, including SEK-1. The importance of this MAP kinase cross-activation was demonstrated in that both ERK and JNK are required for VEGF-induced EC proliferation. The general importance of ERK in this setting has been established recently (10). Unanticipated was our finding that JNK is the final mediator for ERK to stimulate cell proliferation, underlying the response to VEGF; i.e., JNK, not ERK, action appears directly responsible for the majority of VEGF-induced proliferation. The role of ERK is mainly to induce the activation of JNK when activated by an EC growth factor such as VEGF. Our identified role of JNK and the importance of ERK/JNK cross-activation is specifically seen for the stimulation of important G1 cell cycle events that lead to progression to S phase (DNA synthesis).

The rapid cross-activation of JNK by ERK in the setting of growth factor stimulation of EC is a novel, but perhaps cell context-specific finding. Limited reported investigations in transfected cell lines and occasional primary cells have not correlated or shown ERK-induced activation of JNK (reviewed in Refs. 38 and 39). It is appreciated that ERK often mediates a transmembrane signal to the transcription of an autologous growth factor, which is then secreted, binds cell membrane receptors, and enacts autocrine JNK activation. However, this process requires several hours, and indirectly involves ERK activation of JNK. The JNK pathway was initially proposed to mediate cytokine and stress pathway-induced apoptosis (24,
A lysate complex was then incubated for 30 min at 30 °C with 40 μM without VEGF for 8 h. Cell lysate was added to Cdk4 antiserum and nontransfected EC were synchronized and then incubated with or without VEGF, followed by addition of p38 MAP kinase activity in EC, while both stimulated JNK activity. This differential effect on p38 probably contributes to VEGF-stimulated production of cyclin D1 and cell cycle progression, since this MAP kinase inhibits cyclin D1 transcription. Furthermore, in our model, activation of Cdk4 by ERK/JNK occurs earlier than cyclin D1 synthesis and perhaps occurs directly, since Cdk4 contains a minimal consensus phosphorylation site for these MAP kinases. This would create recognition sites for proteins such as Pin1, further modifying the structure and hence the activity of Cdk4.

40), where ERK was often not induced. Consistent with this idea, we found that inhibition of ERK had no effect on UV irradiation-induced JNK activity in the EC.

It has been more recently appreciated that various growth factor receptors stimulate both ERK and JNK (41–44), leading to the proposal that both may contribute to cell proliferation. ERK is essential for induced cell division (45, 46), and although the role of JNK is less well defined, c-Jun is necessary for fibroblast proliferation, perhaps as one substrate for Ras and JNK-related signaling (47–49). Here, we found that many EC growth factors, both of the tyrosine kinase and G protein-coupled varieties, activate JNK through ERK cross-talk. Thus, it is likely that the activation of both MAP kinases is necessary for specific cell biologic actions induced by a variety of EC growth factors. We have recently found that inhibition of ERK or JNK activation stimulated by bFGF also inhibits EC proliferation induced by this growth factor.  

However, the interactive roles for each MAP kinase are not well understood in the context of cell proliferation. Previously, activated Rho GTPases Rac and Cdc42 were found to promote cell cycle progression through G1 and activate JNK (but not ERK). Hence, activation of signaling through the JNK pathway possibly contributes to cell cycle progression in that model (50). This might occur upstream of JNK as well, since Rho-activated PAK1 phosphorylates MEK-1 and probably contributes to growth factor signaling to the nucleus (51). We found that VEGF stimulation of ERK induces JNK activation, which serves as a primary effector for ERK (i.e. increasing cyclin D1 synthesis) but may also augment the ERK signal to substrates such as Elk-1 (52, 53). In other contexts, however, the signals diverge, and ERK opposes JNK-induced apoptosis. This may reflect contributions from additional signaling molecules, activated in cell- or stimulus-specific fashion. In preliminary studies, we found that VEGF inhibited, while UV irradiation activated, p38 MAP kinase activity in EC, while both stimulated JNK activity. This differential effect on p38 probably contributes to VEGF-stimulated production of cyclin D1 and cell cycle progression, since this MAP kinase inhibits cyclin D1 transcription (54). Also, in our model, activation of Cdk4 by ERK/JNK occurs earlier than cyclin D1 synthesis and perhaps occurs directly, since Cdk4 contains a minimal consensus phosphorylation site for these MAP kinases. This would create recognition sites for proteins such as Pin1, further modifying the structure and hence the activity of Cdk4.

The mechanism by which ERK induces JNK is of interest. We showed that ERK cannot directly phosphorylate JNK, but rather VEGF (or ERK in the absence of VEGF) can increase the activity of this kinase via JNK kinase stimulation. This was shown using the dominant negative SEK-1 construct, implicating this kinase in the signal transduction pathway for ERK to JNK. This could occur by dominant negative SEK interfering with upstream kinase phosphorylation/activation of endogenous SEK-1 by members of the MAP kinase kinase or mixed lineage kinase families or various other kinases that have been shown in some cells to activate SEK-1 (reviewed in Ref. 56). This pathway would probably originate from the activation of further upstream signaling molecules, a challenge for additional investigation. Alternatively, the dominant negative SEK-1 could titrate endogenous JNK-1, not allowing access to wild type SEK-1 or other JNK-kinases. The incomplete inhibition of VEGF or ERK-triggered JNK probably reflects incomplete transfection/expression of dominant negative SEK-1, but it also points out that other JNK-kinases such as JNK kinase-2 (32) could contribute to this ERK-initiated pathway to JNK. In addition, the inhibition by dominant negative SEK-1 of p38 activation (32) could modify the ERK-JNK interaction. Based upon expression of wild type Jip-1 in the EC, we conclude that the translocation of activated JNK to the nucleus is critical for the proliferation-enhancing effects of ERK.

Rapid onset and brief ERK expression underlies cell proliferation, while prolonged activation of ERK has been observed during cell differentiation (57). It is likely that cross-talk between members of the MAP kinase family contributes to the decision by a cell to divide or terminally differentiate. For instance, in some cells, JNK stimulates ERK-directed phosphatase synthesis (MKP-1), which could limit the duration and perhaps magnitude of ERK activation (58). However, we found that dominant negative SEK-1, JNK-1, or wild type Jip-1 expression did not affect the magnitude or duration of VEGF stimulation of ERK activity. In the EC, various growth factors were found to enact ERK/JNK cross-activation. Therefore, many of the angiogenesis-inducing effects of VEGF or other vascular growth factors may result from currently unappreciated MAP kinase interactions. It is also possible that other primary cells may demonstrate MAP kinase cross-talk leading to essential cell functions.

In summary, a novel rapid cross-activation of JNK by ERK is

2 A. Pedram, M. Razandi, and E. R. Levin, unpublished observations.
ERK Stimulates JNK Activation

ERK is a key regulator of cell proliferation and survival, and its activation is crucial for the growth and angiogenesis of endothelial cells (ECs). The expression of ERK in ECs is essential for the activation of signaling pathways that control cell proliferation. This activation is mediated by the mitogen-activated protein kinase (MAPK) pathway, which includes the extracellular-signal-regulated kinase (ERK) and the c-Jun NH2-terminal kinase (JNK) pathways. The ERK-JNK signaling pathway is critical for the regulation of cell proliferation and survival, and its dysregulation is associated with various types of cancer.

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