ERK5 and the regulation of endothelial cell function

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Abstract

ERK5 (extracellular-signal-regulated kinase 5), also termed BMK1 [big MAPK1 (mitogen-activated protein kinase 1)], is the most recently discovered member of the MAPK family. It is expressed in a variety of tissues and is activated by a range of growth factors, cytokines and cellular stresses. Targeted deletion of *Erk5* in mice has revealed that the ERK5 signalling cascade is critical for normal cardiovascular development and vascular integrity. *In vitro* studies have revealed that in endothelial cells, ERK5 is required for preventing apoptosis, mediating shear-stress signalling, regulating hypoxia, tumour angiogenesis and cell migration. This review focuses on our current understanding of the role of ERK5 in regulating endothelial cell function.

Introduction

MAPKs (mitogen-activated protein kinases) play an essential role in regulating many cellular processes including growth, differentiation and apoptosis. MAPKs are activated by a range of growth factors and chemical stimuli such as oxidative stress and osmotic imbalance, and are responsible for transducing extracellular signals to the cytoplasm and nucleus. In mammalian cells, the MAPK signalling system consists of four distinct linear signalling cascades terminating in ERK1/2 (extracellular-signal-regulated kinase 1 and 2), JNK1–3 (c-Jun N-terminal kinases 1, 2 and 3), p38 MAPKs (p38 α, β, γ and δ) or the most recently discovered MAPK, ERK5 [1,2]. Each of these terminal kinases phosphorylates a variety of cellular targets ranging from cytoplasmic enzymes to transcription factors [3].

Identification of ERK5

ERK5 was cloned by two independent research groups in 1995. Dixon and co-workers first identified MEK5 and then utilized a yeast two-hybrid assay to identify binding partners, resulting in the discovery of ERK5 [4]. In a separate study, Lee et al. [5] used degenerate PCR to screen a human placenta cDNA library and isolated a novel MAPK, which, due to its relatively large size when compared with ERK1 and ERK2, they termed BMK1 (big MAPK1). It later became apparent that BMK1 and ERK5 were in fact the same protein. In vertebrates, ERK5 is expressed in a variety of tissues, showing high abundance in heart, brain, lung, skeletal muscle, placenta and kidneys [4,5]. ERK5 is also widely expressed in a number of different cell lines [6].

Key words: angiogenesis, big mitogen-activated protein kinase 1 (BMK1), endothelial cell, extracellular-signal-regulated kinase 5 (ERK5), mitogen-activated protein kinase (MAPK), signal transduction.

Abbreviations used: BMK1, big mitogen-activated protein kinase 1; BLMEC, bovine lung microvascular endothelial cell; CREB, cAMP-response-element-binding protein; DUSP, dual-specificity phosphatase; E, embryonic day; ERK5, extracellular-signal-regulated kinase 5; FAK, focal adhesion kinase; FGF-2, fibroblast growth factor-2; HIF-1α, hypoxia-inducible factor-1α; HUVEC, human umbilical vein endothelial cell; KLF2, Krüppel-like factor 2; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; MEF2, myocyte enhancer factor 2; MEK, MAP/ERK kinase; MEKK, MAP/ERK kinase kinase; NLS, nuclear localization signal; rpS6, ribosomal protein S6; RSK, ribosomal S6 kinase; p90^{RSK}, p90 RSK; TNFα, tumour necrosis factor α; VEGF, vascular endothelial growth factor.

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Structure of ERK5

The human *ERK5* gene (also termed *MAPK7*) is present on chromosome 17p11.2 and spans 5.79 kb. It has an open reading frame of 2445 bp encoding a protein of 816 amino acids with a predicted molecular mass of 98 kDa (Figure 1). ERK5 shares 66% sequence homology with ERK1/2 within the kinase domain, which contains the TEY dual phosphorylation motif in the activation loop [4]. The N-terminal domain of ERK5 contains the kinase domain (amino acids 78–406). In addition, it is important for cytoplasmic targeting (amino acids 1–77), interaction with MEK5 (amino acids 78–139) and oligomerization (amino acids 140–406) [7,8]. The large size of ERK5 is attributable to its long C-terminal tail of approx. 400 amino acids, which is unique among the MAPKs. The C-terminal domain contains an NLS (nuclear localization signal) (amino acids 505–539) and two proline-rich domains (amino acids 434–465 and 578–701) that are proposed to serve as binding sites for SH3 (Src homology 3)-domain-containing proteins [4,8]. The C-terminal region also contains a MEF2 (myocyte enhancer factor 2)-interacting region (amino acids 440–501) and a transcriptional activation domain (amino acids 664–789) that regulates MEF2 transcription factor activity [7]. Truncation of the C-terminal tail results in increased ERK5 kinase activity, revealing that the C-terminal tail of ERK5 has an autoinhibitory function [6].

Activation of the ERK5 signalling axis

ERK5 was originally identified as a stress-activated MAPK, activated by both osmotic and oxidative stresses [1]. Subsequent studies have revealed that it is also activated by serum

Figure 1 | **Structure and function of ERK5**

The ERK5 protein consists of 816 amino acids and contains a kinase domain, NLS and two proline-rich domains. The ERK5 protein also contains a unique and relatively large C-terminal tail of approx. 400 amino acids. ERK5 is activated by phosphorylation on Thr^{218} and Tyr^{220} within the activation loop of the kinase domain by its upstream activator MEK5. ERK5 is then able to phosphorylate a number of intracellular targets.

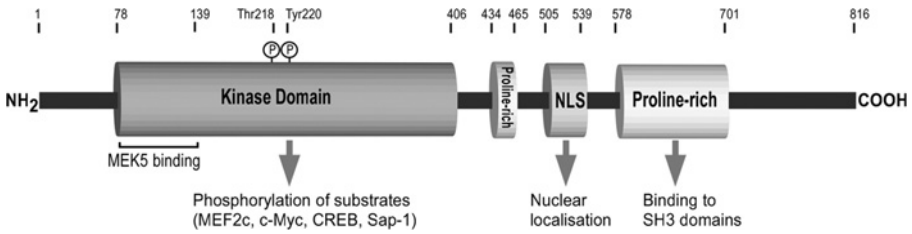
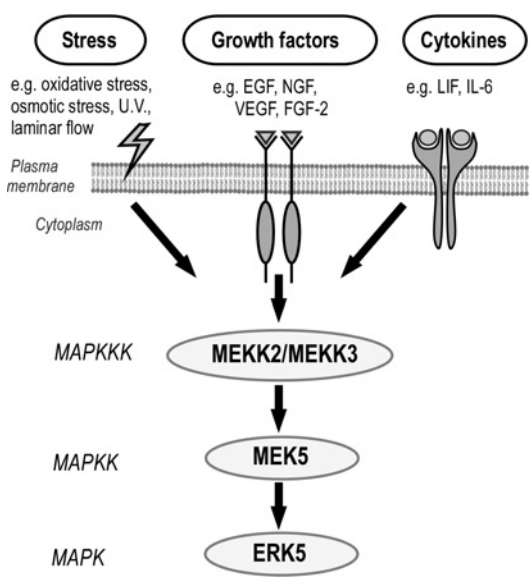


Figure 2 | **The ERK5 signalling axis**

ERK5 is activated by a linear signalling cascade. MEKK2/MEKK3 (*MAPKKK*) phosphorylate MEK5 (*MAPKK*), which in turn phosphorylates ERK5 (*MAPK*). The signalling axis is activated by a range of cellular stresses, growth factors and cytokines.



[9] and a range of growth factors including EGF (epidermal growth factor) [10], FGF-2 (fibroblast growth factor-2) [11] and VEGF (vascular endothelial growth factor) [12] and by cytokines such as LIF (leukaemia inhibitory factor) [13] and IL-6 (interleukin 6) [14] (Figure 2). ERK5 is also activated by a range of physiological and pathological conditions such as fluid shear stress [15], hypoxia [16] and ischaemia [17].

Activation of a MAPK signalling module consists of the initial activation of a MAPKKK (MAPKK kinase), resulting in the sequential activation of MAPKK and ultimately MAPK [18] (Figure 2). MEKK2 (MAP/ERK kinase kinase 2) and MEKK3 phosphorylate MEK5 on Ser^{311} and Thr^{315}, resulting in an increase in MEK5 activity [9,19]. ERK5 contains a dual phosphorylation motif (TEY) in its activation loop and is phosphorylated on Thr^{218}/Tyr^{220} by the upstream kinase MEK5, resulting in an increase in the catalytic activity of ERK5 [4,5,20]. MEK5 preferentially phosphorylates ERK5 on Thr^{218}, which is believed to induce a conformational change facilitating the subsequent phosphorylation of Tyr^{220} leading to full catalytic activity [21]. Active ERK5 is able to undergo autophosphorylation on a number of residues and can also phosphorylate MEK5 [21]. A recent study has identified a number of residues within the C-terminal tail of ERK5, which are autophosphorylated, leading to an enhancement of ERK5 transcriptional activity [22].

Dephosphorylation of MAPKs on the TXY motif by an MKP (MAP kinase phosphatase) subfamily of DUSPs (dual-specificity phosphatases) leads to their inactivation [23]. Currently, no DUSP has been identified that dephosphorylates ERK5. However, ERK5 is dephosphorylated by the phosphotyrosine-specific phosphatase PTP-SL (protein tyrosine phosphatase STEP-like), which interacts with ERK5 and impedes its translocation to the nucleus [24]. ERK5 is also regulated by other post-translational modifications in addition to phosphorylation. It has recently been reported that ERK5 undergoes SUMOylation by SUMO3 (small ubiquitin-related modifier 3) on Lys^{6} and Lys^{22} after treatment with H_2O_2 and AGEs (advanced glycation end-products) in HUVECs (human umbilical vein endothelial cells) [25].

Similar to other MAPKs, ERK5 belongs to a family of evolutionarily conserved proline-directed protein kinases that phosphorylate substrates on serine and threonine residues immediately preceding a proline residue. However, certain serine and threonine autophosphorylation sites in ERK5 are not followed by proline [21,22], suggesting that the specificity of ERK5 may differ from other MAPK family members. Activation of the ERK5 signalling axis stimulates both distinct and similar pathways to the classical ERK1/2 pathway [26]. Downstream targets of ERK5 include the MEF2 transcription factor family members MEF2A, MEF2C and MEF2D [9,27,28]. Other targets include the Ets domain transcription factor Sap1a [29], c-Myc [30] and CREB (cAMP-response-element-binding protein) [31].

Table 1 | The ERK5 signalling axis and associated phenotype in knockout mice

Gene knockout of specific components of the ERK5 signalling axis reveals a range of phenotypes in mice.

Genotype	Phenotype	Reference
Mekk2$^{-/-}$	Mice are viable, develop normally and are fertile	[11]
	Mice are viable and develop normally, but exhibit altered cytokine expression in thymocytes	[63]
	Mice are viable and develop normally, but exhibit reduced cytokine expression in embryonic stem-cell-derived mast cells	[64]
Mekk3$^{-/-}$	Embryonically lethal at E11.0 with severe defects in early angiogenesis. Did not prevent early formation of blood islands, suggesting that vasculogenesis was not affected	[36]
Mek5$^{-/-}$	Embryonically lethal at E10.5 due to defective cardiac development, increased apoptosis and decreased proliferation in the heart, head and dorsal regions	[35]
Erk5$^{-/-}$	Embryonically lethal at E9.5–E10.5 due to defects in normal heart looping, cardiac development, vascular maturation and angiogenesis	[33]
	Embryonically lethal at E10.5–E11.5. Embryos displayed stunted growth, especially in the head and lower trunk with dilated pericardial sacs. Impaired angiogenesis in the embryo and placenta	[16]
	Embryonically lethal at E10.5–E11.0 due to impaired angiogenesis in the embryo and the placenta. Embryonic endothelial cell apoptosis evident	[34]
	Embryonically lethal at E9.5–E10.5 with growth retardation and underdeveloped yolk sac vasculature	[12]
Erk5$^{-/-}$ endothelial cell	Embryonically lethal at E9.5–10.5 due to cardiovascular defects. Identical phenotype to that of global *Erk5*$^{-/-}$ mice	[12]
Erk5$^{-/-}$ cardiomyocyte	Mice are viable and develop normally	[12]
Erk5$^{-/-}$ hepatocyte	Mice are viable and develop normally	[32]
Erk5$^{-/-}$ inducible knockout	Lethality of adult mice within 2 weeks after induced ablation of *Erk5*. Mice display degeneration of the cardiovascular system with endothelial cell apoptosis	[12]
Mef2c$^{-/-}$	Embryonically lethal at E9.5 due to failure of the heart tube to undergo rightward loop morphogenesis leading to right ventricle malformation. Endothelial cells fail to organize normally into a vascular plexus	[65,66]
	Embryonically lethal at E9.5 due to cardiac and vascular malformations.	[67]

Role of ERK5 *in vivo*

To address the physiological role of the ERK5 signalling axis, researchers have utilized gene targeting in mice to ablate specific genes (Table 1; [32]). *Erk5*-deficient mice die at approximately E10.5 (embryonic day 10.5) due to cardiovascular defects and angiogenic failure in embryonic and extraembryonic tissues. In these mice, the developing vasculature fails to mature, with endothelial cells becoming disorganized and rounded, leading to a loss of vascular integrity [16,33,34]. Similar phenotypic abnormalities are seen in mice lacking *Mek5* [35] and *Mekk3* [36], suggesting that the ERK5 signalling axis is critical to vasculogenesis and angiogenesis. In an attempt to determine the primary defect on ERK5 gene ablation, researchers have generated conditional tissue-specific ERK5-knockout mice. Endothelial-specific *Erk5*-knockout mice show cardiovascular defects and die at approx. E10.0, similar to the conventional *Erk5*-knockout mice [12]. However, knockout of *Erk5* specifically in cardiomyocytes does not affect development [12]. These important data suggest that whereas global *Erk5* knockout affects cardiovascular development, the initial defect occurs in the endothelium and that ERK5 is critical for endothelial cell function. The requirement of ERK5 in the maintenance of vascular integrity is highlighted by the fact that induced ablation of *Erk5* in adult mice is lethal within 2–3 weeks as blood vessels become leaky due to endothelial cell apoptosis [12].

ERK5 and endothelial cell physiology

Inhibition of endothelial apoptosis

Targeted deletion of the ERK5 signalling axis in mice suggests that ERK5 plays an essential role in endothelial cell physiology (Table 1). Initial studies using HUVECs stimulated with H_2O_2 showed that ERK5 was a redox-sensitive kinase [1]. Further studies demonstrated that flow-induced shear stress and osmotic stress could stimulate ERK5 activity in BAECs (bovine aortic endothelial cells) [15]. Given the known atheroprotective effects of laminar flow [37], Pi et al. [38] subsequently demonstrated that ERK5 is required for mediating flow-stimulated survival in BLMECs (bovine lung microvascular endothelial cells). This study [38] revealed

that ERK5 induced the phosphorylation and inactivation of the pro-apoptotic protein Bad at Ser^{112} and Ser^{136}, thus sequestering Bad in the cytoplasm and preventing the subsequent activation of caspase 3 and cell death [38]. Bad does not contain a MAPK consensus sequence, suggesting that ERK5 does not phosphorylate Bad directly. Surprisingly, other candidate kinases such as Akt/PKB (protein kinase B), PKA (protein kinase A) and $p90^{RSK}$ (RSK is ribosomal S6 kinase), which are known to phosphorylate Bad, were not responsible for mediating ERK5-induced phosphorylation of Bad in these cells [38]. However, recent results have shown that in murine fibroblasts, ERK5 protects against osmotic stress by inducing Akt phosphorylation on Ser^{473} and Thr^{308}, leading to inactivation of the Foxo3a (forkhead box O3a) transcription factor and down-regulation of FasL expression [39].

Mediation of shear-stress signalling

Fluid shear-stress-mediated ERK5 activation has been shown to confer an atheroprotective effect by negatively-regulating TNFα (tumour necrosis factor α)-stimulated expression of adhesion molecules in endothelial cells [40]. A more recent study utilizing a MEK5 inhibitor has revealed that the MEK5–ERK5 pathway mediates flow-dependent inhibition of TNFα signalling in BLMECs [41]. Analysis of laminar shear-stress-induced transcriptional responses in endothelial cells has identified KLF2 (Krüppel-like factor 2) as a mechano-stress-induced gene [42,43]. KLF2 is responsible for negatively regulating inflammation and angiogenesis and maintaining vascular quiescence [43–45]. KLF2 has subsequently been identified as an ERK5 responsive gene in mouse embryonic fibroblasts in a pathway requiring MEF2 transcription factor [46]. In addition, recent studies have shown that ERK5 is required for flow-induced expression of KLF2 in HUVECs [47], and the subsequent increased cell-surface expression of CD59 [48].

Regulation of hypoxia

ERK5 is activated under hypoxic conditions and has been reported to negatively regulate VEGF expression in mouse embryonic fibroblasts [16]. Furthermore, increased VEGF expression was observed in the $Erk5^{-/-}$ embryos compared with wild-type and $Erk^{+/-}$ mice [16]. VEGF is critical for vasculogenesis and angiogenesis [49]. However, it is unlikely that increased VEGF is the primary defect leading to death in the $Erk5^{-/-}$ mice at E11 [16], as overexpression of *Vegf* in mouse embryos results in normal development up to E12.5, with lethality due to cardiovascular abnormalities only evident at E12.5–14.5 [50]. ERK5 has been shown to regulate HIF-1α (hypoxia-inducible factor-1α) levels by promoting the ubiquitination and subsequent proteolysis of HIF-1α in BLMECs, leading to a decrease in hypoxia-induced VEGF mRNA levels [51]. It has been shown that HIF-1α is directly phosphorylated by ERK1/2, leading to an increase in transcriptional activity [52,53]. It remains to be determined whether HIF-1α is also a direct substrate for phosphorylation by ERK5, possibly antagonizing activation by ERK1/2.

Tumour angiogenesis

Angiogenesis is defined as the formation of new blood vessels from pre-existing vessels and plays a critical role in normal physiological development and in the pathology of diseases such as cancer [54]. Hayashi et al. [55] have provided evidence that ERK5 regulates tumour angiogenesis. After the establishment of melanoma and Lewis lung carcinoma tumour xenografts in mice, induced ablation of *Erk5* in $Erk5^{flox/flox}$ mice carrying an inducible Mx1-Cre transgene resulted in a regression of the tumour vasculature and a concomitant reduction in tumour volume by 63 and 72% respectively. Furthermore, screening of potential ERK5 targets using a Pepchip array revealed that ablation of *Erk5* in mouse lung endothelial cells prevented phosphorylation of rpS6 (ribosomal protein S6) on Ser^{235}/Ser^{236} by p90RSK [55]. Interestingly, ablation of *Erk5* in mouse fibroblasts did not affect phosphorylation of rpS6, suggesting that activation of this signalling pathway may be cell type specific.

The precise role of ERK5 in regulating VEGF-mediated angiogenesis still remains to be determined. It is known that both VEGF and FGF-2 stimulate ERK5 activity in HUVECs and MLCECs (mouse lung capillary endothelial cells) [12]; therefore it remains a distinct possibility that ERK5 is an important component of the VEGF signalling cascade in endothelial cells, responsible for regulating endothelial cell survival, permeability, proliferation and differentiation [49].

Cell migration

Recent results point to a role for the MEK5–ERK5 pathway in regulating endothelial cell migration and focal contact turnover [56]. Expression of constitutively active MEK5 (MEK5DD, in which Ser^{313} and Thr^{317} are replaced by aspartate) led to hyperphosphorylation of FAK (focal adhesion kinase). Another recent report has implicated ERK5 in integrin-mediated cell adhesion and FAK phosphorylation in cancer cells [57]. Taken together, these results suggest that ERK5 plays an important role in cell attachment to the extracellular matrix and cell migration.

Conclusions and perspectives

The ERK5 signalling axis was the most recent of the MAPK modules to be discovered. However, research over the past decade has revealed a vital role for this kinase cascade in normal physiology. Whereas ERK5 appears to be almost ubiquitously expressed in different tissues, the phenotype of the ERK5-knockout mice indicates that it is critical for endothelial cell physiology. However, conditional knockout in other cell types suggests a degree of redundancy with other signalling pathways [32]. *In vitro* studies have revealed that ERK5 is important for endothelial and neuronal cell survival [58–60], suggesting that under certain conditions, these cell types have a critical dependence on ERK5 activity and may express specific ERK5 substrates not expressed in other cells. It is possible that certain diseases may be amenable to pharmacological intervention with modulators of the ERK5 signalling axis. Aberrant activation of ERK5

during tumour development in both the tumour [61] and vascular compartments [55] may present a therapeutic window for the use of ERK5 signalling inhibitors such as the recently developed MEK5 inhibitor [62]. Conversely, stimulating ERK5 activity by gene therapy may offer a way of stimulating endothelial cell survival and revascularization under conditions such as ischaemia.

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