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How ERK1/2 Activation Controls Cell Proliferation and Cell Death Is Subcellular Localization the Answer?

Yohannes Mebratu and **Yohannes Tesfaigzi**
Lovelace Respiratory Research Institute, Albuquerque, NM 87108, USA

Abstract

Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) are members of the mitogen-activated protein kinase super family that can mediate cell proliferation and apoptosis. The Ras–Raf–MEK–ERK signaling cascade controlling cell proliferation has been well studied but the mechanisms involved in ERK1/2-mediated cell death are largely unknown. This review focuses on recent papers that define ERK1/2 translocation to the nucleus and the proteins involved in the cytosolic retention of activated ERK1/2. Cytosolic retention of ERK1/2 denies access to the transcription factor substrates that are responsible for the mitogenic response. In addition, cytosolic ERK1/2, besides inhibiting survival and proliferative signals in the nucleus, potentiates the catalytic activity of some proapoptotic proteins such as DAP kinase in the cytoplasm. Studies that further define the function of cytosolic ERK1/2 and its cytosolic substrates that enhance cell death will be essential to harness this pathway for developing effective treatments for cancer and chronic inflammatory diseases.

Keywords

MAP kinases; activation; epithelial cells; nuclear translocation; cancer; lung diseases

Background

Protein kinases are crucial components of the signalling network that allows cells to function as an integral part of an organism. A family of protein kinases, the mitogen-activated protein kinases (MAPKs) with conserved function in all eukaryotes have been the subject of intense investigation since first discovered 20 years ago. Abnormal regulation of the MAPK pathways have been reported for a wide range of diseases including many cancers [1], obesity [2], diabetes [2], polycystic kidney diseases [3], cardiovascular diseases [4]5, Alzheimer's diseases [6], and pulmonary diseases, such as asthma [7, 8], emphysema [9], and COPD [10]. As a result, drugs targeting the MAPKs are being tested for a variety of disease conditions.

Extracellular stimuli such as growth factors, cytokines, mitogens, hormones, and oxidative or heat stress [11] trigger a signal by interacting with a multimolecular complex of receptors such as receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs) or epidermal growth factor receptor (EGFR). These receptors transmit activating signals by recruiting SOS (son of sevenless), a Ras-activating guanine nucleotide exchange factor through the adaptor protein growth-factor-receptor-bound-2 (Grb 2) to stimulate Ras and convert GDP to GTP. This conversion activates Ras and initiates the interaction with a wide range of downstream effector proteins, including isoforms of the serine/threonine kinase Raf [12]. The binding of Ras to Raf, a MAPK kinase kinase (MAPKKK), results in a conformational change of Raf

Corresponding author and for reprint requests: Yohannes Tesfaigzi, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive, SE, Albuquerque, NM 87108, Tel: (505) 348-9495, Fax: (505) 348-8567, ytesfaig@LRRI.org.

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increasing its kinase activity or providing the proper environment for Raf signaling [13, 14, 15]. MAPKKK activation leads to the phosphorylation of two families of kinases — the MAPKK and then the MAPK activity on threonine and tyrosine residues [16, 17] (Figure 1). Once activated, MAPKs primarily phosphorylate a multitude of target substrates on serine or threonine residues followed by a proline residue, and regulate cellular activities ranging from gene expression, mitosis, embryogenesis, cell differentiation, movement, metabolism, and programmed death. At least four members of the MAPK family have been identified: extracellular-signal-regulated kinase 1/2 (ERK1/2), c-Jun-amino-terminal kinase (JNK), p38, and ERK5 [18, 19, 20].

Excellent reviews have focused on the role of ERK1/2 in phosphorylating activities [21], on the scaffolds and inhibitors that coordinate ERK1/2 signaling [22], the possibilities of targeting this pathway for the treatment of cancer [1], or in comparison with other MAPK pathways such as ERK5 [23]. However, the mechanisms how ERK1/2 modulates cell proliferation and death responses have not been reviewed extensively. We have tried to summarize the most important proteins that are considered to be relevant for the downstream effects of ERK1/2 activation from *in vitro* findings that have been validated in primary cell cultures and various mouse models.

The ERK1 and ERK2 Cascades

Activation of MEK1/2 leads to the phosphorylation of threonine and tyrosine residues of ERK1 and ERK2 (referred to as ERK1/2) with the recognition sites being Thr–Glu–Tyr (TEY) [24, 25, 26, 27]. ERK1 and ERK2 are homologous isoforms that share the same substrate-specificities *in vitro* 28, [29, 30]. These 44- and 42-kDa proteins that phosphorylated a multitude of protein substrates [31, 32] have nearly 85% amino acid identity with much greater identity in the core regions and are expressed in almost all tissues [33].

ERK1/2 activation appears to be responsible for proper development of the fetal lung because inhibition by U0126 diminishes branching morphogenesis, characterized by increased mesenchymal apoptosis and decreased epithelial proliferation in fetal lung explants [34]. Targeted deletion studies have identified the roles of ERK1 and ERK2 in the development of whole organisms [23]. ERK2 and MEK1, rather than ERK1 and MEK2, are essential for embryonic development: ERK2- or MEK1-deficient mice show defects in development of the placenta, whereas ERK1- or MEK2-deficient mice are viable, fertile, and normal in size [35, 36, 37, 38, 39]. However, another line of MEK2-deficient mice lacked mesoderm differentiation, suggesting that ERK2 may have a key role in mesoderm formation [40].

ERK1/2 Activation and Cell Proliferation

In resting conditions, ERK is anchored in the cytoplasm by its association with MEK [41], the microtubule network [42], or with phosphatases. For example, MKP3, a member of the MAP kinase phosphatase family has a nuclear export sequence (NES) and anchors ERK1/2 in the cytoplasm under non-stimulated conditions [43]. Mitogens induce a biphasic activation of ERK1 and ERK2, with a rapid and strong burst of kinase activity peaking at 5–10 min followed by a second wave of lower but sustained activity that persists throughout the G1 phase for up to 6 h [44, 45, 46]. Nuclear translocation of ERK1/2 occurs within 15 min of activation, persists during the entire G1 phase, and can be reversed upon removing the mitogenic stimulus. ERK1/2 activation must be sustained until late G1 for successful S-phase entry [47] and ERK1/2 translocation to the nucleus is essential for G1 to S phase progression [48], although it is nonetheless insufficient to drive cells into S phase [49, 50, 51]. ERK1/2 are rapidly inactivated at the G1/S transition [45].

Various mechanisms have been reported that facilitate nuclear translocation of phospho-ERK1/2. Integrin-mediated organization of the actin cytoskeleton [52] is essential for the proper

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localization and translocation of activated ERK1/2 and, in turn, the ability of ERK to efficiently phosphorylate nuclear substrates [53, 52]. Upon stimulation, ERK1/2 becomes phosphorylated at threonine and tyrosine residues and the latter results in the dissociation of ERK1/2 from MEK1/2. ERK1/2 then translocates to the nucleus by passive diffusion of the monomer, active transport of the dimer, or by a direct interaction of ERK1/2 with the nuclear pore complex [54, 55, 56, 57, 58]. The rapid and persistent nuclear transfer of ERK1/2 during the entire G0-G1 period is crucial for the function of these kinases in mediating the growth response [59]. Upon translocation to the nucleus, activated ERK1/2 phosphorylates the ternary complex factors Elk-1, Sap-1a, and TIF-IA [60, 30, 59, 61]. Phosphorylation of Elk-1 on the C-terminus [62] increases its affinity for the serum response factor and enhances transcription of growth related proteins, such as c-Fos [62, 63] (Figure 1).

Because cell cycle regulatory proteins that are activated by ERK1/2 are localized in the nucleus, access of the ERKs to their substrates is a potential point of regulation. Phosphoprotein enriched in astrocytes 15 (PEA-15) and Sef are also spatial regulators of ERK1/2 [64, 65, 66]. Sef, a MAPK scaffold protein that resides on the Golgi apparatus binds active MEK/ERK complexes and permits signaling to cytosolic substrates but not nuclear targets [65]. PEA-15 contains a nuclear export sequence that mediates the relocation of ERK to the cytoplasm. PEA-15 binds ERK1/2, abolishes their nuclear translocation, and blocks the phosphorylation of Elk-1 [64]. Therefore, genetic deletion of PEA-15 markedly stimulates ERK-dependent proliferation and gene transcription; while PEA-15 overexpression blocks the proliferation and thereby invasion of cancer cells via its ability to bind and sequester ERK1/2 in the cytoplasm [67]. Once dephosphorylated in the nucleus, ERK1/2 are rapidly exported out of the nucleus via an active mechanism that is mediated, at least in part, by MEK that enters the nucleus independently from ERK [68]. MEK1/2 enters the nucleus by passive diffusion [68, 69] or a stimulus-dependent rapid transport mechanism [70, 69].

Aside from regulating the activation of cell cycle regulatory transcription factors such as Elk-1 and Sep-1a, ERK1/2 signalling pathway promotes cell survival by a dual mechanism comprising the posttranslational modification and inactivation of a component of the cell death machinery and the increased transcription of pro-survival genes [22]. ERK1/2 can affect the FOXO transcription factors that activate multiple target genes involved in tumor suppression including *Bim* and *FasL* for inducing apoptosis [71, 72] and p27kip1 [73] and *cyclin D*74 for cell cycle regulation (Figure 2). FOXO3a expression is associated with suppression of tumor progression and inhibiting FOXO3a expression promotes cell transformation, tumor progression and angiogenesis [75, 76, 77]. Phosphorylation of FOXO3a by ERK1/2 at residues Ser 294, Ser 344 and Ser 425 increases FOXO3a–MDM2 interaction and enhances FOXO3a degradation via an MDM2-dependent ubiquitin-proteasome pathway. A non-phosphorylated FOXO3a-mimic mutant exhibits more resistance to the interaction and degradation by MDM2 compared to wild-type FOXO3a and strongly inhibits cell proliferation *in vitro* and tumorigenesis in mice [78]. These studies highlight the possible therapeutic efficacy of ERK inhibitors by concurrent stabilization of FOXO3a to inhibit cell proliferation (Figure 2).

ERK-activates the RSK (ribosomal s6 family kinases) family of serine/threonine kinases, RSK1, RSK2, and RSK3 that is usually present in the cytoplasm of quiescent cells. Upon stimulation, a significant portion of these proteins translocates to the nucleus [79, 80] (Figure 1). The RSKs catalyze the phosphorylation of the pro-apoptotic protein BAD at serine 112 and phosphorylate the transcription factor CREB (cAMP response element-binding protein) at serine 133 to promote cell survival [81].

Furthermore, the BH-3 only protein Bim is phosphorylated on multiple sites by members of the MAP kinase family and targeted for polyubiquitination and subsequent degradation via the proteosome pathway. This was substantiated by generating mutations of the phosphorylation

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sites Ser-55, Ser-65, and Ser-73 to cause increased apoptosis because of reduced proteosome degradation of Bim [82]. Serum withdrawal leads to decreased ERK activation and consequent dephosphorylation and accumulation of Bim [83]84, while ectopic expression of a constitutively active Raf-1 leads to phosphorylation and degradation of Bim [83] (Figure 2). In addition, phosphorylation at Thr-112 of Bim decreases binding of Bim to the antiapoptotic protein Bcl-2 and can increase cell survival (Figure 2).

Rather than proteasome-mediated destruction, ERK-mediated phosphorylation of Bad is linked to sequestration by the phosphoserine-binding 14-3-3-proteins [85, 81]. However, under certain pro-apoptotic conditions, such as IL-3 withdrawal, Bad becomes dephosphorylated and is available for displacing anti-apoptotic Bcl-2 family members from Bax and/or Bak, thereby lowering the threshold for apoptosis [85] (Figure 2). Constitutively active B-RafV600E mutant can promote robust ERK-dependent phosphorylation and destabilization of both Bim and Bad [81], suggesting that melanoma-associated B-Raf mutations may contribute to chemoresistance in part through ERK-mediated inactivation of the BH3-only proteins Bim and Bad. Together, these studies show that phosphorylation of Bim and/or Bad by ERK1/2 through multiple mechanisms can contribute to reduced sensitivity of cells to apoptosis and promote cell proliferation.

ERK1/2 Activation and Cell Death

Although ERK activation has generally been associated with cell survival and proliferation, a number of studies show that depending on the stimuli and cell types involved, activation of ERK can mediate cell death. Some studies suggest that the balance among the intensity and duration of pro- versus anti-apoptotic signals transmitted by ERK1/2 determines whether a cell proliferates or undergoes apoptosis [86]. However, the molecular mechanisms that define the conditions for ERK-mediated cell death remain poorly understood.

ERK and DNA Damage-induced Cell Death

How cells sense DNA damage is yet to be completely understood, but it is clear that two members of the phosphatidylinositol 3-kinase (PI3K) family, ATM and ATR, are major DNA damage signal transducers [87]. DNA damage-inducing agents, including etoposide, adriamycin, and ionizing or ultraviolet irradiation activate ERK1/2 in a variety of primary, immortalized and transformed cells [88]. The MEK1 inhibitor PD98059 prevents ERK activation but not p53 stabilization, and maximal ERK activation in response to DNA damage is not attenuated in p53-deficient mouse embryonic fibroblasts (MEFs). Furthermore, ERK1/2 activation in response to etoposide is abolished in $ATM^{-/-}$ fibroblasts suggesting that ERK activation takes place downstream of ATM and is independent of p53.

Similar to what is known for p53, low intensity DNA damage-induced ERK activation causes cell cycle arrest, while extensive DNA damage-induced ERK activation causes apoptosis [88]. Cisplatin, another DNA damaging agent, activates ERK [89, 90, 91], and inhibition of ERK improves cell survival by inhibiting apoptosis in renal cell lines and primary cultures of renal proximal tubular cells [92, 93]. These studies propose a possible link of ERK activation and p53 phosphorylation. One study reported that ERK directly interacts and phosphorylates p53 on Ser-15 [89]. However, it remains unclear how ERK, a proline-directed kinase, could phosphorylate Ser-15 of p53, because this residue is not followed by a proline but rather by a Gln residue. Another study showed that overexpression of wild-type p53 caused ERK activation [94] with the underlying possible mechanism being that activation of a DNA damage-response pathway results in ATM kinase activation. ATM kinase in turn could lead to ERK activation, consistent with the observation that ERK activation depends on ATM after DNA damage [88].

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Reactive oxygen species (ROS), such as oxygen ions, free radicals, and hydrogen peroxide (H_2O_2), are generated in cells as by-products of electron transfer reactions in response to ionizing radiation and arachidonic acid metabolism [95] ROS can induce oxidative damage of DNA, including DNA strand breaks and base and nucleotide modifications, particularly in sequences with high guanosine content [96]. Oxidative modification activate DNA repair enzymes, including ATM and ATR that phosphorylate and activate specific checkpoint kinases, such as chk2 and hCDS1, with subsequent phosphorylation of p53. p53 stimulates base excision repair but also coordinates the cell's response to damage by inducing both growth arrest and apoptosis. Because inhibition of ERK using the MEK1 inhibitor PD98059 rescues many cell types from ROS-induced cell death [97, 91, 98] and ERK activation is associated with cell death induced by ROS [99], ERK activation may be mediating signaling pathways downstream of p53 activation.

ERK and IFNγ-induced Cell Death

IFNγ causes cell death in a variety of cell types such as HeLa [100], keratinocytes [101], lung epithelial cells [102], colon adenocarcinoma cells [103], oligodendroglial progenitor cells [104], and human breast tumor cells [105]. Unraveling the role of IFNγ in apoptosis remains a challenge, because IFNγ may prime cells to apoptosis but through induction of many genes can concomitantly elicit an anti-proliferative and a proliferative state [106]. Evidence for the involvement of ERK1/2 in IFNγ-induced death was first described in oligodendroglial progenitor cells (OP) [104], because inhibition of ERK1/2 activation by U0126 reversed the cytotoxic effect of IFNγ. Simultaneous activation of MEK-ERK and STAT pathways was proposed to account for the vulnerability of OP cells to IFNγ because IFNγ activates the STAT1 pathway in both oligodendroglial progenitor and mature oligodendrocytes (OD) but ERK1/2 is not activated in mature OD cells [104]. However, these studies did not show the signaling proteins downstream of ERK and the mechanism by which ERK activation leads to cell death. Recently, we reported the mechanism of how ERK1/2 may be involved in IFNγ-induced apoptotic cell death in airway epithelial cells [107]. Screening of the Bcl-2 family proteins identified Bik to be a specific mediator for IFNγ-induced death of airway epithelial cells. Bik directly interacts with activated ERK1/2 and sequesters it to the cytoplasm by blocking the translocation to the nucleus (Figure 3). Suppression of IFNγ-induced Bik expression, targeted deletion of Bik, or expression of a Bik mutant in which the conserved Leu residue in the BH3 domain was substituted with a Gly residue, was accompanied with nuclear ERK1/2 translocation and cell survival [107].

Death associated protein kinase (DAPK) was isolated from HeLa cells as a mediator of IFNγ-induced cell death [100, 108]. DAPK sequesters ERK1/2 in the cytoplasm by interacting with ERK through a D-domain within its death domain [109]. DAPK–ERK interplay promotes the proapoptotic function of DAPK through two mechanisms. First, ERK functions as an upstream activating kinase for DAPK by phosphorylating DAPK at Ser 735. Second, DAPK promotes the cytoplasmic retention of ERK to further potentiate the phosphorylation and activation of the cytosolic DAPK and possibly impair ERK survival signals, and/or may establish a positive feedback loop to promote the apoptotic effect of DAPK [109] (Figure 3).

Role of ERK1/2 in Suppressing Survival Signaling

Withdrawal of soluble survival factors from primary cultures of mouse renal proximal tubular cells leads to ERK1/2 activation-induced apoptosis that is inhibited by U0126 or PD98059 [110]. In these cells, ERK1/2 decreases Akt activity and, because the phosphatidylinositol 3-kinase/Akt pathway regulates cell survival [111], ERK1/2 promotes cell death by suppressing survival signaling pathways. When Akt is activated by phorbol 12-myristate 13-acetate [112, 113] or epidermal growth factor [114] Raf activity is inhibited, leading to suppression of the

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ERK1/2.pathway. However, inhibition of Ras, Raf, or MEK by overexpressed Akt cannot account for this negative regulation because ERK1/2 phosphorylation is not affected in cells overexpressing Akt [115], suggesting that Akt acts downstream of ERK1/2 activation in the cytosol. While Akt does not modify ERK1/2 phosphorylation [116], it phosphorylates and stabilizes PEA-15 [117] and thereby retains active ERK1/2 in the cytosol and downregulates Elk-1–dependent transcription and cFos expression [115]. Pretreatment with the PI3K inhibitor LY294002, which blocks Akt phosphorylation, restores ERK1/2 nuclear translocation and cell proliferation.

Role of ERK in Fas-mediated Cell Death

Fas crosslinking activates ERK in glioma cells [118] and in SH-SY5Y neuroblastoma cells [119], and interference with the ERK pathway by expression of a dominant-negative MEK1 results in inhibition of Fas-mediated apoptosis [120]. Activation of ERK prevents Fas-induced apoptosis in activated T cells [121] and, conversely, inhibition of ERK prevents Fas-induced proliferation [122]. However, in contrast to the well-characterized Fas-mediated apoptotic pathway, relatively little is known about the mechanism of how ERK1/2 activation may block Fas-mediated cell death that may contribute to the signaling pathways involved in Fas-mediated growth induction.

Conclusions and Future Directions

Despite the progress in identifying the mechanisms that control ERK1/2-mediated cell proliferation and the strong evidence supporting a distinct role for the MEK-ERK signaling cassette in cell death, additional studies are required to define the conditions that allow ERK1/2 activation to be responsible for both cell proliferation and apoptosis. The ERK1/2-induced signaling that elicits proliferation or apoptosis appears to be dependent on the type of stimuli and the cell type which defines the available ERK1/2 substrates. The substrates in turn may be defined by conditions facilitating protein-protein interactions including subcellular distribution of pathway components and the spatial and temporal changes and fluctuations in ERK1/2 activity. For instance, inhibition of ERK translocation to the nucleus denies access to the transcription factor substrates and abrogates the mitogenic response. Cytosolic ERK1/2, besides inhibiting survival and proliferative signals in the nucleus, may further potentiate the catalytic activities of some pro-apoptotic proteins in the cytoplasm.

The possible interaction of DAPK, PEA-15, and Bik to inhibit nuclear localization of ERK and to promote cell death needs further investigation. Together with the finding that Bik sequesters activated ERK1/2 in the cytosole, future studies should investigate the interplay between Bik, activated ERK1/2, and DAPK and whether a scaffolding of these proteins ultimately initiates the demise of the cell. Moreover, the kinetics and duration of ERK activation may play an important role in influencing cell fate. It has been reported that prolonged ERK activation is accompanied by the proapoptotic effect of ERK [123], whereas a transient activation of ERK protects cells from death [124]. Further studies are necessary to elucidate the activated signal transduction upstream and downstream of the ERK cascades to define the cross-talk among the Ras-Raf-MEK-ERK cascade and the PI3-kinase-Akt, IFNγ-STAT, or other signaling pathways. Most of the data generated to understand the molecular mechanisms of ERK-mediated cell death are based on the surviving cells rather than the cells which have in fact died. Thus, it is not clear whether the cells in which the analyses are based on represent the behavior of the apoptotic or already dead cells. Further studies should employ inhibitors of downstream caspases to generate a model system that will enable detailed analysis of the molecular mechanisms of ERK-mediated cell death in apoptotic cells. Such studies should use primary cells and *in vivo* animal models to reliably define this pathway to better allow harnessing the knowledge for developing effective treatments of chronic inflammatory

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diseases and cancer. Furthermore, gene disruption studies using tissue-specific or conditional knockout mice deficient in ERK in different organ systems may provide better insight on the specific role of ERK in organ development and in cell-type-specific effects of ERK activation.

Analysis of lung tissues from emphysema patients reveals significantly increased ERK activity compared to lungs from control subjects [9]. ERK1/2 activation is implicated in the airway inflammation and cell death mechanisms associated with emphysema [125] 126 127 128. In mice, overexpressing IL-13 in the lung induces inflammation, stimulates production of chemokines, MMP-2, and cathepsin B, and inhibition of β1-antitrypsin, and ultimately leads to alveolar destruction and the development of emphysema. Systemic administration of the MEK1 inhibitor PD98059 or use of dominant-negative ERK1/2- expressing transgenic mice in which a dominant-negative MEK1 construct was expressed, inhibits the IL-13-induced effects, demonstrating that IL-13 is a potent and selective stimulator of ERK1/2 activation [129]. Because cell death is facilitated by ERK1/2 activation in non-transformed pulmonary epithelial cells, it is possible that PEA-15, DAPK, and/or Bik may be present in these cells and merely ERK1/2 activation is sufficient to cause cell death and the associated emphysematous changes.

The significance of the ERK1/2 signaling in cancer biology was first identified when Ras proteins were found as the transforming component of oncogenic viruses for K-Ras and H-Ras, whereas N-Ras was identified as the transforming component of a neuroblastoma [130]; [131]. Additional support for the importance of the ERK pathway in oncogenesis comes from the prevalence of activating mutations among family members in multiple cancer types. Ras mutations are found in up to 30% of all cancers and are particularly common in pancreatic (90%), colon (50%), thyroid (50%), lung (30%), and melanoma (25%) cancers [132, 133]. Mutant Ras proteins are GAP insensitive, rendering the proteins constitutively GTP bound and activated, leading to stimulus-independent, persistent activation of downstream effectors, in particular, the Ras–Raf–MEK–ERK cascade [132]. Somatic B-Raf missense mutations have been reported in 60% of malignant melanomas and at lower frequency in a wide range of human cancers [134]. Mutations in Ras or Raf lead to persistent activation of ERK1/2 and contribute to increased tumor cell proliferation. Despite the absence of MEK and ERK mutations in human cancers, studies using genetic or pharmacologic approaches have shown that MEK and ERK are required for the transforming activities of Ras and other oncogenes. Such studies have led to the development of a number of inhibitors of the ERK MAPK cascade as potential anticancer agents [135]. Many inhibitors of EGFR, Ras, Raf, and MEK have been developed that target different components of ERK signaling, with a handful of agents already approved and added to the collection of anticancer agents available [1]. However, whether inhibitors of ERK signaling will provide drugs that significantly advance cancer treatment is still uncertain. As these efforts continue, research efforts have also revealed a considerably greater complexity to the linear Raf–MEK–ERK signaling cascade. These complexities suggest that targeting this pathway will not be as straightforward as once imagined and the recent understanding in the mechanisms of how ERK1/2 regulate cell proliferation and apoptosis may provide important insight into improving the efficacy of these inhibitors. Mechanisms described in this review would suggest that blocking nuclear ERK translocation may enhance the cell death-inducing activity of ERK1/2 and provide a better means to kill tumor cells and thereby control the development of cancer.

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List of Abbreviations

AECs, airway epithelial cells, BALF, bronchial lavage fluid; MCM, mucous cell metaplasia; NHBEs, normal human airway epithelial cells.

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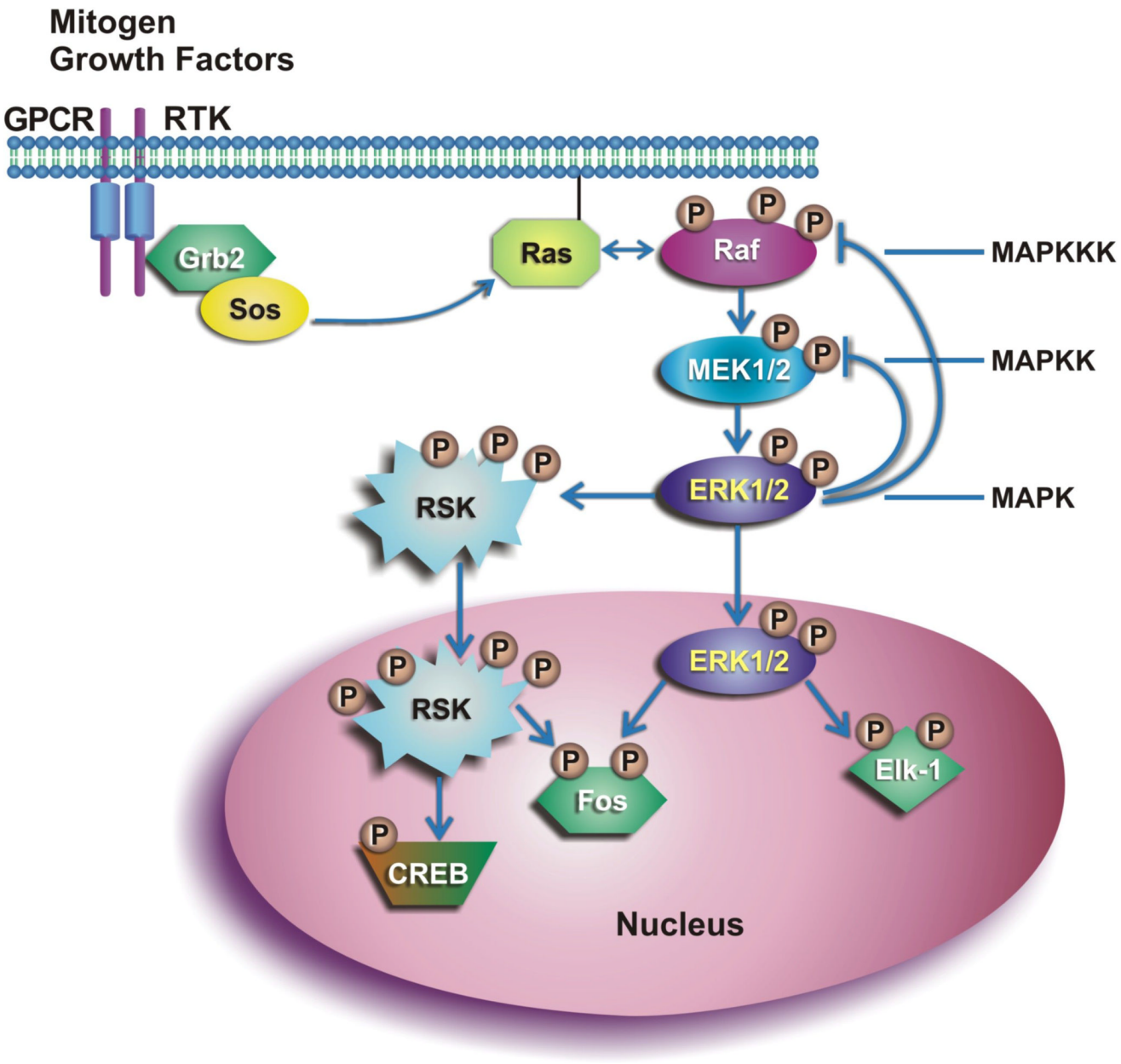

Figure 1. Mechanism of ERK activation and cell proliferation

Activation of receptor tyrosine kinases (RTKs) or G protein-coupled receptors (GPCRs) by growth factors or mitogens leads to the recruitment of an adaptor protein Grb2 (growth factor receptor bound protein) and the guanine nucleotide exchange factor (SOS). The SOS activates Ras to recruit and activate Raf at the plasma membrane by phosphorylation at multiple sites. MEK1/2 is which then phosphorylated at two serine residues that subsequently phosphorylates ERK1/2 on both threonine and tyrosine. Activated ERK1/2 phosphorylates RSK and both RSK and ERK translocate to the nucleus where they activates multiple transcription factors ultimately resulting in effector protein synthesis and causing changes in cell proliferation and survival. ERK phosphorylation of MEK and possibly Raf can inactivate the pathway at those steps creating a negative feedback loop.

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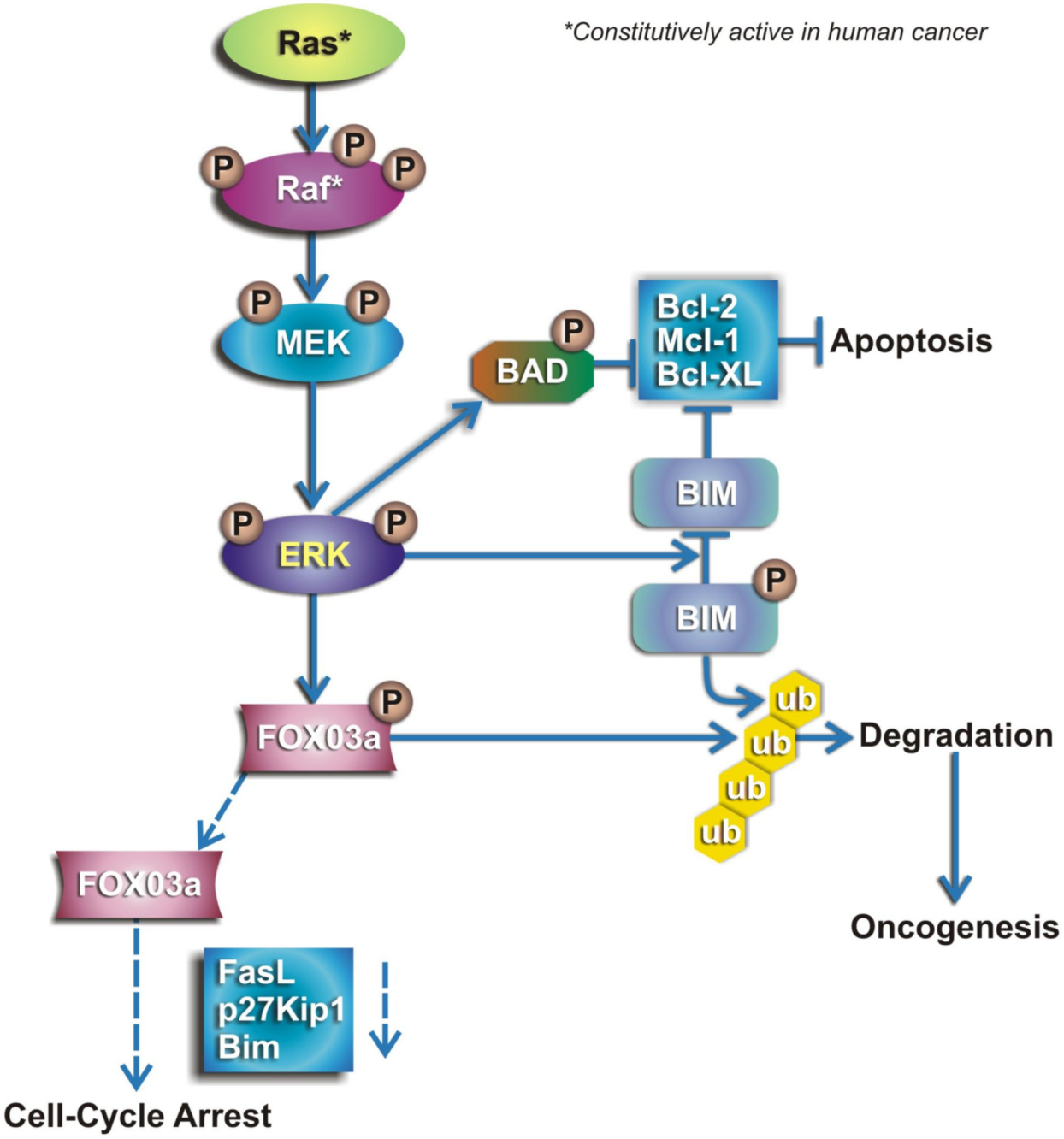

Figure 2. Mechanisms of ERK1/2-mediated oncogenesis

ERK1/2 activation promotes metaplasia and tumor development by phosphorylating Bim and Bid and causing the proteosomal degradation of Bim and the sequestration of Bad to the phosphoserine-binding proteins and, thereby, inhibiting apoptosis. In a separate pathway, ERK1/2 activation phosphorylates FOXO3a at Ser 294, Ser 344, and Ser 425 and facilitates FOXO3a-MDM2 interaction. This interaction enhances FOXO3a degradation through a MDM2-dependent ubiquitin-proteosome pathway, leading to tumor development.

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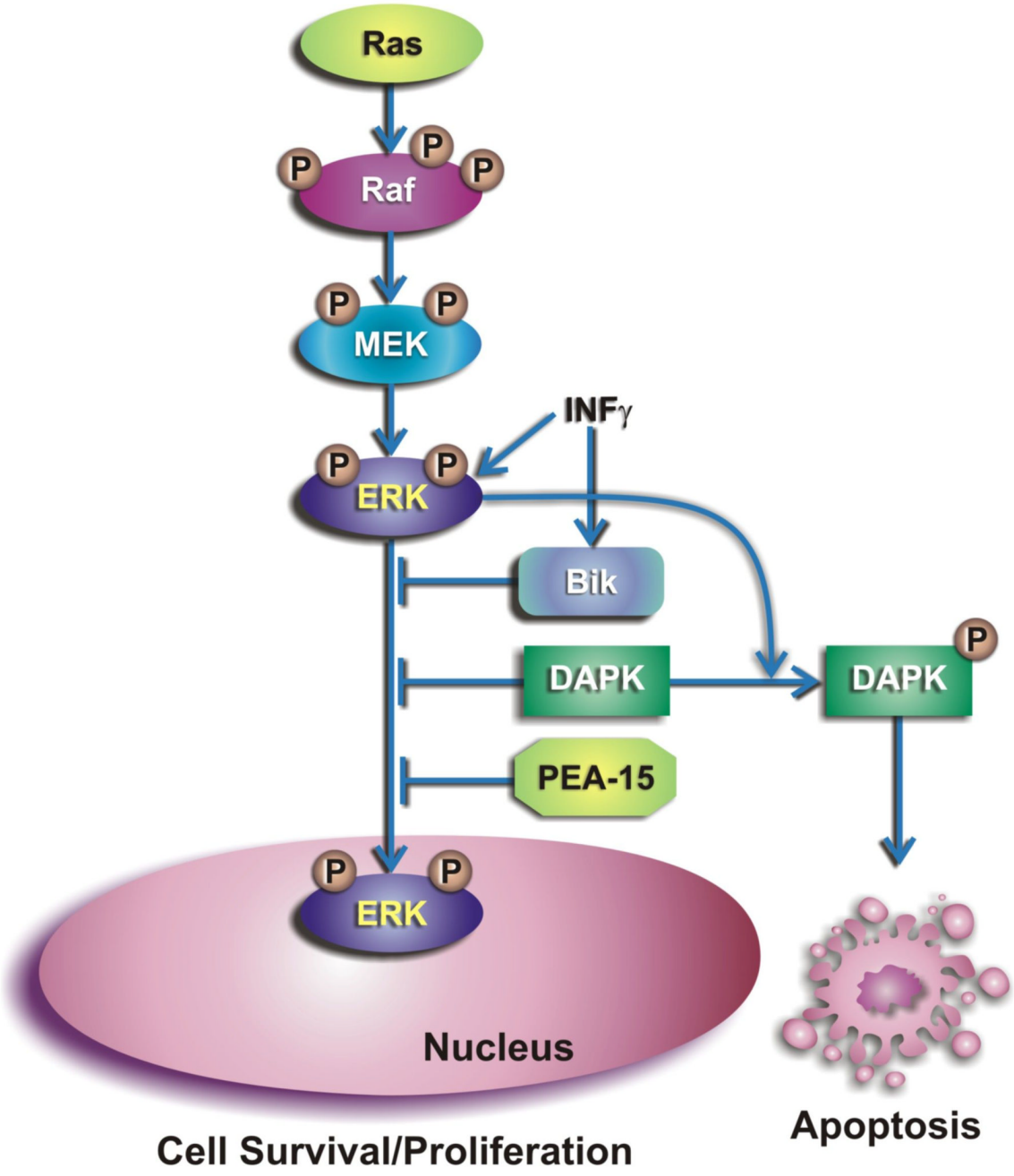

Figure 3. Mechanism of ERK1/2-mediated cell death

The cytoplasmic of ERK1/2 by Bik, PEA-15 or DAPK plays a major role in ERK1/2-mediated cell death. Activated ERK1/2 interacts with PEA-15, Bik, and DAPK and is sequestered in the cytoplasm. Inhibition of ERK1/2 nuclear localization impairs ERK1/2-mediated survival signals and in addition augments the proapoptotic signals of DAPK by phosphorylating the cytoplasmic DAPK.