

# Mechanisms shaping the role of ERK1/2 in cellular senescence (Review)

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**Abstract.** Senescence is a result of cellular stress and is a potential mechanism for regulating cancer. As a member of the mitogen-activated protein kinase family, ERK1/2 (extracellular signal-regulated protein kinase) has an important role in delivering extracellular signals to the nucleus, and these signals regulate the cell cycle, cell proliferation and cell development. Previous studies demonstrated that ERK1/2 is closely associated with cell aging; however other previous studies suggested that ERK1/2 exerts an opposite effect on aging models and target proteins, even within the same cell model. Recent studies demonstrated that the effect of ERK1/2 on aging is likely associated with its target proteins and regulators, negative feedback loops, phosphorylated ERK1/2 factors and ERK1/2 translocation from the cytoplasm to the nucleus. The present review aims to examine the mechanism of ERK1/2 and discuss its role in cellular outcomes and novel drug development.

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## 1. Introduction

The Hayflick limit is a response to cellular lesions, which are triggered by multiple mechanisms, including replicative senescence, oncogene activation, telomerase dysfunction and DNA lesions (1-3). Senescent cells arrested at the G<sub>1</sub> phase demonstrated more properties associated with dysfunctional cells compared with normal cells (4). Although senescence is an undesirable stress for normal cells, it is beneficial for the body as it restrains excessive proliferation of tumor cells. Therefore, senescence is used as a means of suppressing cancer and is an important cancer treatment method (5-8).

Extracellular signal-regulated protein kinase (ERK)1/2 is a mitogen-activated protein kinase (MAPK) family protein with typical cascade signaling characteristics and serves an important role in signal transduction pathways and the function of transcription factors, including activator protein-1, proto-oncogene c-Fos (c-Fos) and ETS domain-containing protein Elk-1 (Elk1) (9). The majority of research has focused on its regulatory effect on cell growth and differentiation (10-14); however, a number of previous studies demonstrated that ERK1/2 promotes cell senescence (15-17). Based on these characteristics, numerous small molecule MAPK/ERK kinase (MEK) inhibitors were examined in early-phase clinical trials, including PD098059, U0126, CI-1040, PD0325901 and AZD6244 (18); however, none of them were approved by The Food and Drug Administration due to adverse side effects or other toxic reactions (18). Many of these inhibitors negatively affected normal and abnormal cells. Notably, these effects may have been the result of the dual roles of ERK1/2 in senescence, as demonstrated by other previous studies (19,20). The

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present review examines the mechanisms regulating the role of ERK1/2 in cell senescence and suggests that ERK1/2 is a potentially useful target in treating cancer.

## 2. Senescence, ERK1/2 and cell fate

**ERK1/2 signaling pathway.** The mammalian MAPKs consist of cytoplasmic serine/threonine kinases that are involved in the transduction of signals from the surface to the interior of the cell. This family includes the ERK family (ERK1-8), the p38 kinase family (p38  $\alpha/\beta/\gamma/\delta$ ) and the c-Jun N-terminal kinase family (JNK1-3, additionally termed stress-activated protein kinase) (9). With a number of substrate docking and enzyme recruitment sites (21), ERK1/2 (MAPK1/3) is a multifunctional serine/threonine kinase that is able to phosphorylate numerous substrates, including protein kinases, signal effectors, receptors, cellular scaffold proteins and nuclear transcriptional regulators (21). At present, five types of ERK isoforms are known. ERK1 and ERK2 are thought to be the most important isoforms with 84% homology for the primary sequences and similar functions (21).

The Ras/Raf/MEK/ERK1/2 signaling pathway is a small GTPase ligation of activated tyrosine receptors and cytoplasmic kinase signal transduction cascades. The key point of activation is to transmit a signal from tyrosine receptors, including epidermal growth factor receptor (EGFR), which subsequently recruit Son of sevenless (SOS) through intracellular Shc and Grb2 domains, ultimately catalyzing the conversion of inactive Ras/guanosine diphosphate to the active Ras/guanosine triphosphate complex (22). As an activator of ERK1/2, MEK1/2 catalyzes the phosphorylation of ERK1/2 at Tyr204/187 and Thr202/185 by casein kinase 2 (CK2) (23). This enzyme subsequently binds to importin7 and translocates ERK1/2 from the cytoplasm to the nucleus (24), where it functions as an upstream regulator of substrate genes that encode for transcription factors, including Elk1, c-Myc, signal transducers and activators of transcription (STATs), c-Jun and c-Fos (21). These transcription factors regulate their counterpart target genes to alter the expression or activity of various proteins and are involved in the regulation of a large variety of processes, including adhesion, cell cycle progression, migration, survival, differentiation, metabolism, proliferation and transcription (21). The Ras/Raf/MEK/ERK cascade is a highly efficient signaling pathway, aided by scaffold proteins, including kinase suppressor of Ras, MEK partner 1/p14 complex,  $\beta$ -arrestins, fibroblast growth factor receptor substrate 2, MAPK organizer 1 and flotillin-1 (25). The function of scaffold proteins is characterized by combinatorial inhibition, which is the stoichiometry of a scaffold and its signaling partners; the expression levels of scaffold proteins should not be too high (the kinase and its substrate may each bind to an individual scaffold protein) or too low (the phosphorylation of the cascade is sub-optimal) (26). With these different scaffold proteins, the phosphorylation of different isoforms is accurately regulated; the scaffold protein MEK protein 1 specifically binds ERK1, not ERK2 (27).

**Role of ERK1/2 in cellular senescence.** Cellular senescence was first observed in cultured fibrocytes, when the Hayflick limit demonstrated that as the cells divided, their cell cycle became

arrested (replicative senescence) (28). Senescent cells have abnormal metabolic activity (3), accompanied by morphological, biological and genetic alterations. When  $\beta$ -galactosidase expression (an important senescence marker) increases (29), the cell cycle is arrested at the G<sub>1</sub>/S phase (30). Cell cycle dependent kinase (CDK) and cyclin A activity additionally decrease with increased activity of cyclin-dependent kinase inhibitors (p16INK4a and p21) (31). Without the protection of histones, mitochondrial reactive oxygen species (ROS) may damage mitochondrial DNA, which induces a series of oxidative stress reactions (32). Telomere shortening, which causes DNA to lose protection from the telomeres, is another feature of senescence and leads to DNA integration and degradation. Oxidative stress reactions and mitochondrial dysfunction (33) accelerate the shortening of telomeres. Cellular senescence is triggered by a number of signaling pathways and mechanisms, including DNA injury, telomerase dysfunction, oncogenes, oxidative stress reactions and mitochondrial dysfunction (34).

ERK1/2 is an important messenger for extracellular and intracellular signals, which serve a vital role in processes, including proliferation, differentiation, cytoskeleton construction and cellular senescence (35). In the majority of cases, ERK1/2 is a regulator of cellular proliferation; however, it has been identified that ERK1/2 may additionally promote senescence. Strong, constitutively active expression of MEK1 (an upstream activator of ERK1/2) in non-immortalized intestinal epithelial cells (HIEC cells) promotes cellular senescence, whereas, in immortalized intestinal cells (IEC-6 cells), it does not, suggesting that cell type may serve a role (36). This phenomenon requires further investigation to improve the clinical use of ERK1/2-associated reagents, including ERK1/2 inhibitors.

## 3. ERK1/2 promotes cellular proliferation

ERK1/2 is associated with cell survival, proliferation and development. To investigate the role of ERK1/2 in different cell types and animal models, a number of previous studies investigating this were reviewed. ERK1/2 functions more frequently as a cellular proliferation marker than as a dual role kinase (Table I) (37-54). Based on these previous studies, cellular proliferation is primarily regulated by the effects of ERK1/2 on cell cycle entry and protein synthesis.

**DNA and protein synthesis.** As a transcription factor regulator, ERK1/2 transduces signals from the cell membrane to the nucleus. ERK1/2 may additionally regulate carbamoyl phosphate synthetase II (55), which catalyzes the initial rate-limiting step in the *de novo* synthesis of pyrimidine nucleotides. Furthermore, it was identified that ERK1/2 phosphorylates high motility group boxes of nucleolar transcription factor 1, an RNA polymerase I factor transcriptional enhancer that enhances ribosomal RNA genes (56).

Mendoza *et al* (57) demonstrated that the MEK1/2-ERK1/2 pathway cross-talks with the phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway via cross-inhibition, cross-activation and pathway convergence on substrates. In mouse uterine epithelial cells, activation of protein kinase C by estradiol-17 $\beta$  promotes protein synthesis by activating the ERK-mTOR-40s

Table I. Models of ERK controlled proliferation.

| Author, year                   | Cellular mode                        | Cell and tissue type                          | Key signaling pathway and mechanism              | (Refs.)           |
|--------------------------------|--------------------------------------|---|--|-------------------|
| Ravasi <i>et al</i> , 2006     | Human bronchi isolated primary cell  | Human airway smooth muscle cells              | LTD4-EGFR-Ras-ERK1/2                             | (37)              |
| Gong <i>et al</i> , 2006       | Mouse isolated primary cell          | Neural progenitor cell                        | SDF1-ERK1/2 or PI3K                              | (38)              |
| Iyengar <i>et al</i> , 2006    | Lens epithelial explants             | Lens epithelial explants                      | AKT-ERK1/2                                       | (39)              |
| Wang <i>et al</i> , 2006       | Oral tongue squamous cell carcinomas | Oral tongue carcinomas                        | ERK1/2-Cyclin D1                                 | (40)              |
| De Rosa <i>et al</i> , 2007    | Human primary isolated Treg cells    | Human Treg cells                              | Leptin inhibits ERK1/2                           | (41) <sup>a</sup> |
| Li <i>et al</i> , 2007         | Panc-1, Panc-03.27, ASPC-1 and PL45  | Human pancreatic cancer cells                 | Thy1-ERK1/2 or JNK                               | (42)              |
| Li <i>et al</i> , 2008         | AGS, RGM-1 cell                      | Rat gastric mucosal cells                     | Fas Ag-FasL coupling activation of ERK1/2        | (43)              |
| He <i>et al</i> , 2008         | C18-4 cells                          | Mouse spermatogonial stem cell                | GNDF-shc-Grb-Ras-ERK1/2-CREB1-C-fos-cyclinA/CDK2 | (44)              |
| Mancinelli <i>et al</i> , 2009 | Cholangiocyte and animal model       | Mouse cholangiocyte                           | FSH-cAMP-ERK1/2                                  | (45)              |
| Sirianni <i>et al</i> , 2010   | MCF-7                                | Human breast cancer cell                      | No data  | (46)              |
| Yang and Han, 2010             | Mouse isolated primary cell          | Mouse immature Sertoli cells in testis tissue | GDNF-NCAM-ERK1/2                                 | (47)              |
| Lee and Kay, 2011              | Rabbit isolated primary cell         | Rabbit corneal endothelial cells              | FGF2-ERK1/2-Cdc25A-CDK2                          | (48)              |
| Gao <i>et al</i> , 2014        | L02                                  | Human hepatocyte                              | HPS/Src/EGFR/ERK                                 | (49)              |
| Tocharus <i>et al</i> , 2014   | Rat isolated primary cell            | Neural stem/progenitor cells                  | Melatonin receptor/c-Raf/ERK1/2                  | (50)              |
| Wu <i>et al</i> , 2014         | NHEKs                                | Human epidermal keratinocytes                 | EGFR/ERK/AQP3                                    | (51)              |
| Liu <i>et al</i> , 2015        | A549, SPCA1 and PC9 cells            | Human non-small cell lung cancer              | PTPIB-src-ERK1/2                                 | (52)              |
| Wang <i>et al</i> , 2016       | Mouse isolated primary Müller cell   | Mouse retinal Müller cells                    | NGF/TrkA-VEGF ERK1/2                             | (53)              |
| Kim <i>et al</i> , 2017        | Human ASM cells                      | Human airway smooth muscle cells              | No data  | (54)              |

<sup>a</sup>Study mentioned dual role of ERK1/2.

ribosomal protein S6 cascade (58). ERK1 and ERK2 enhance protein translation by increasing the ability of eukaryotic translation factor-4E (59) to recruit protein-synthesizing ribosomes and other protein synthesis initiation factors to the mRNA. This recruitment includes nuclear substrates (ternary complex factor, Elk1, and c-Fos), cytoplasmic substrates [40s ribosomal protein S6 kinase (RSK) family], cytoskeletal proteins and proteins of the nuclear pore complex, many of which serve a direct role in cellular proliferation and development (21). A previous study additionally demonstrated that ERK1/2 may functionally dephosphorylate the tuberous sclerosis 1 and 2 proteins (TSC1/2) complex via its downstream RSK in HEK293 cells (60).

*Cell cycle entry.* ERK1/2 is involved in G<sub>1</sub>/S and G<sub>2</sub>/M transitions (61). During G<sub>1</sub>/S, ERK1/2 regulates cyclin D1 transcription through the Fos family of proteins (62) and Myc (63,64). In G<sub>2</sub>/M, ERK1/2 is involved in the nuclear translocation of cyclin B1 by phosphorylating two of four sites within the cytoplasmic retention sequence of cyclin B1 (65) and inhibiting the negative phosphorylation of cell division control protein 2 homolog by myelin transcription factor 1 via RSK2 (66).

In addition to the substrates involved in cell proliferation, ERK1/2 additionally regulates cellular tumor antigen p53 (p53) phosphorylation. p53 is a tumor suppressor protein and functions as a transcription factor by binding to a number of genes, including cyclin-dependent kinase inhibitor 1A, which encodes p21. p21 binds and inactivates CDKs, which are crucial for cell entry into the G<sub>1</sub>/S phase (67,68). The association between ERK1/2 and p53 remains unclear. A previous study suggested that p53 functions upstream of ERK1/2 (69); however, the most widely accepted hypothesis is that ERK1/2 regulates p53 by activating STAT3 (70) and other transcription factors. ERK1/2 and p53 have hundreds of substrates, thus, it is easy for them to engage in crosstalk, as is the case with dual-specificity phosphatases (DUSPs) (71). The effect of ERK1/2 on its downstream substrates may accelerate the degradation of p53 (72). ERK1/2 regulates p53 phosphorylation [a form that protects p53 from E3 ubiquitin-protein ligase Mdm2 (73)] through the forkhead box M1/c-myc/polycomb complex protein BMI-1 pathway, which inhibits p19 phosphorylation, attenuating cellular senescence (74).

*ERK1/2 regulates mitochondria.* Mitochondria not only provide energy to cells; however, additionally serve a decisive role in cell fate. Mitochondria within the respiratory chain are responsible for maintaining the proton gradient and providing various respiratory enzymes; it was demonstrated that the proton gradient is not just associated with adenosine triphosphate synthesis. Rasola *et al* (75) identified that ERK1/2 phosphorylates glycogen synthase kinase-3 $\beta$ , inhibiting permeability transition pore opening by regulating cyclophilin D and preventing the release of apoptotic substances, including mitochondrial cytochrome C, ROS, Ca<sup>2+</sup> and free radicals. The number of mitochondria is additionally an important hallmark of cellular proliferation. A previous study demonstrated that ERK2 may phosphorylate dynamin-1-like protein (an important regulator of mitochondrial fission) on serine 616 in several tumor models (76), resulting in tumor growth.

#### 4. ERK1/2 promotes cellular senescence through several mechanisms

Based on extensive investigations in a variety of cell types, previous studies identified that ERK1/2 may additionally facilitate cellular senescence under certain circumstances. In the present review, a number of previous studies are discussed to gain a better understanding of the role of ERK1/2 during cellular senescence and the underlying mechanisms behind its control. In contrast, the role of ERK1/2 in cellular proliferation was only studied in numerous cell types, primarily fibroblasts, providing limited information. The previous studies investigating ERK1/2 involvement in cellular senescence (Table II) (77-90) identified a number of possible mechanisms for the role of ERK1/2 that are associated with abnormal signaling of negative feedback loops, caused by constitutive and overexpressed ERK1/2 (20,77,78), and ERK1/2 cellular localization (79).

##### *Crosstalk and negative feedback loops associated with ERK1/2-induced cellular senescence*

*Regulation of the MAPK signaling pathway.* A number of previous studies demonstrated that negative regulation of ERK1/2 within MAPK signaling cascades regulate ERK1/2 signaling. ERK1/2 phosphorylates proteins within this cascade at alternate sites, which interrupts the normal binding behavior of their respective downstream substrates (91). ERK1/2 phosphorylates EGFR at T669 (92) and decreases constitutive tyrosine phosphorylation activity, decreasing the ability of the phosphorylated loop to cross-activate other adaptors (93). ERK1/2 may additionally phosphorylate dual specificity Cdc25C at T48, which dephosphorylates EGFR at Y1068 (94). Furthermore, ERK1/2 was identified to phosphorylate MAPK signaling components, including fibroblast growth factor receptor (FGFR) at S777 (95); SOS1 at S1132, S1167, S1178 and S1193 (96); fibroblast growth factor receptor substrate 2 (FRS2) $\alpha$  at T132, T135, T138, T376, T452, T455, T458 and T463 (97,98); RAF proto-oncogene serine/threonine-protein kinase (Raf-1) at S29, S289, S296, S301 and S642 (99-103); serine/threonine-protein kinase B-raf (B-Raf) at S151, T401, S750, T753 and S642 (104,105); MEK1 at T292 and T386 (106,107); and kinesin suppressor or Ras 1 at T260, T274, S320, S443 and S463 (108,109) (Fig. 1). The phosphorylation of all these components results in a disruption of binding to downstream substrates.

In other previous study, Fey *et al* (110) developed a dynamic model of the multiple MAPK cascade interactions and feedback systems of specific proteins of the MAPK pathway using mathematical analysis. Based on their model, p38 inhibits ERK through Ser/Thr protein phosphatase-2A, and JNK restrains p38 and ERK through induction of DUSPs (JNK upregulates transcription of DUSP1; Fig. 1).

*Dephosphorylation of ERK1/2.* ERK1/2 requires dual phosphorylation of threonine and tyrosine residues to acquire its biological kinase function. Dual-specificity Thr/Tyr phosphatases [DUSPs; additionally termed MAPK phosphatases (MKP)] represent a large family that regulates the activity of MAPKs by dephosphorylating threonine and tyrosine residues within the activation loop of MAPKs, which in turn regulates



Table II. Models of ERK-induced senescence.

| Author, year                         | Cellular mode                         | Cell and tissue type                            | Key signaling pathway and mechanism   | (Refs.)           |
|--------------------------------------|---------------------------------------|---|---|-------------------|
| Wang <i>et al</i> , 2002             | BJ human foreskin fibroblasts         | Human fibroblasts                               | ERK-MKK3/6-p38-p53-p16  | (19) <sup>a</sup> |
| Lin <i>et al</i> , 1998              | IMR90                                 | Human fibroblasts                               | Constitutive activation active p53/p16  | (20,77,78)        |
| Zhu <i>et al</i> , 1998              |                                       |   |   |                   |
| Cammarano <i>et al</i> , 2005        |                                       |   |   |                   |
| Kim <i>et al</i> , 2000              | TIG-3 and TIG-7                       | Human fibroblasts                               | Translocation from nucleus of p-ERK1/2 which was helped by E1A                              | (79)              |
| Lim <i>et al</i> , 2000              | Primary isolated and PA137            | Human and mouse fibroblasts                     | H-rasV12S35/MEK/Rac1/RhoA   | (80)              |
| Chaturvedi <i>et al</i> , 2003       | KCs                                   | Human neonatal foreskin                         | Ras/Raf/ERK elevate p16 and p14 <sup>ARF</sup>  | (81)              |
| Kim <i>et al</i> , 2003              | Human diploid fibroblasts             | Human fibroblasts                               | ROS inactivation PPP1/2A (resensitise Raf-1), lead to ERK1/2 constitutive activation        | (82)              |
| Todd <i>et al</i> , 2004             | CC139, RAT-1, 3T3L1                   | Human/mouse fibroblast and preadipocyte         | p38 and ERK1/2 cooperate to induce p21 <sup>CIP1</sup> expression                           | (83)              |
| Klein <i>et al</i> , 2005            | A549                                  | Human non-small cell lung cancer cell           | Sustained activation of ERK1/2 by discodermolide  | (84)              |
| Albrecht <i>et al</i> , 2007         | PC-3                                  | Prostate cancer cell                            | EGCG (drug) activate ERK1/2 via MEK1/2 independently  | (85)              |
| Deschênes-Simard <i>et al</i> , 2013 | 293T, HeLa, IMR90                     | Human embryonic kidney cell, etc.               | Sustained activation of ERK1/2 induce senescence associated protein degradation             | (86)              |
| Zhu <i>et al</i> , 2014              | Primary keratinocytes, HaCat cell     | Primary mouse keratinocytes and human skin cell | PPAR $\beta/\delta$ increases ERK1/2 activation, which upregulate expression of p53 and p21 | (87)              |
| Wang <i>et al</i> , 2013             | HCT116                                | Human epithelial cells                          | ASPP2 stimulate HRas induced ERK1/2 activation  | (88)              |
| El Bezawy <i>et al</i> , 2017        | MesoII, STO, MP115, MP4 and MP8 cells | Human diffuse malignant peritoneal mesothelioma | miR-34a induced persistent activation of ERK1/2   | (89)              |
| Del Nogal <i>et al</i> , 2014        | Human glomerular mesangial cells      | Human primary renal glomerular cell             | Constitutive activation of Ras, and ROS production elevated                                 | (90)              |

<sup>a</sup>Study mentioned dual role of ERK1/2.

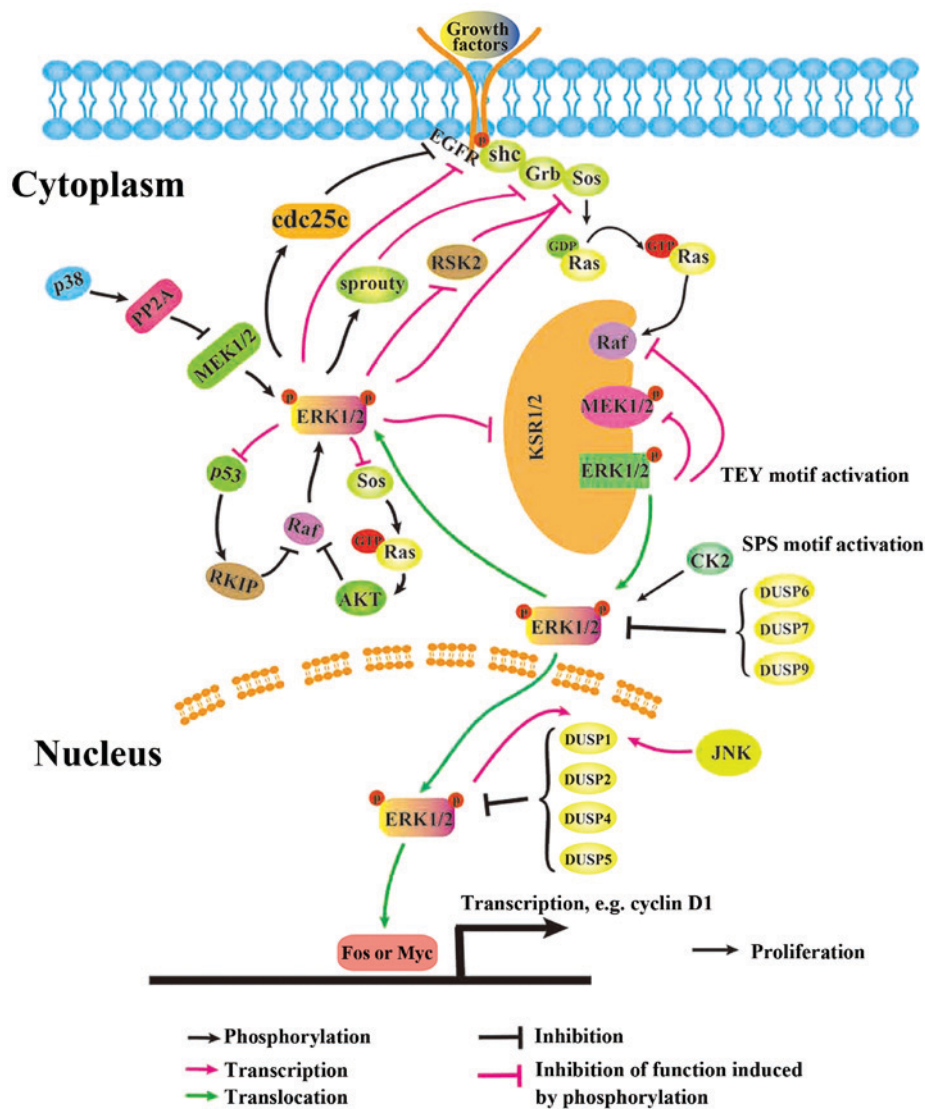


Figure 1. Regulatory system of ERK1/2 signaling. ERK1/2 has a number of substrates, including the MAPK cascade kinases. ERK1/2 may phosphorylate these proteins at numerous sites, which disrupts their normal binding to downstream substrates. Furthermore, ERK1/2 engages in crosstalk with the AKT signaling pathway, p53, DUSPs and Sprys, all of which form a massive regulatory network to tightly regulate ERK1/2 signaling. ERK, extracellular signal-regulated protein kinase; MAPK, mitogen-activated protein kinase; AKT, protein kinase B; DUSPs, dual-specificity phosphatases; Sprys, Sprouty proteins.

the biologically active form of ERK1/2 in the cytoplasm and the nucleus (111). DUSP1, DUSP2, DUSP4 and DUSP5 are located in the nucleus, whereas DUSP6, DUSP7 and DUSP9 are located in the cytoplasm (112). Their binding to ERK1/2 is regulated by a conserved motif within the amino-terminal non-catalytic domain (kinase-interacting motif) of the protein (113,114) and results in a significant increase in catalytic activity, which deprives ERK1/2 of the phosphate group. The interaction between MAPKs and DUSPs is a two-way regulation; MAPKs are able to upregulate transcription of DUSPs (113), primarily those in the cytoplasm (115), in a delayed manner following MAPK activation, whereas, DUSPs strictly regulate MAPK signaling.

Sprouty [protein sprout homolog (Spry) 1-4] is another ERK1/2 regulator family that is not well-studied. A previous Japanese study demonstrated that Spry1 and Spry2 are phosphorylated at Y53 and Y55, which creates a docking site for growth factor receptor-bound protein 2 at the Src homology 2 domain and consequently disrupts association with the FGFR

adaptor FRS2 in C2C12 cells (116). Other previous studies suggested that the spry protein inhibits receptor tyrosine kinase signaling, which suppresses the activation of Ras in BRA<sup>FV600E</sup> melanomas (117,118). Another previous study demonstrated that in 293T cells, Spry1 and Spry2 regulate Raf-1 by directly binding to it (119). Lake *et al* (91) suggested that Sprouty serves its functions at multiple nodes in a context-specific manner.

**Crosstalk between Ras/ERK1/2 and PI3K/AKT signaling.** In contrast to signaling that regulates cell proliferation, AKT/mTOR and ERK1/2 engage in crosstalk that sustains cell proliferation and survival, which in turn helps cells escape from either PI3K/AKT or ERK1/2 suppression (120). Crystal structure analysis demonstrated that astrocytic phosphoprotein PEA-15 (PEA-15) may efficiently bind to the ERK2 activation loop at the Thr-X-Tyr region (121), activating transportation of ERK1/2 from the nucleus. Sinha *et al* (122) observed that ERK1/2 decreases phosphorylated (p)-AKT expression levels in mouse renal proximal tubular cells via Ras/PI3K through a

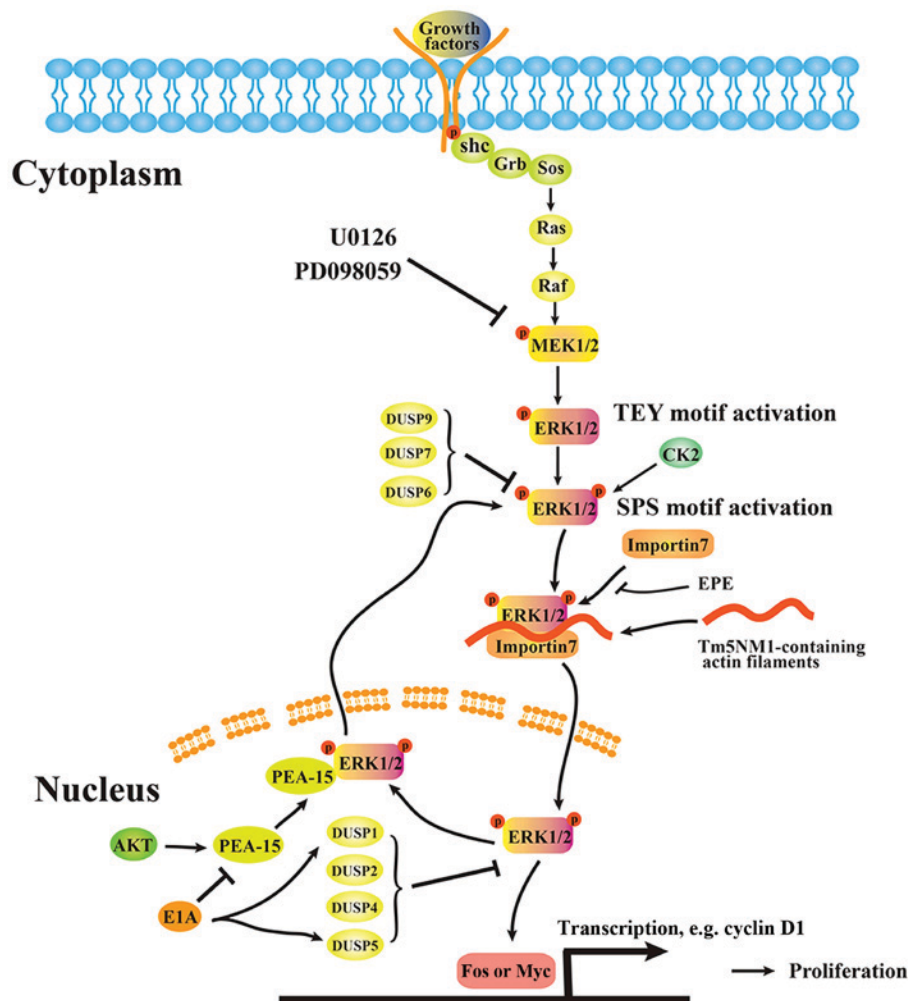


Figure 2. Schema of dynamic translocation of ERK1/2. ERK1/2 translocation from the cytoplasm to the nucleus requires dual activation of the TEY and SPS motifs, followed by binding to Imp7. Subsequently, with the help of Tm5NM1-containing actin filaments, the pSPS-pERK/Imp7/Tm5NM1-containing actin filament complex translocates into the nucleus. Although the cellular localization of ERK1/2 is dynamically regulated, ERK1/2 is exported directly by PEA-15. ERK, extra-cellular signal-regulated protein kinase; TEY, Thr-Glu-Tyr; SPS, Ser-244/Pro-245/Ser-246; Imp7, importin7; p, phosphorylated; PEA-15, astrocytic phosphoprotein PEA-15.

negative feedback pathway, whereas AKT phosphorylates and stabilizes PEA-15, which subsequently decreases the nuclear localization of ERK1/2 (123) and induces cellular senescence. Although the regulatory nodes shared by Ras/MAPK and PI3K/AKT signaling are complicated, the two signaling pathways are influenced by co-effectors, including TSC1/2, mTOR, ER and S6, and regulate each other in concentration- and context-dependent manners (124).

**Crosstalk between ERK1/2 and p53.** As a messenger of extracellular and intracellular proteins, ERK1/2 has many substrates. MEK1/2 functions upstream of ERK1/2 and serves a vital regulatory role in ERK1/2 activation. MEK1/ERK signaling promotes cell proliferation, whereas MEK2/ERK signaling promotes G<sub>1</sub>/S cell cycle arrest (125). In different tissues and cell types, including mouse embryos and fibroblasts, cellular senescence induced by the Ras/Raf/MEK/ERK signaling pathway is dependent on the integrity of p16/INK4A, p21 and p53; in human primary fibroblasts, inhibition of either p16 or p53 is not able to reverse ERK1/2-induced senescence (17,20,78). However, a previous study demonstrated that

the matrix cell protein G1/S-specific cyclin CCN1 induces senescence through the p53/p21 pathway and inhibits lung cancer growth (36). p53 and ERK1/2 have two-way regulation, which means there is a negative feedback loop between ERK1/2 and p53. Lee *et al* (126) suggested that a novel p53 target protein, Raf kinase inhibitor protein, inhibits ERK1/2 by affecting Raf proteins and promoting senescence. Notably, p53 may regulate the transcription of all nuclear DUSPs (DUSP1/2/4/5) (127-129).

**Chemicals or gene mutations lead to ERK1/2-associated senescence.** Exposure of cells to certain bioactive chemicals, including the natural ethanolic *Rhus coriaria* extract may lead to activation of ERK1/2 and p21 upregulation (130). The microtubule stabilizing agent discodermolide was identified to induce cellular senescence due to overexpressed ERK1/2 in A549 cells (66). Administration of epigallocatechin-3-gallate leads to cellular senescence during PC-3 prostate cancer cell proliferation via MEK-independent ERK1/2 activation (85). In addition, a Ras or B-Raf mutation is common in tumors, particularly in malignant tumors, which may lead to sustained

activation of ERK1/2 signaling. In specific cases, downstream proteins are activated by ERK1/2 aberrantly, resulting in different physiological effects (103). Expression of proteins downstream of ERK1/2, including BRAF-induced insulin-like growth factor-binding protein 7 (IGFBP7) in normal melanoma cells, is low and primarily controlled by autocrine or paracrine functions that influence cell proliferation (103). Examples of such phenomenon include BRAFV600E-positive nevi, which contain high BRAF expression; the continuously activated RAF/MEK/ERK pathway, which increases IGFBP7 expression; and high expression levels of IGFBP7, which inhibit the RAF/MEK/ERK pathway within cells. However, in melanoma cells, IGFBP7 expression is lost, which results in uncontrolled proliferation (131).

*Relationship between ERK1/2 and micro (mi)RNAs.* miRNAs are important for regulating cell biology. Previous studies demonstrated that miRNA-34a induces persistent activation of ERK1/2, leading to cellular senescence via inhibition of p53 signaling (89) and MEK1/2 (132). miR-21 increases the expression level of p-ERK1/2 by inhibiting Spry1 (116) and Spry2 (133).

*Cellular localization and duration influence ERK1/2-associated cell fate*

*Negative feedback occurs when ERK1/2 is overexpressed.* A number of previous studies suggest that ERK1/2-induced cellular senescence may be associated with the strength of ERK1/2 signals and the duration of its activation (20,77,78,86,90,134). As ERK1/2 has many substrates, different biological effects may occur when the signal proteins compete for the same target protein. As discussed above, many negative feedback loops and regulatory nodes shape ERK1/2 signaling, and when ERK1/2 is overexpressed, it may activate those negative loops. However, this regulatory system is dependent on the cellular context.

*Translocation from the cytoplasm to the nucleus determines the role of ERK1/2.* Scaffold proteins facilitate protein translocation, and translocation of ERK1/2 from the cytoplasm to the nucleus is essential for regulation of the cell cycle and cellular proliferation (135). This process requires dual-phosphorylation of specific residues within the activation loop. ERK is initially phosphorylated by MAPK/ERK kinase (MEK) on the Thr-Glu-Tyr motif, with subsequent phosphorylation on the Ser-244/Pro-245/Ser-246 (SPS) nuclear translocation sequence (NTS) (108,109). This is achieved primarily by CK2 to generate pSPS-pERK, which binds to the shuttling protein importin7 (Imp7) (136,137). A previous study investigating mouse embryo fibroblasts demonstrated that Tm5NM1-containing actin filaments facilitate the binding of pSPS-pERK (Ser-244/Pro-245/Ser-246) and Imp7, possibly by functioning as a scaffold and/or recruiting myosin motors to assist in the physical transportation of pSPS-pERK from the cytoplasm to the nucleus (136). Inhibiting the binding of pSPS-pERK and Imp7 appears to be an effective way of blocking the translocation of dual-phosphorylated ERK. Plotnikov *et al* (135) developed a myristoylated, NTS-derived phosphomimetic peptide (EPE peptide) that competes with the binding of Imp7 and blocks this process in a number of cell lines.

ETS translocation variant 4 (E1A) is additionally a negative regulator of activated ERK1/2 translocation from the cytoplasm to the nucleus (79,138). A previous study examining normal human diploid fibroblast IMR90 cells demonstrated that E1A decreases expression levels of PEA-15 (139), an ERK1/2 nuclear export factor, and increases expression levels of MKP1/DUSP1 and DUSP5 (138). The translocation regulation model is presented in Fig. 2.

## 5. Conclusion

ERK1/2 serves a vital role in cellular outcomes, which involve numerous substrates, regulators and scaffolding proteins. If a small dose of a MEK1/2 inhibitor, including U0126 or PD098059, is applied to a cell, the cell will recover by utilizing a compensatory pathway to regain its proliferation capacity. Even in the same type of cell or animal model, ERK1/2 serves a dual role in cellular senescence under different circumstances, which are dose- and duration-dependent. Cells subjected to drugs against ERK1/2 may additionally contain mutations in upstream (AKT, MEK and Ras/Raf) or downstream (p21 and p53) proteins. Analyzing cells for Ras/Raf/MAPK mutations and testing cell sensitivity may help to determine the proper dosage and duration of drug administration. In terms of drug development, altering the translocation of ERK1/2 from the cytoplasm to the nucleus (135), which is primarily required for induction of cell proliferation, may help to decrease the proliferation of cancer cells. However, as cells may shift their proliferation signals between a number of different proteins, a combination of numerous anti-proliferation tactics require consideration.

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## Availability of data and materials

Not applicable.

## Authors' contributions

JZ and TL contributed equally in reviewing the publications and writing this manuscript. PG and DH drafted the manuscript. JY, QX, YL and RK designed the schema of ERK1/2 signaling. All authors read and approved the final version of the manuscript.

## Ethics approval and consent to participate

Not applicable.



## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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