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Growth arrest signaling of the Raf/MEK/ERK pathway in cancer

Jong-In Park
Department of Biochemistry, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA

Abstract

The Raf/MEK/extracellular signal-regulated kinase (ERK) pathway has a pivotal role in facilitating cell proliferation, and its deregulated activation is a central signature of many epithelial cancers. However paradoxically, sustained activity of Raf/MEK/ERK can also result in growth arrest in many different cell types. This anti-proliferative Raf/MEK/ERK signaling also has physiological significance, as exemplified by its potential as a tumor suppressive mechanism. Therefore, significant questions include in which cell types and by what mechanisms this pathway can mediate such an opposing context of signaling. Particularly, our understating of the role of ERK1 and ERK2, the focal points of pathway signaling, in growth arrest signaling is still limited. This review discusses these aspects of Raf/MEK/ERK-mediated growth arrest signaling.

Keywords

Raf; MEK1/2; ERK1/2; proliferation; growth arrest; non-kinase effect

1. Introduction

Since the discovery of Fus3p, a component of the pheromone response pathway in the yeast *S. cerevisiae*, in 1989 and its mammalian counterpart extracellular signal-regulated kinase (ERK) 1 in 1991 (Courchesne et al., 1989; Boulton et al., 1990), a vast amount of knowledge has expanded the biological significance of the mitogen-activated protein kinase (MAPK) pathway spanning from early development to various diseases with tremendous implication in cancer. In response to the signals from different receptor tyrosine kinases and other cell surface receptors, the Raf/MEK/ERK pathway regulates cell survival, cell cycle progression and differentiation, and its deregulated signaling is a central signature of many epithelial cancers [reviewed in (Dhillon et al., 2007; Roberts and Der, 2007; Lawrence et al., 2008; McCubrey et al., 2012)]. Raf/MEK/ERK is a highly specific three-layered kinase cascade that consists of the Ser/Thr kinase Raf (i.e., A-Raf, B-Raf, or C-Raf/Raf-1), the highly homologous dual-specificity kinases MEK1/MAP2K1 and MEK2/MAP2K2 (collectively referred to MEK1/2), and the ubiquitously expressed Ser/Thr kinase ERK1/MAPK3 and its homolog ERK2/MAPK1 (collectively referred to ERK1/2). These molecular switches are controlled by a complex network of regulators, including the small GTPases

Correspondence: Jong-In PARK, jipark@mcw.edu.

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Ras and Rap, phosphatases, scaffolds, and other kinases, which affects the magnitude, duration, and compartmentalization of the pathway activity. Detailed mechanisms of these regulations have been extensively reviewed elsewhere (Pearson et al., 2001; Shaul and Seger, 2007; Wortzel and Seger, 2011; Roskoski, 2012).

Although mainly known for its critical role in promoting cell survival and driving cell cycle progression in response to mitogenic signals, Raf/MEK/ERK can also mediate growth inhibitory signaling, including cell death and cell cycle arrest in response to a variety of signals [reviewed in (Cagnol and Chambard, 2010; Mebratu and Tesfaigzi, 2009; Subramaniam and Unsicker, 2009)]. This anti-proliferative pathway signaling has significance in different physiological settings, including early development, neuronal differentiation, and tumor response to chemotherapy. Most notably, substantial attention has recently been paid to the potential of Raf/MEK/ERK-mediated growth arrest as a tumor suppressive mechanism. This review discusses cell types in which aberrant Raf/MEK/ERK activation can induce growth arrest and some of recent updates regarding how Raf/MEK/ERK mediates the opposing context of signal transduction.

2. Cell types in which aberrant Raf/MEK/ERK activation can induce growth arrest

Oncogenic Ras- or Raf-mediated growth inhibition was initially proposed as a potential tumor suppressive response based upon the observations made in primary normal human diploid fibroblast cells. In these cells, oncogenic Ras could induce senescence-like growth arrest, which was associated with induction of the tumor suppressor TP53 and the cyclin-dependent kinase inhibitors $p16^{INK4A}$ and $p21^{CIP1}$ (Serrano et al., 1997). Many of these effects were reproduced by constitutively active Raf mutants, establishing Raf as a major effector of Ras-induced growth inhibition (Lin et al., 1998; Zhu et al., 1998). It was then demonstrated that oncogenic Ras and Raf could also induce growth arrest in different primary cultured normal epithelial cells, including keratinocytes (Roper et al., 2001) and mammary epithelial cells (Olsen et al., 2002). Subsequently, cells in senescence-like growth arrest state were indeed detected in premalignant lesions of Ras/Raf-transformed tumors from animal models and human patients, including K-Ras^{G12V}-induced pancreatic cancer and lymphoma in mice (Braig et al., 2005; Collado et al., 2005) and B-Raf^{V600E}-mutated human melanoma (Michaloglou et al., 2005). These phenomena, referred to as “oncogene-induced senescence,” are now interpreted in a context such that proliferative programs in mammalian cells are interfaced with a variety of innate tumor-suppressive mechanisms, which trigger cell death or senescence-like growth arrest in response to aberrant cell proliferation signals [reviewed in (Mooi and Peeper, 2006; Courtois-Cox et al., 2008; McDuff and Turner, 2011)]. These mechanisms should be inactivated for carcinogenesis to occur (a conceptual model depicted in Fig. 1).

Intriguingly, growth inhibitory effects of Raf/MEK/ERK are not limited to normal cell types. It has long been known that expression of constitutively active Ras, Raf, or MEK mutants could induce irreversible growth arrest in different malignant tumor cells which were not transformed by Ras or Raf. These cell lines were derived from diverse tumor types, including small cell lung carcinoma (Mabry et al., 1989; Ravi et al., 1998, 1999a),

medullary thyroid carcinoma (MTC) (Nakagawa et al., 1987; Carson et al., 1995; Carson-Walter et al., 1998; Park et al., 2003; Vaccaro et al., 2006), glioma (Fanton et al., 2001), pheochromocytoma (Wood et al., 1993; Park et al., 2005b), gastrointestinal carcinoid (Sippel et al., 2003), prostate carcinoma (Ravi et al., 1999b; Hong et al., 2011), hepatocarcinoma (Guégan et al., 2013b) and breast carcinoma (Taylor et al., 2011). In some of these tumor cell lines, Raf/MEK/ERK activation could lead to expression of senescence-associated β-galactosidase (Ravi et al., 1999b; Arthan et al., 2010; Taylor et al., 2011), a key marker used for senescence determination (Gupta and Wajapeyee, 2013). Moreover, Raf-induced growth arrest was reproducible in *in vivo* microenvironments, as demonstrated by the MTC cell line, TT, xenograft in mice (Vaccaro et al., 2006). Notably, the growth inhibitory context of Ras/Raf signaling in MTC was supported by a genetically engineered mouse model (Rb [−/+]) of MTC tumorigenesis, in which the loss of N-Ras was followed by an increased rate of spontaneous MTC development, although suppression of concurrent pituitary tumor development was observed simultaneously (Takahashi et al., 2006).

Of note, along with growth arrest, Raf/MEK/ERK could induce dramatic suppression of the oncogenes to which these tumor cells were addicted. For example, in response to Raf/MEK/ERK activation, the highly malignant human MTC cell lines TT and MZ-CRC-1 exhibited silenced expression of oncogenically mutated rearranged during transfection (*RET*) while undergoing G_0/G_1 phase cell cycle arrest and expressing increased levels of the neuroendocrine peptide hormone calcitonin (Nakagawa et al., 1987; Carson et al., 1995; Carson-Walter et al., 1998). The proto-oncogene RET encodes a receptor tyrosine kinase whose alterations are mainly etiological to the development of MTC (Pinchot et al., 2009). Investigation of the underlying mechanism for RET downregulation and growth arrest identified a leukemia inhibitory factor/gp130/JAK/STAT/IFI16-mediated autocrine/paracrine pathway (Park et al., 2003; Kim et al., 2005; Park et al., 2005a; Arthan et al., 2010), and revealed the potential of recombinant leukemia inhibitory factor to suppress MTC xenografts in mice (Starenki et al., 2013).

Similarly in the human prostate cancer line LNCaP and CWR22R*v1*, sustained Raf/MEK/ERK activation was sufficient to substantially downregulate full length as well as hormone binding domain-deficient isoforms of androgen receptor (AR) at mRNA and protein levels, which was accompanied by growth arrest (Hong et al., 2011). AR is a member of the nuclear receptor superfamily that controls the growth regulatory and differentiation pathways in prostate epithelial cells, and its dysregulation is pivotal to prostate carcinogenesis (Balk and Knudsen, 2008). Of note, ectopic expression of a constitutively active AR could inhibit Raf/MEK/ERK-mediated expression of the cyclin-dependent kinase inhibitors, $p16^{INK4A}$ and $p21^{CIP1}$, suggesting that Raf/MEK/ERK can specifically inhibit AR-mediated proliferation in certain prostate cancer types (Hong et al., 2011). These studies demonstrate that growth inhibitory signaling of Raf/MEK/ERK can occur in a broad spectrum of cell types, even including malignant tumor cells, and may be targeted to suppress some of the key oncogenic events in cancer.

How is it possible that Raf/MEK/ERK can induce growth arrest in these cancer cells? These tumor lines are generally derived from the tumor types in which Ras or Raf mutations, or deregulated MEK/ERK activity is rarely detected. It was also demonstrated in some of these

tumor cells that their basal levels of MEK/ERK activity are substantially lower than those detected in primary normal fibroblasts (Hong et al., 2009). It is therefore conceivable that Raf/MEK/ERK activation does not provide growth advantage to these tumor types and, thus, the pathway-associated tumor suppressive mechanism(s) were not inactivated by an oncogenic selection pressure in these tumor types. These characteristics were conducive to study some of the challenging questions in Raf/MEK/ERK-mediated growth arrest signaling (discussed more below).

3. The role of Raf, MEK1/2, and ERK1/2 in growth arrest signaling

Each layer of the Raf/MEK/ERK pathway consists of multiple isoforms, i.e., A-Raf, B-Raf, and c-Raf-1 at MAP3K level, MEK1 and MEK2 at MAP2K level, and ERK1 and ERK2 at MAPK level. Although additional isoforms, i.e., MEK1b and ERK1c, have been recently identified and designated for their subcellular location-specific roles, e.g., golgi fragmentation and mitotic progression (Shaul et al., 2009), the distinct roles and biochemical characteristics in the context of cell proliferation and growth arrest have been questioned mainly for these classic members of the pathway.

3.1. Raf

Although only B-Raf oncogenic mutations are detected in cancer, all three Raf kinases can induce oncogenic responses when constitutively activated, as determined earlier using their kinase domain (Samuels et al., 1993; Pritchard et al., 1995). Similarly, expression of any of all three Raf kinases was sufficient to induce growth inhibition accompanied by $p21^{CIP1}$ expression (Woods et al., 1997). Therefore, all three Raf proteins have the intrinsic property to signal cellular transformation as well as growth inhibition. Of note, titrated activation of MEK/ERK using the tamoxifen-inducible ΔRaf-1:ER, a CR3 catalytic domain of C-Raf fused to hormone binding domain of the estrogen receptor could induce biphasic responses in NIH3T3 cells, i.e., proliferation at low Raf activity whereas growth arrest at its high activity, suggesting that the magnitude of Raf/MEK/ERK activity is an important factor in determining the physiological output (Woods et al., 1997). Therefore, there is an upper threshold where increasing Raf/MEK/ERK activity switches its signaling context from proliferation to growth arrest. This upper threshold is contrasted with the generally known lower threshold which determines the minimum pathway activity required for cell proliferation (Fig. 2). This phenomenon is consistent with the notion that different duration and strength of the kinase cascade signal can lead to distinct, and even opposing, cellular processes because different levels of active ERK1/2 proteins in cells would results in the activation/inactivation of various substrates with different affinity (Shaul and Seger, 2007).

3.2. MEK1/2

MEK1 and MEK2 are activated by Raf-mediated phosphorylation of two Ser residues (i.e., Ser217/221 for MEK1 and Ser222/226 for MEK2). While single phosphorylation can induce kinase activity of MEK, dual phosphorylation increases MEK activity to its maximum capacity. MEK1 and MEK2 are abundant in cells, present around 1 μM depending upon cell types, similarly to the levels of ERK1/2 (Ferrell, 1996). In contrast, Raf is not as abundant as MEK. Therefore, signal amplification in the Raf/MEK/ERK pathway occurs at the Raf-

MEK step due to the greater molar ratio between Raf-MEK than between MEK-ERK (Ferrell, 1996). MEK1 and MEK2 are > 86% identical at the amino acid level, and early evaluation of constitutively active mutants, MEK1-ΔN3/S218E/S222D and MEK2-ΔN4/S222D/S226D revealed their functional redundancy (Mansour et al., 1996). Nevertheless, increasing evidences indicate that MEK1 and MEK2 can have distinct functions in determining the physiological output of pathway signaling. For example, MEK1 and MEK2 have been known for being subject to a selective regulation by ERK1/2 (Eblen et al., 2004), A-Raf (Wu et al., 1996), and the scaffold MP1 (Schaeffer et al., 1998). Gene deletion in mice also revealed a critical difference in their requirement at an early developmental stage (Giroux et al., 1999; Bélanger et al., 2003; Nadeau et al., 2009). Further, their unique functions in epidermal neoplasia or hepatocarcinoma growth have been reported (Scholl et al., 2009; Guégan et al., 2013b), although activation of MEK1 or MEK2 was equally sufficient to transform intestinal epithelial cells and to induce the formation of metastatic tumors (Voisin et al., 2008). It is noteworthy that determination of functional specificity of MEK1 and MEK2 can be affected by their intrinsic properties as well as gene dosage effects.

Although MEK1/2 accounts for most of Raf effects, Raf can mediate MEK1/2-independent growth inhibitory signaling, e.g., Raf-mediated apoptotic cell death (Chen et al., 2001; Dhillon et al., 2003). However, Raf-induced cell cycle arrest was abrogated by the MEK1/2 specific inhibitors or RNA interference, indicating that MEK1/2 activation is necessary for Raf-induced growth arrest (Carson-Walter et al., 1998; Ravi et al., 1998; Zhu et al., 1998; Park et al., 2003; Hong et al., 2009). In addition, ectopic expression of constitutively active MEK1 or MEK2 mutant was equally sufficient to induce growth arrest in different cell types (Hong et al., 2009; Guégan et al., 2013b), suggesting that MEK1 and MEK2 have redundant roles in the context of growth arrest signaling.

3.3. ERK1/2

ERK1 and ERK2 are activated by MEK1/2 via sequential phosphorylation of Tyr and Thr residues on the activation loop, which induces activation conformational changes. Although functioning as a Ser/Thr kinase, ERK can also autophosphorylate the Tyr residue in the activation loop (Rossomando et al., 1992; Robbins et al., 1993). ERK1 and ERK2 account for most, if not all, effects mediated by MEK1/2, and thus MEK1/2 and ERK1/2 are usually inseparable in addressing their physiological effects. This high specificity between MEK and ERK is a typical characteristic of the MAPK pathways due to the strikingly high affinity between MEK and ERK relative to a typical enzyme-substrate interaction (Fukuda et al., 1997). Upon activation, ERK1/2 can phosphorylate more than 160 substrates identified to date, which contain including transcription factors, kinases, phosphatases, cytoskeletal proteins, scaffolds, receptors and other molecular switches [reviewed in (Yoon and Seger, 2006)]. ERK substrates contain the signature, Ser/Thr-Pro (preferentially Pro-Xaa-Ser/Thr-Pro in which Xaa is any amino acid), which is found in approximately 80% of cellular proteins. Therefore, more ERK targets are to be identified. While essential biochemistry of ERK1/2 has been established, our understanding of how ERK1/2 process signals for proliferation or growth arrest is still limited. Some of the prominent questions recently addressed include:

3.3.1. Do ERK1 and ERK2 have redundant roles in mediating growth arrest signaling?—Serving as the focal point of the Raf/MEK/ERK pathway signaling, ERK1 and ERK2 have been investigated for their potential distinct role in a variety of physiological contexts. ERK1 and ERK2 are > 84% identical at the amino acid level and have highly overlapping functions under most physiological conditions. Nevertheless, gene deletion studies in mice have revealed distinct roles of ERK1 and ERK2 in developmental biology, including embryonic stem cell lineage commitment, T cell development, thymocyte maturation, and trophoblast development, with the characterization of ERK2 as being more important (Pagès et al., 1999; Saba-El-Leil et al., 2003; Fischer et al., 2005; Binétruy et al., 2007). In vitro studies of cell lines have also distinguished the role of ERK1 and ERK2 (Bessard et al., 2008; Krens et al., 2008; Lefloch et al., 2008; Shin et al., 2010; Guégan et al., 2013a; Hamilton et al., 2013; Radtke et al., 2013; Shin et al., 2013).

In the context of growth arrest signaling, ERK1 and ERK2 showed redundancy in different cell lines models. For example, in LNCaP, TT, and the glioma line U251 cells, RNA interference of ERK1 or ERK2 had only partial effects whereas simultaneous knockdown of ERK1 and ERK2 was required to effectively inhibit Raf/MEK-induced growth arrest (Hong et al., 2009). In contrast, it was suggested that ERK2, but not ERK1, is necessary for oncogenic Ras-induced senescence based upon the effects of shRNA-mediated depletion of ERK1 and ERK2 in mouse embryonic fibroblasts (Shin et al., 2013). This discrepancy may be due to the differences in cell type-specific expression levels of ERK1 and ERK2. In support of this notion, activation of ERK1, but not ERK2, had predominant role in mediating cisplatin-induced death effects in hepatocarcinoma cells (Guégan et al., 2013a). A similar situation is encountered when studying the opposite context of ERK1/2 signaling. For example, although the significance of ERK2 over ERK1 for cell proliferation and survival was suggested based upon the effects of RNA interference of ERK1/2 in NIH3T3 cells (Vantaggiato et al., 2006), later studies concluded that ERK1 and ERK2 activities are indistinguishable and that the expression levels of ERK1 and ERK2 determine their biological differences *in vitro* and *in vivo* (Lefloch et al., 2008; Voisin et al., 2010). Determination of ERK1 and ERK2 for their specific function also requires evaluation of exogenously expressed ERK1 or ERK2. A good example was demonstrated when exogenously expressed ERK2, but not ERK1, displayed sufficient effects on epithelial-to-mesenchymal transformation (Shin et al., 2010; von Thun et al., 2012). Indeed, evaluation of exogenously expressed ERK1 and ERK2 revealed their redundant roles, and unexpected effects, in Raf/MEK-induced growth arrest signaling, as discussed below.

3.3.2. Does ERK1/2 have non-kinase effects on growth arrest signaling?—Although kinase activity of ERK1/2 is central in activation or inactivation of these ERK targets, it was also demonstrated that ERK, in an in vitro reaction, can mediate non-catalytic activation of DNA topoisomerase Iα (Shapiro et al., 1999). Consistent with this, recent reports have demonstrated that ERK1/2 can mediate kinase-independent effects in cells [reviewed in (Rodríguez and Crespo, 2011)]. For example, profiling the human protein-DNA interactome revealed the ability of kinase-inactive ERK2 to interact with DNA and act as a transcriptional repressor of interferon signaling (Hu et al., 2009). It was also demonstrated that ERK2 can stabilize dual-specificity phosphatase 5 via its physical

interaction but independently of its kinase activity (Kucharska et al., 2009). In addition, ERK1/2 could promote cell cycle entry via kinase-independent disruption of retinoblastoma-lamin A complexes (Rodríguez et al., 2010). These results are consistent with a notion that ERK interactions with proteins are not necessarily predictive of whether efficient phosphoryl transfer will occur (Burkhard et al., 2011).

Kinase-independent effects of ERK1/2 were also determined in the context of Raf/MEK-induced growth arrest signaling (Hong et al., 2009; Guégan et al., 2013b). Briefly, decreases in ERK1/2 activity can arrest proliferation of many cell types, as determined by expression of kinase-deficient ERK mutants (Pagés et al., 1993; Kortenjann et al., 1994) or gene knockdown (Vantaggiato et al., 2006; Bessard et al., 2008; Lefloch et al., 2008). In contrast, some of those aforementioned Ras/Raf-responsive tumor lines mentioned above (i.e., LNCaP, TT, and U251) could tolerate substantial ERK1/2 knockdown. In these ERK1/2-knocked down yet proliferating cells, Raf could no longer induce growth arrest (Hong et al., 2009). Surprisingly, upon expression of active site-disabled ERK1 or ERK2 mutant, these cells could selectively restore Raf-induced growth arrest responses. Under this condition, overexpression of kinase-deficient ERK further depleted cells of residual ERK kinase activity, as determined by the ERK substrates $p90^{RSK}$ and Elk1, strongly supporting the presence of a non-kinase ERK effect. Intriguingly, expression of the ERK mutants with disabled activation loop was not effective in restoring the growth arrest signaling, suggesting that phosphorylation-mediated conformational changes are still required for this ERK effect (Hong et al., 2009). These effects are in contrast with the effects of kinase-deficient ERK on Raf-induced transformation or growth factor-stimulated cell proliferation, for which the necessity of ERK kinase activity was obvious (Pagés et al., 1993; Kortenjann et al., 1994). Therefore, a key mechanistic distinction between Raf/MEK/ERK pathway-mediated proliferation and growth arrest signaling appears to be determined at the level of ERK1/2.

It is important to understand the mechanism underlying these intriguing non-kinase ERK effects. It appears that kinase-deficient ERK1/2 has specific but limited effects in mediating Raf/MEK-induced growth arrest signaling. Most notably, kinase-deficient ERK1/2 could upregulate $p21^{CIP1}$ levels and subsequently induce G0/G1 phase cell cycle arrest in response to Raf/MEK activation, although it could not mediate other effects of Raf/MEK activation relevant to growth arrest signaling, e.g., c-MYC downregulation in LNCaP, and RET downregulation in TT cells (Hong et al., 2009). A recent study also demonstrated similar non-kinase ERK-mediated $p21^{CIP1}$ regulation in different cell types, including the hepatocarcinoma lines Huh-7D12 and HepG2, and the breast cancer cell line MCF7 (Guégan et al., 2013b). Moreover, this study demonstrated that kinase-deficient ERK could regulate $p21^{CIP1}$ translation by regulating p70 S6 kinase, a key effector of mTOR complex 1 (mTORC1), suggesting an involvement of mTORC1-mediated translational regulation in this ERK effect. Importantly, in the context of cell proliferative signaling, ERK2, albeit not ERK1, phosphorylated Thr57 and Ser130 of $p21^{CIP1}$, which subsequently induced nuclear export, ubiquitination, and proteasomal degradation of $p21^{CIP1}$ (Hwang et al., 2009). These effects of ERK1/2 on $p21^{CIP1}$ in mediating growth arrest versus proliferation are in stark contrast, suggesting that a distinct mode of ERK1/2 signaling is involved in the opposing contexts of signal transduction (Fig. 3).

Noteworthy is that interpretation of the results in the context of non-kinase ERK function is limited by the presence of residual endogenous ERK in the ERK1/2-knocked down cell models. It may be possible that overexpression of kinase-deficient ERK facilitates subcellular location-specific activation of the residual ERK1/2 despite the decreases in net ERK kinase activity in cells. Indeed, it was reported that not all ERK in active state mediate catalytic reaction but substantial portion of them serve as the adaptor for those that phosphorylate substrates (Casar et al., 2008). Currently, the model to address this issue is not available because cells cannot tolerate complete ablation of ERK1/2 (Pagés et al., 1999; Saba-El-Leil et al., 2003).

5. Concluding remarks

Although mechanistic dichotomy of Raf/MEK/ERK pathway-mediated proliferation and growth arrest is still incomplete, it has been rigorously studied by what mechanism(s) growth arrest signaling is inactivated in the course of tumorigenesis. While a number of carcinogenic molecular alterations occur at upstream and downstream levels of the pathway [reviewed in (Courtois-Cox et al., 2008; McDuff and Turner, 2011)], growing evidences suggest that alteration of Raf/MEK/ERK activity through other kinases, scaffolds, and molecular chaperones also contribute to bypassing growth arrest (Cheung et al., 2008; Duhamel et al., 2012; Wu et al., 2013). Remarkably, it seems possible to reactivate Raf/MEK/ERK-mediated senescence-like growth arrest signaling in cancer by targeting an alteration in these regulators (Wu et al., 2013). Of note, activation of Raf/MEK/ERK can induce growth arrest even in the cancer cells defective of the key tumor suppressor(s), Rb, TP53, $p16^{INK4A}$, or $p21^{WAF}$, suggesting that multiple parallel independent tumor suppressive mechanisms are networked to Raf/MEK/ERK. Better understanding of the connection between Raf/MEK/ERK and these mechanisms may allow the development of a novel therapeutic strategy.

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References

Arthan D, Hong SK, Park JI. Leukemia inhibitory factor can mediate Ras/Raf/MEK/ERK-induced growth inhibitory signaling in medullary thyroid cancer cells. Cancer Lett. 2010; 297(1):31–41. PMID:20570039. [PubMed: 20570039]

Balk SP, Knudsen KE. AR, the cell cycle, and prostate cancer. Nucl Recept Signal. 2008; 6:e001. PMID:18301781. [PubMed: 18301781]

Bélanger LF, Roy S, Tremblay M, Brott B, Steff AM, Mourad W, Hugo P, Erikson R, Charron J. Mek2 is dispensable for mouse growth and development. Mol Cell Biol. 2003; 23(14):4778–4787. PMID:12832465. [PubMed: 12832465]

Bessard A, Frémin C, Ezan F, Fautrel A, Gailhouste L, Baffet G. RNAi-mediated ERK2 knockdown inhibits growth of tumor cells *in vitro* and *in vivo* . Oncogene. 2008; 27(40):5315–5325. PMID: 18521085. [PubMed: 18521085]

Binétruy B, Heasley L, Bost F, Caron L, Aouadi M. Concise review: regulation of embryonic stem cell lineage commitment by mitogen-activated protein kinases. Stem Cells. 2007; 25(5):1090–1095. PMID:17218395. [PubMed: 17218395]

Boulton TG, Yancopoulos GD, Gregory JS, Slaughter C, Moomaw C, Hsu J, Cobb MH. An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control. Science. 1990; 249(4964):64–67. PMID:2164259. [PubMed: 2164259]

Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dörken B, Jenuwein T, Schmitt CA. Oncogene-induced senescence as an initial barrier in lymphoma development. Nature. 2005; 436(7051):660–665. PMID:16079837. [PubMed: 16079837]

Burkhard KA, Chen F, Shapiro P. Quantitative analysis of ERK2 interactions with substrate proteins: roles for kinase docking domains and activity in determining binding affinity. J Biol Chem. 2011; 286(4):2477–2485. PMID:21098038. [PubMed: 21098038]

Cagnol S, Chambard JC. ERK and cell death: mechanisms of ERK-induced cell death—apoptosis, autophagy and senescence. FEBS J. 2010; 277(1):2–21. PMID:19843174. [PubMed: 19843174]

Carson EB, McMahon M, Baylin SB, Nelkin BD. Ret gene silencing is associated with Raf-1-induced medullary thyroid carcinoma cell differentiation. Cancer Res. 1995; 55(10):2048–2052. PMID: 7743500. [PubMed: 7743500]

Carson-Walter EB, Smith DP, Ponder BA, Baylin SB, Nelkin BD. Post-transcriptional silencing of RET occurs, but is not required, during raf-1 mediated differentiation of medullary thyroid carcinoma cells. Oncogene. 1998; 17(3):367–376. PMID:9690518. [PubMed: 9690518]

Casar B, Pinto A, Crespo P. Essential role of ERK dimers in the activation of cytoplasmic but not nuclear substrates by ERK-scaffold complexes. Mol Cell. 2008; 31(5):708–721. PMID:18775330. [PubMed: 18775330]

Chen J, Fujii K, Zhang L, Roberts T, Fu H. Raf-1 promotes cell survival by antagonizing apoptosis signal-regulating kinase 1 through a MEK-ERK independent mechanism. Proc Natl Acad Sci USA. 2001; 98(14):7783–7788. PMID:11427728. [PubMed: 11427728]

Cheung M, Sharma A, Madhunapantula SV, Robertson GP. Akt3 and mutant V600E B-Raf cooperate to promote early melanoma development. Cancer Res. 2008; 68(9):3429–3439. PMID:18451171. [PubMed: 18451171]

Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguría A, Zaballos A, Flores JM, Barbacid M, Beach D, Serrano M. Tumour biology: senescence in premalignant tumours. Nature. 2005; 436(7051):642. PMID:16079833. [PubMed: 16079833]

Courchesne WE, Kunisawa R, Thorner J. A putative protein kinase overcomes pheromone-induced arrest of cell cycling in S. cerevisiae. Cell. 1989; 58(6):1107–1119. PMID:2673544. [PubMed: 2673544]

Courtois-Cox S, Jones SL, Cichowski K. Many roads lead to oncogene-induced senescence. Oncogene. 2008; 27(20):2801–2809. PMID:18193093. [PubMed: 18193093]

Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. Oncogene. 2007; 26(22):3279–3290. PMID:17496922. [PubMed: 17496922]

Dhillon AS, Meikle S, Peyssonnaux C, Grindlay J, Kaiser C, Steen H, Shaw PE, Mischak H, Eychène A, Kolch W. A Raf-1 mutant that dissociates MEK/extracellular signal-regulated kinase activation from malignant transformation and differentiation but not proliferation. Mol Cell Biol. 2003; 23(6):1983–1993. PMID:12612072. [PubMed: 12612072]

Duhamel S, Hébert J, Gaboury L, Bouchard A, Simon R, Sauter G, Basik M, Meloche S. Sef downregulation by Ras causes MEK1/2 to become aberrantly nuclear localized leading to polyploidy and neoplastic transformation. Cancer Res. 2012; 72(3):626–635. PMID:22298595. [PubMed: 22298595]

Eblen ST, Slack-Davis JK, Tarcsafalvi A, Parsons JT, Weber MJ, Catling AD. Mitogen-activated protein kinase feedback phosphorylation regulates MEK1 complex formation and activation during cellular adhesion. Mol Cell Biol. 2004; 24(6):2308–2317. PMID:14993270. [PubMed: 14993270]

Fanton CP, McMahon M, Pieper RO. Dual growth arrest pathways in astrocytes and astrocytic tumors in response to Raf-1 activation. J Biol Chem. 2001; 276(22):18871–18877. PMID:11278920. [PubMed: 11278920]

Ferrell JE Jr. Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. Trends Biochem Sci. 1996; 21(12):460–466. PMID:9009826. [PubMed: 9009826]

Fischer AM, Katayama CD, Pagès G, Pouysségur J, Hedrick SM. The role of erk1 and erk2 in multiple stages of T cell development. Immunity. 2005; 23(4):431–443. PMID:16226508. [PubMed: 16226508]

Fukuda M, Gotoh Y, Nishida E. Interaction of MAP kinase with MAP kinase kinase: its possible role in the control of nucleocytoplasmic transport of MAP kinase. EMBO J. 1997; 16(8):1901–1908. PMID:9155016. [PubMed: 9155016]

Giroux S, Tremblay M, Bernard D, Cardin-Girard JF, Aubry S, Larouche L, Rousseau S, Huot J, Landry J, Jeannotte L, Charron J. Embryonic death of Mek1-deficient mice reveals a role for this kinase in angiogenesis in the labyrinthine region of the placenta. Curr Biol. 1999; 9(7):369–372. PMID:10209122. [PubMed: 10209122]

Guégan JP, Ezan F, Gailhouste L, Langouët S, Baffet G. MEK1/2 Overactivation can Promote Growth Arrest by Mediating ERK1/2-Dependent Phosphorylation of p70S6K. J Cell Physiol. 2013b n/a, PMID:24501087.

Guégan JP, Ezan F, Théret N, Langouët S, Baffet G. MAPK signaling in cisplatin-induced death: predominant role of ERK1 over ERK2 in human hepatocellular carcinoma cells. Carcinogenesis. 2013a; 34(1):38–47. PMID:23042098. [PubMed: 23042098]

Gupta R, Wajapeyee N. Induction of cellular senescence by oncogenic RAS. Methods Mol Biol. 2013; 1048:127–133. PMID:23929102. [PubMed: 23929102]

Hamilton WB, Kaji K, Kunath T. ERK2 suppresses self-renewal capacity of embryonic stem cells, but is not required for multi-lineage commitment. PLoS ONE. 2013; 8(4):e60907. PMID:23613754. [PubMed: 23613754]

Hong SK, Kim JH, Lin MF, Park JI. The Raf/MEK/extracellular signal-regulated kinase 1/2 pathway can mediate growth inhibitory and differentiation signaling via androgen receptor downregulation in prostate cancer cells. Exp Cell Res. 2011; 317(18):2671–2682. PMID:21871886. [PubMed: 21871886]

Hong SK, Yoon S, Moelling C, Arthan D, Park JI. Noncatalytic function of ERK1/2 can promote Raf/MEK/ERK-mediated growth arrest signaling. J Biol Chem. 2009; 284(48):33006–33018. PMID:19805545. [PubMed: 19805545]

Hu S, Xie Z, Onishi A, Yu X, Jiang L, Lin J, Rho HS, Woodard C, Wang H, Jeong JS, Long S, He X, Wade H, Blackshaw S, Qian J, Zhu H. Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. Cell. 2009; 139(3):610–622. PMID: 19879846. [PubMed: 19879846]

Hwang CY, Lee C, Kwon KS. Extracellular signal-regulated kinase 2-dependent phosphorylation induces cytoplasmic localization and degradation of p21Cip1. Mol Cell Biol. 2009; 29(12):3379–3389. PMID:19364816. [PubMed: 19364816]

Kim EJ, Park JI, Nelkin BD. IFI16 is an essential mediator of growth inhibition, but not differentiation, induced by the leukemia inhibitory factor/JAK/STAT pathway in medullary thyroid carcinoma cells. J Biol Chem. 2005; 280(6):4913–4920. PMID:15572361. [PubMed: 15572361]

Kortenjann M, Thomae O, Shaw PE. Inhibition of v-raf-dependent c-fos expression and transformation by a kinase-defective mutant of the mitogen-activated protein kinase Erk2. Mol Cell Biol. 1994; 14(7):4815–4824. PMID:8007980. [PubMed: 8007980]

Krens SF, He S, Lamers GE, Meijer AH, Bakkers J, Schmidt T, Spaink HP, Snaar-Jagalska BE. Distinct functions for ERK1 and ERK2 in cell migration processes during zebrafish gastrulation. Dev Biol. 2008; 319(2):370–383. PMID:18514184. [PubMed: 18514184]

Kucharska A, Rushworth LK, Staples C, Morrice NA, Keyse SM. Regulation of the inducible nuclear dual-specificity phosphatase DUSP5 by ERK MAPK. Cell Signal. 2009; 21(12):1794–1805. PMID:19666109. [PubMed: 19666109]

Lawrence MC, Jivan A, Shao C, Duan L, Goad D, Zaganjor E, Osborne J, McGlynn K, Stippec S, Earnest S, Chen W, Cobb MH. The roles of MAPKs in disease. Cell Res. 2008; 18(4):436–442. PMID:18347614. [PubMed: 18347614]

Lefloch R, Pouysségur J, Lenormand P. Single and combined silencing of ERK1 and ERK2 reveals their positive contribution to growth signaling depending on their expression levels. Mol Cell Biol. 2008; 28(1):511–527. PMID:17967895. [PubMed: 17967895]

Lin AW, Barradas M, Stone JC, van Aelst L, Serrano M, Lowe SW. Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. Genes Dev. 1998; 12(19):3008–3019. PMID:9765203. [PubMed: 9765203]

Mabry M, Nakagawa T, Baylin S, Pettengill O, Sorenson G, Nelkin B. Insertion of the v-Ha-ras oncogene induces differentiation of calcitonin-producing human small cell lung cancer. J Clin Invest. 1989; 84(1):194–199. PMID:2544624. [PubMed: 2544624]

Mansour SJ, Candia JM, Gloor KK, Ahn NG. Constitutively active mitogen-activated protein kinase kinase 1 (MAPKK1) and MAPKK2 mediate similar transcriptional and morphological responses. Cell Growth Differ. 1996; 7(2):243–250. PMID:8822208. [PubMed: 8822208]

McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Montalto G, Cervello M, Nicoletti F, Fagone P, Malaponte G, Mazzarino MC, Candido S, Libra M, Bäsecke J, Mijatovic S, Maksimovic-Ivanic D, Milella M, Tafuri A, Cocco L, Evangelisti C, Chiarini F, Martelli AM. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades which alter therapy response. Oncotarget. 2012; 3(9):954–987. PMID:23006971. [PubMed: 23006971]

McDuff FK, Turner SD. Jailbreak: oncogene-induced senescence and its evasion. Cell Signal. 2011; 23(1):6–13. PMID:20633638. [PubMed: 20633638]

Mebratu Y, Tesfaigz iY. How ERK1/2 activation controls cell proliferation and cell death: Is subcellular localization the answer? Cell Cycle. 2009; 8(8):1168–1175. PMID:19282669. [PubMed: 19282669]

Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005; 436(7051):720–724. PMID:16079850. [PubMed: 16079850]

Mooi WJ, Peeper DS. Oncogene-induced cell senescence—halting on the road to cancer. N Engl J Med. 2006; 355(10):1037–1046. PMID:16957149. [PubMed: 16957149]

Nadeau V, Guillemette S, Bélanger LF, Jacob O, Roy S, Charron J. Map2k1 and Map2k2 genes contribute to the normal development of syncytiotrophoblasts during placentation. Development. 2009; 136(8):1363–1374. PMID:19304888. [PubMed: 19304888]

Nakagawa T, Mabry M, de Bustros A, Ihle JN, Nelkin BD, Baylin SB. Introduction of v-Ha-ras oncogene induces differentiation of cultured human medullary thyroid carcinoma cells. Proc Natl Acad Sci USA. 1987; 84(16):5923–5927. PMID:3112776. [PubMed: 3112776]

Olsen CL, Gardie B, Yaswen P, Stampfer MR. Raf-1-induced growth arrest in human mammary epithelial cells is p16-independent and is overcome in immortal cells during conversion. Oncogene. 2002; 21(41):6328–6339. PMID:12214273. [PubMed: 12214273]

Pagès G, Guérin S, Grall D, Bonino F, Smith A, Anjuere F, Auberger P, Pouysségur J. Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. Science. 1999; 286(5443):1374–1377. PMID:10558995. [PubMed: 10558995]

Pagès G, Lenormand P, L'Allemain G, Chambard JC, Meloche S, Pouysségur J. Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation. Proc Natl Acad Sci USA. 1993; 90(18):8319–8323. PMID:8397401. [PubMed: 8397401]

Park JI, Powers JF, Tischler AS, Strock CJ, Ball DW, Nelkin BD. GDNF-induced leukemia inhibitory factor can mediate differentiation via the MEK/ERK pathway in pheochromocytoma cells derived from nf1-heterozygous knockout mice. Exp Cell Res. 2005b; 303(1):79–88. PMID:15572029. [PubMed: 15572029]

Park JI, Strock CJ, Ball DW, Nelkin BD. The Ras/Raf/MEK/extracellular signal-regulated kinase pathway induces autocrine-paracrine growth inhibition via the leukemia inhibitory factor/JAK/STAT pathway. Mol Cell Biol. 2003; 23(2):543–554. PMID:12509453. [PubMed: 12509453]

Park JI, Strock CJ, Ball DW, Nelkin BD. Interleukin-1beta can mediate growth arrest and differentiation via the leukemia inhibitory factor/JAK/STAT pathway in medullary thyroid carcinoma cells. Cytokine. 2005a; 29(3):125–134. PMID:15613280. [PubMed: 15613280]

Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 2001; 22(2):153–183. PMID:11294822. [PubMed: 11294822]

Pinchot SN, Kunnimalaiyaan M, Sippel RS, Chen H. Medullary thyroid carcinoma: targeted therapies and future directions. J Oncol. 2009; 2009:183031. PMID:20069043. [PubMed: 20069043]

Pritchard CA, Samuels ML, Bosch E, McMahon M. Conditionally oncogenic forms of the A-Raf and B-Raf protein kinases display different biological and biochemical properties in NIH 3T3 cells. Mol Cell Biol. 1995; 15(11):6430–6442. PMID:7565795. [PubMed: 7565795]

Radtke S, Milanovic M, Rossé C, De Rycker M, Lachmann S, Hibbert A, Kermorgant S, Parker PJ. ERK2 but not ERK1 mediates HGF-induced motility in non-small cell lung carcinoma cell lines. J Cell Sci. 2013; 126(Pt 11):2381–2391. PMID:23549785. [PubMed: 23549785]

Ravi RK, McMahon M, Yangang Z, Williams JR, Dillehay LE, Nelkin BD, Mabry M. Raf-1-induced cell cycle arrest in LNCaP human prostate cancer cells. J Cell Biochem. 1999b; 72(4):458–469. PMID:10022606. [PubMed: 10022606]

Ravi RK, Thiagalingam A, Weber E, McMahon M, Nelkin BD, Mabry M. Raf-1 causes growth suppression and alteration of neuroendocrine markers in DMS53 human small-cell lung cancer cells. Am J Respir Cell Mol Biol. 1999a; 20(4):543–549. PMID:10100985. [PubMed: 10100985]

Ravi RK, Weber E, McMahon M, Williams JR, Baylin S, Mal A, Harter ML, Dillehay LE, Claudio PP, Giordano A, Nelkin BD, Mabry M. Activated Raf-1 causes growth arrest in human small cell lung cancer cells. J Clin Invest. 1998; 101(1):153–159. PMID:9421477. [PubMed: 9421477]

Robbins DJ, Zhen E, Owaki H, Vanderbilt CA, Ebert D, Geppert TD, Cobb MH. Regulation and properties of extracellular signal-regulated protein kinases 1 and 2 in vitro. J Biol Chem. 1993; 268(7):5097–5106. PMID:8444886. [PubMed: 8444886]

Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007; 26(22):3291–3310. PMID:17496923. [PubMed: 17496923]

Rodríguez J, Calvo F, González JM, Casar B, Andrés V, Crespo P. ERK1/2 MAP kinases promote cell cycle entry by rapid, kinase-independent disruption of retinoblastoma-lamin A complexes. J Cell Biol. 2010; 191(5):967–979. PMID:21115804. [PubMed: 21115804]

Rodríguez J, Crespo P. Working without kinase activity: phosphotransfer-independent functions of extracellular signal-regulated kinases. Sci Signal. 2011; 4(196):re3. PMID:22028468. [PubMed: 22028468]

Roper E, Weinberg W, Watt FM, Land H. p19ARF-independent induction of p53 and cell cycle arrest by Raf in murine keratinocytes. EMBO Rep. 2001; 2(2):145–150. PMID:11258707. [PubMed: 11258707]

Roskoski R Jr. ERK1/2 MAP kinases: structure, function, and regulation. Pharmacol Res. 2012; 66(2): 105–143. PMID:22569528. [PubMed: 22569528]

Rossomando AJ, Wu J, Michel H, Shabanowitz J, Hunt DF, Weber MJ, Sturgill TW. Identification of Tyr-185 as the site of tyrosine autophosphorylation of recombinant mitogen-activated protein kinase p42mapk. Proc Natl Acad Sci USA. 1992; 89(13):5779–5783. PMID:1378617. [PubMed: 1378617]

Saba-El-Leil MK, Vella FD, Vernay B, Voisin L, Chen L, Labrecque N, Ang SL, Meloche S. An essential function of the mitogen-activated protein kinase Erk2 in mouse trophoblast development. EMBO Rep. 2003; 4(10):964–968. PMID:14502223. [PubMed: 14502223]

Samuels ML, Weber MJ, Bishop JM, McMahon M. Conditional transformation of cells and rapid activation of the mitogen-activated protein kinase cascade by an estradiol-dependent human raf-1 protein kinase. Mol Cell Biol. 1993; 13(10):6241–6252. PMID:8413224. [PubMed: 8413224]

Schaeffer HJ, Catling AD, Eblen ST, Collier LS, Krauss A, Weber MJ. MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. Science. 1998; 281(5383):1668–1671. PMID:9733512. [PubMed: 9733512]

Scholl FA, Dumesic PA, Barragan DI, Harada K, Charron J, Khavari PA. Selective role for Mek1 but not Mek2 in the induction of epidermal neoplasia. Cancer Res. 2009; 69(9):3772–3778. PMID: 19383924. [PubMed: 19383924]

Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell. 1997; 88(5):593–602. PMID:9054499. [PubMed: 9054499]

Shapiro PS, Whalen AM, Tolwinski NS, Wilsbacher J, Froelich-Ammon SJ, Garcia M, Osheroff N, Ahn NG. Extracellular signal-regulated kinase activates topoisomerase IIalpha through a mechanism independent of phosphorylation. Mol Cell Biol. 1999; 19(5):3551–3560. PMID: 1020707877. [PubMed: 10207078]

Shaul YD, Gibor G, Plotnikov A, Seger R. Specific phosphorylation and activation of ERK1c by MEK1b: a unique route in the ERK cascade. Genes Dev. 2009; 23(15):1779–1790. PMID: 19651986. [PubMed: 19651986]

Shaul YD, Seger R. The MEK/ERK cascade: from signaling specificity to diverse functions. Biochim Biophys Acta. 2007; 1773(8):1213–1226. PMID:17112607. [PubMed: 17112607]

Shin J, Yang J, Lee JC, Baek KH. Depletion of ERK2 but not ERK1 abrogates oncogenic Ras-induced senescence. Cell Signal. 2013; 25(12):2540–2547. PMID:23993963. [PubMed: 23993963]

Shin S, Dimitri CA, Yoon SO, Dowdle W, Blenis J. ERK2 but not ERK1 induces epithelial-to-mesenchymal transformation via DEF motif-dependent signaling events. Mol Cell. 2010; 38(1): 114–127. PMID:20385094. [PubMed: 20385094]

Sippel RS, Carpenter JE, Kunnimalaiyaan M, Lagerholm S, Chen H. Raf-1 activation suppresses neuroendocrine marker and hormone levels in human gastrointestinal carcinoid cells. Am J Physiol Gastrointest Liver Physiol. 2003; 285(2):G245–G254. PMID:1285121682. [PubMed: 12851216]

Starenki D, Singh NK, Jensen DR, Peterson FC, Park JI. Recombinant leukemia inhibitory factor suppresses human medullary thyroid carcinoma cell line xenografts in mice. Cancer Lett. 2013; 339(1):144–151. PMID:23856028. [PubMed: 23856028]

Subramaniam S, Unsicker K. ERK and cell death: ERK1/2 in neuronal death. FEBS J. 2010; 277:22–29. PMID:19843173. [PubMed: 19843173]

Takahashi C, Contreras B, Iwanaga T, Takegami Y, Bakker A, Bronson RT, Noda M, Loda M, Hunt JL, Ewen ME. Nras loss induces metastatic conversion of Rb1-deficient neuroendocrine thyroid tumor. Nat Genet. 2006; 38(1):118–123. PMID:16369533. [PubMed: 16369533]

Taylor JR, Lehmann BD, Chappell WH, Abrams SL, Steelman LS, McCubrey JA. Cooperative effects of Akt-1 and Raf-1 on the induction of cellular senescence in doxorubicin or tamoxifen treated breast cancer cells. Oncotarget. 2011; 2(8):610–626. PMID:2188116786. [PubMed: 21881167]

Vaccaro A, Chen H, Kunnimalaiyaan M. In-vivo activation of Raf-1 inhibits tumor growth and development in a xenograft model of human medullary thyroid cancer. Anticancer Drugs. 2006; 17(7):849–853. PMID:16926634. [PubMed: 16926634]

Vantaggiato C, Formentini I, Bondanza A, Bonini C, Naldini L, Brambilla R. ERK1 and ERK2 mitogen-activated protein kinases affect Ras-dependent cell signaling differentially. J Biol. 2006; 5(5):14. PMID:16805921. [PubMed: 16805921]

Voisin L, Julien C, Duhamel S, Gopalbhai K, Claveau I, Saba-El-Leil MK, Rodrigue-Gervais IG, Gaboury L, Lamarre D, Basik M, Meloche S. Activation of MEK1 or MEK2 isoform is sufficient to fully transform intestinal epithelial cells and induce the formation of metastatic tumors. BMC Cancer. 2008; 8(1):337. PMID:19014680. [PubMed: 19014680]

Voisin L, Saba-El-Leil MK, Julien C, Frémin C, Meloche S. Genetic demonstration of a redundant role of extracellular signal-regulated kinase 1 (ERK1) and ERK2 mitogen-activated protein kinases in promoting fibroblast proliferation. Mol Cell Biol. 2010; 30(12):2918–2932. PMID:20368360. [PubMed: 20368360]

von Thun A, Birtwistle M, Kalna G, Grindlay J, Strachan D, Kolch W, von Kriegsheim A, Norman JC. ERK2 drives tumour cell migration in three-dimensional microenvironments by suppressing expression of Rab17 and liprin-b2. J Cell Sci. 2012; 125(Pt 6):1465–1477. PMID:22328529. [PubMed: 22328529]

Wood KW, Qi H, D'Arcangelo G, Armstrong RC, Roberts TM, Halegoua S. The cytoplasmic raf oncogene induces a neuronal phenotype in PC12 cells: a potential role for cellular raf kinases in neuronal growth factor signal transduction. Proc Natl Acad Sci USA. 1993; 90(11):5016–5020. PMID:8389463. [PubMed: 8389463]

Woods D, Parry D, Cherwinski H, Bosch E, Lees E, McMahon M. Raf-induced proliferation or cell cycle arrest is determined by the level of Raf activity with arrest mediated by p21Cip1. Mol Cell Biol. 1997; 17(9):5598–5611. PMID:927143593. [PubMed: 9271435]

Wortzel I, Seger R. The ERK Cascade: Distinct Functions within Various Subcellular Organelles. Genes Cancer. 2011; 2(3):195–209. PMID:21779493. [PubMed: 21779493]

Wu PK, Hong SK, Veeranki S, Karkhanis M, Starenki D, Plaza JA, Park JI. A mortalin/HSPA9-mediated switch in tumor-suppressive signaling of Raf/MEK/extracellular signal-regulated kinase. Mol Cell Biol. 2013; 33(20):4051–4067. PMID:23959801. [PubMed: 23959801]

Wu X, Noh SJ, Zhou G, Dixon JE, Guan KL. Selective activation of MEK1 but not MEK2 by A-Raf from epidermal growth factor-stimulated Hela cells. J Biol Chem. 1996; 271(6):3265–3271. PMID:8621729. [PubMed: 8621729]

Yoon S, Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors. 2006; 24(1):21–44. PMID:16393692. [PubMed: 16393692]

Zhu J, Woods D, McMahon M, Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. Genes Dev. 1998; 12(19):2997–3007. PMID:9765202. [PubMed: 9765202]

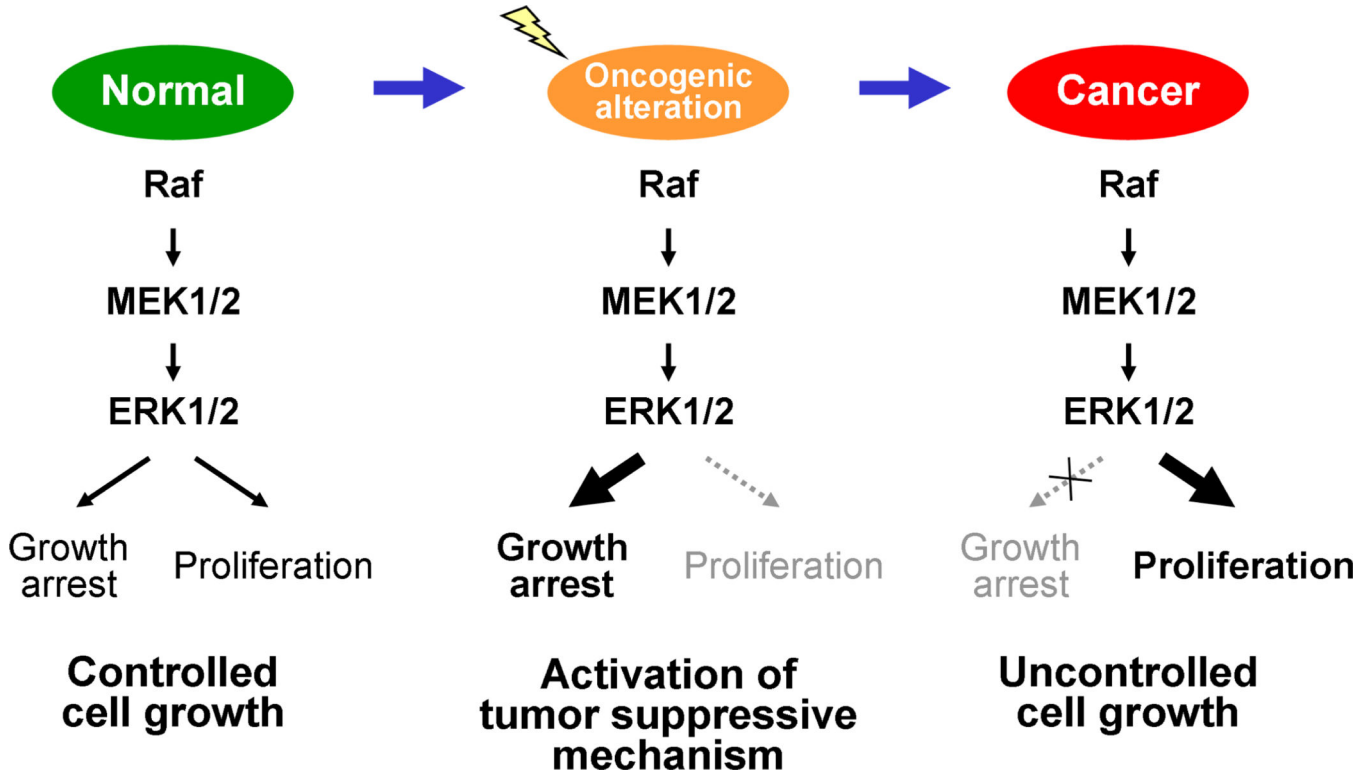

Figure 1.

A conceptual model for tumor suppressive signaling of Raf/MEK/ERK. Oncogenic alterations leading to aberrant activation of Raf/MEK/ERK can direct the pathway signaling to mediate growth arrest. Progression of tumorigenesis requires inactivation of this tumor suppressive response. In the absence of this mechanism, deregulated Raf/MEK/ERK activity is exploited to stimulate uncontrolled cell growth.

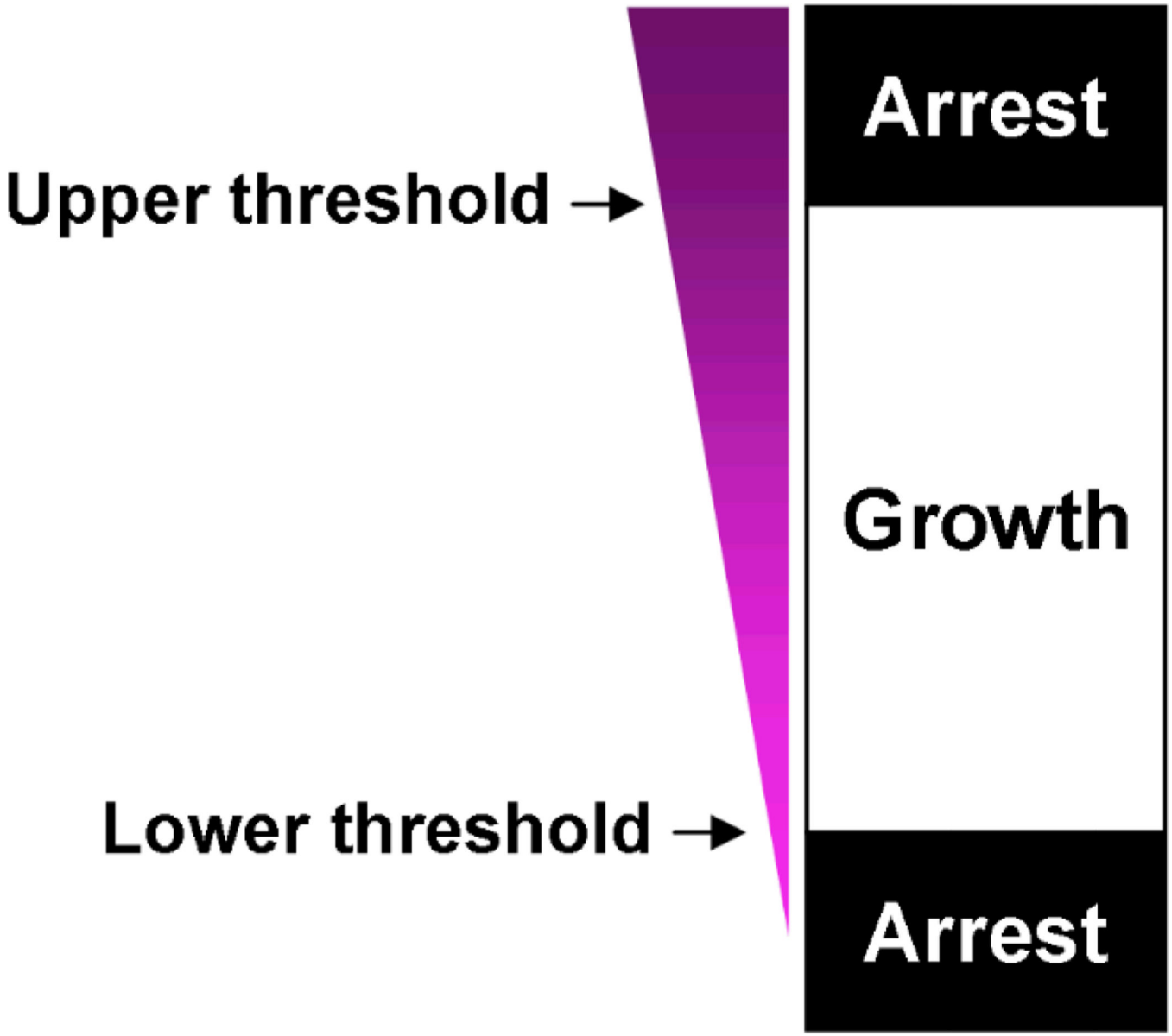

Figure 2.

Two different thresholds of Raf/MEK/ERK activity determine cell fate to proliferation or growth arrest. In this model, not only too low but also too high Raf/MEK/ERK activity restricts cell proliferation. Different cell types may maintain different extent of pathway activity between these thresholds, displaying heterogeneous sensitivity to pathway activity.

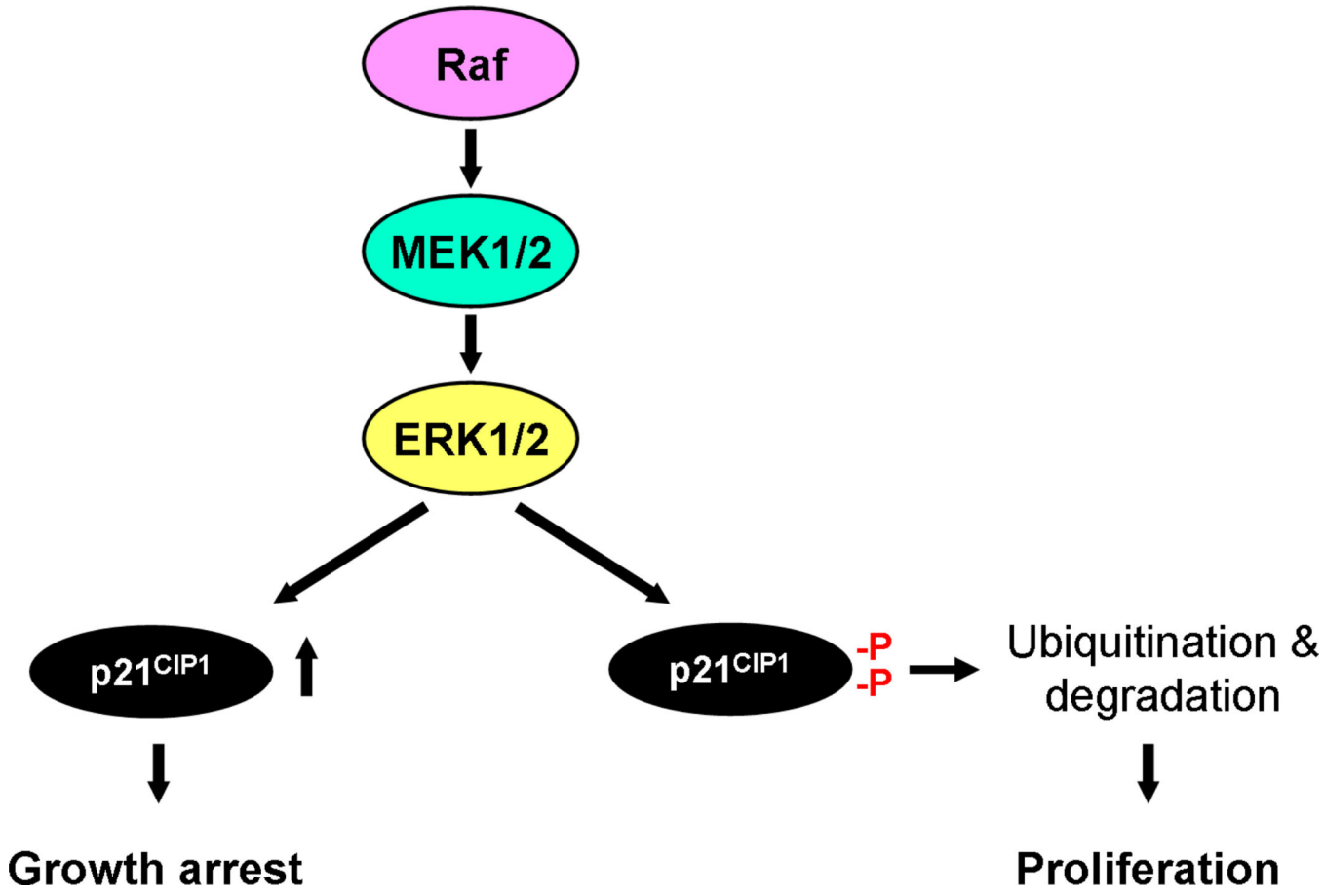

Figure 3.

Regulation of $p21^{CIP1}$ contrasts ERK1/2 function in the opposing contexts of Raf/MEK/ERK signaling, i.e., proliferation versus growth arrest. To promote cell proliferation, ERK2 phosphorylates Thr57 and Ser130 of $p21^{CIP1}$, which induces $p21^{CIP1}$ ubiquitination and subsequent proteasomal degradation. In contrast, to promote growth arrest, ERK1/2 upregulates $p21^{CIP1}$ levels in cells for which ERK1/2 has non-kinase effects. These mechanisms highlight different mode of ERK1/2 signaling for the opposing physiological outputs.