REVIEW ARTICLE

Role of the Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway in Cancer

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Abstract

The Ras-ERK mitogen-activated protein kinase (MAPK) pathway is hyperactive in >30% of all human cancers, prompting the development of RAS, RAF, MEK, and ERK inhibitors. The identification of intracellular signaling cascades, which promote the growth and survival of cancer cells, is critical for developing targeted cancer therapeutics aimed at blocking these signals. Currently, there are various FDA-approved drugs to inhibit RAF and MEK mutations for cancer treatment, but patients rapidly develop resistance to these drugs within several months, necessitating the need to develop new drugs against other targets in the MAPK pathway. Developing RAS inhibitors has been challenging due to the high affinity of RAS for its natural ligands (GDP and GTP) and the lack of a druggable binding cavity. As an alternative to targeting RAS, ERK inhibitors, which have also been shown to work on RAF/MEK-resistant cell lines, can block the activation of ERK and act as an effective cancer treatment, causing tumor regression. However, to maximize therapeutic effectiveness, it seems unlikely that any monotherapy would be particularly useful. Future treatment strategies should be designed on a patient-by-patient basis to ensure the most rapid reduction in tumor growth and the minimization of off-target effects by using a combination of two (or more) inhibitors within this MAPK pathway that lead to tumor regression and positive patient outcomes.

Key words: mitogen-activated protein kinases; MAPKs; cancer; signaling; feedback regulation; activators; amplifiers; negative feedback; therapeutic targets; RAS; RAF; BRAF; $BRAF^{V600E}$; MEK; ERK; RTK; NF1

1. MAPK Signaling

Extracellular signal-regulated kinases are members of the mitogen-activated protein kinases known as MAPKs, which are a series of serine/threonine kinases involved in numerous physiological processes and affect various cellular activities related to growth, such as gene expression, differentiation, proliferation, and cell survival.[1] This pathway[2] (Figure 1) is commonly found to be upregulated in a number of human cancers, including numerous carcinomas, sarcomas, and melanoma.[3] Serine/threonine protein kinases are activated via phosphorylation within their activation-process loop.[4] Although mutations to RAS, an upstream protein in this series, are the most frequent MAPK mutations, researchers reported[5] that less frequent mutations in downstream proteins in the pathway, such as RAF and MEK, offer more realistic and responsive therapeutic opportunities.[6]

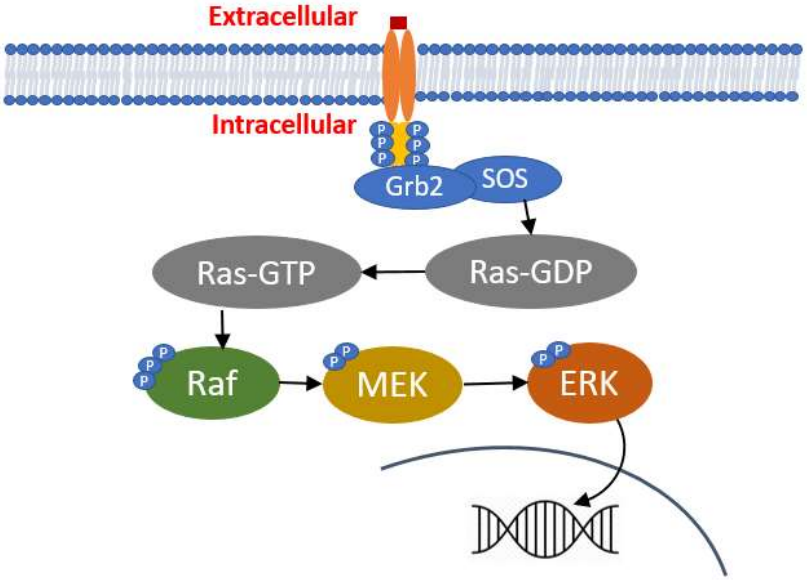

Figure 1: The MAPK pathway

The first classical MAPKs were discovered in the early 1980s by Cooper and colleagues.[7] They identified and reported ERK1/2 (known as MAPK2 and MAPK3 paralogs of serine/threonine kinases) as two structurally-related protein kinases that function[8] as mitogen-stimulated proteins of approximately 42 kDa, which later became known as ERK p44 and ERK p42, respectively.[9]

ERK is downstream of cell surface receptors that regulate cell growth and proliferation, activated by MEK1/2 via Thr-Glu-Tyr (TEY) motifs. ERK activation leads to negative feedback suppression of upstream signaling, caused by phosphorylation of transcription factors that regulate phosphatase genes and also direct phosphorylation of SOS and RAF. However, multiple studies have shown[10] that understanding and exploring the functional

characteristics of RAF and MEK can aid in developing effective therapeutic approaches to target RAS and ERK in dual-inhibitor or multi-inhibitor drug therapy.[11]

There are numerous reports[12] that MAPK inhibitors (also known as RAS/ERK inhibitors) have shown promising clinical efficacy as anticancer drugs, but most cancers eventually develop resistance to these drug therapies via reactivation of the pathway, which may proceed by a number of different mechanisms. One of the most important factors limiting the efficacy of current-generation MAPK inhibitors is the difficulty of inhibiting the ERK pathway while also preventing significant off-target effects and toxicities.

As one moves further downstream in the MAPK pathway, the frequency of gene mutations decreases.[13] Across human tumors, the frequency of mutation is 22% in RAS, 7% in BRAF, and less than 1% in MEK; ERK mutations are exceptionally rare, potentially due to its pleiotropic effects, meaning that mutated ERK would induce cell death.[5] ERK hyperactivation involves the alteration of one of the upstream proteins in the pathway, such as RAS, which is often susceptible to constraint by negative feedback signals. Downstream activation can limit the negative feedback regulation mechanism and lead to activation of the pathway.[13]

2. RAS Mutations

RAS consists of closely-related monomeric globular proteins of 189 amino acids (21 kDa molecular weight) associated with the plasma membrane.[14] This protein is a small GTPase and serves as the principal upstream activating component of the cascade, making it a high-interest target for therapeutic intervention.[5] When RAS is bound to GTP, the RAS-GTP complex is active and activates various signaling pathways, of which the MAPK pathway is best characterized; hydrolysis of RAS-GTP via auto-hydrolysis or the GAP protein returns RAS to its inactive RAS-GDP conformation. This GTP-GDP cycle is regulated by RAS guanine nucleotide exchange factors (GEFs) such as Son of Sevenless (SOS1).[5] Acting as binary molecular switches, Ras proteins cycle between active GTP-bound and inactive GDP-bound states. Active RAS-GTP binds to the N-terminus of RAF and leads to the activation and dimerization.[15] The dimerized RAF protein phosphorylates and activates MEK1/2 (MAPKK), the central component of the MAPK signaling cascade.[16] Activated MEK1/2 phosphorylates ERK1/2 (MAPK), which regulates downstream effectors that control differentiation, proliferation, and cell survival.

RAS mutations are the most common MAPK alterations observed in human cancers. Over the last three decades, targeting RAS has become an important goal of research into the MAPK signaling cascade[17] and has inspired many scientists to identify or develop compounds to inhibit RAS mutations. Mutations in the three main isoforms of RAS (HRAS, NRAS, and KRAS) are among the most common in human tumorigenesis.[18] Approximately 30% of human tumors carry mutations in RAS genes, with KRAS mutations detected in 25-30% of tumors. The KRAS isoform is prevalent among the top three most deadly cancer types: pancreatic (95%), colorectal (45%), and lung (35%).[19] More specifically, the KRAS-G12C mutation (glycine to cysteine at amino acid position 12) has been found[14] in 13% of all cancers, 43% of lung cancers, and in almost 100% of MYH-associated polyposis (familial colon cancer syndrome[20]). The rate of oncogenic mutations in KRAS is 85%, while rates in NRAS and HRAS are much lower, at 12%

and 3%, respectively. In addition, most RAS mutations occur at amino acid residues 12, 13, and 61, with the vast majority of KRAS mutations occurring at residue 12.[21] The mutation at residue 12 sterically hinders GAP proteins (GTPase-activating proteins) from accessing KRAS, inhibiting GTP hydrolysis and resulting in an increase in the GTP-bound active form of KRAS.[22] Therefore, due to the prevalence of the KRAS-G12C mutation in a variety of cancers, the study of RAS proteins has been a target of interest over the past decade. However, targeting the protein has been challenging; there are currently no drugs on the market that are FDA-approved that target RAS.

Recent studies have illustrated that downstream mutations in the MAPK pathway can be categorized in two groups: activators and amplifiers. As shown in Figure 2, activators lead to strongly activated ERK and cause negative feedback of upstream signaling. In contrast, amplifier mutations depend on upstream activity and lead to modest activation of ERK and consequently result in minimal negative feedback inhibition of upstream signaling.[13]

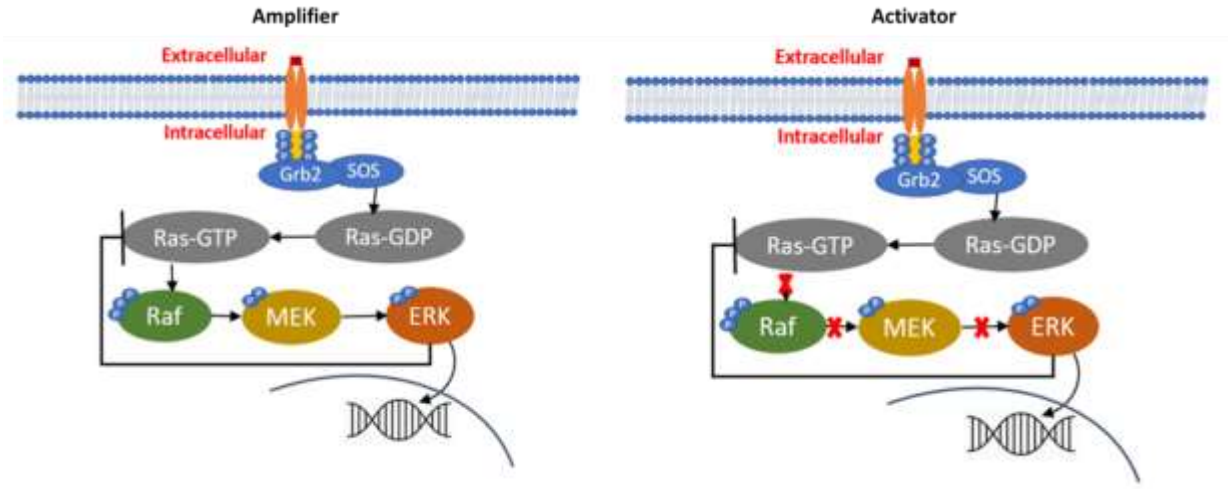

Figure 2: Amplifier and activator mutants in the MAPK pathway

2.1 RAS as a Therapeutic Target

The RAS protein is the principal upstream activating small GTPase of the MAPK signaling cascade, making RAS a high-interest target for therapeutic intervention.[23] As a result, many small molecule inhibitors have been developed attempting to target RAS proteins, specifically KRAS-G12C, but few have proved effective. However, recently, the irreversible covalent RAS inhibitor approach has shown some promise.[24,25] Yet, irreversible covalent inhibitors permanently modify the target protein and off-target binding as well resulting in increased toxicity.[26] Developing competitive reversible RAS inhibitors have been challenging due to the high affinity of RAS for its natural ligands (GDP and GTP) and the lack of an ideal binding cavity for inhibitors.[27] As mentioned above, in the past decade, targeting the KRAS-G12C mutation (i.e., glycine replaces cysteine at position 12) became a focus of scientific research due to the mutation's frequent abundance in lung cancers, corresponding to roughly 50%

of RAS-driven lung cancer tumors.[28] Novel methods targeting KRAS proteins have shown promising results, leading to clinical development of four KRAS-G12C covalently linked inhibitors[29]: AMG510 from Amgen, MRTX849 from Mirati Therapeutics, JNJ-74699157 (ARS-3248) from Wellspring Biosciences and Janssen, and LY3499446 from Eli Lilly. However, no KRAS compounds are as of yet FDA-approved.

3. RAF Mutations

RAF (rapidly accelerated fibrosarcoma) is the most frequently mutated protein in the MAPK pathway downstream of RAS, being mutated in 7-10% of all types of cancers.[30] Generally, RAF proteins dimerize and activate upon RAS activation. The RAF protein family includes three main isoforms: CRAF, BRAF, and ARAF.[31] The most common mutation in this family is found in BRAF; specifically, the mutation is $BRAF^{V600E}$, which is the result of a replacement of valine by glutamic acid at position 600. This V600E (or Val600Glu) mutation results in a new form of the BRAF gene that is abnormally active, can interrupt regulation of cell multiplication, and may lead to uncontrollable division and growth of histiocytes. This uncontrollable abnormal growth of histiocytes results in different types of non-inherited cancers and disorders such as Erdheim-Chester disease.[32] Under certain physiological conditions, this pathway is firmly regulated and shows negative feedback. However, the BRAF mutation can freely lead to uncontrollable division and growth of cells, resulting in cancer. RAF mutations have been classified generally into three distinct functional groups based upon their mechanism of action in the cancer pathway, with class I and II mutants constituting "activators" and class III mutants representing "amplifiers" (Figure 3).[33]

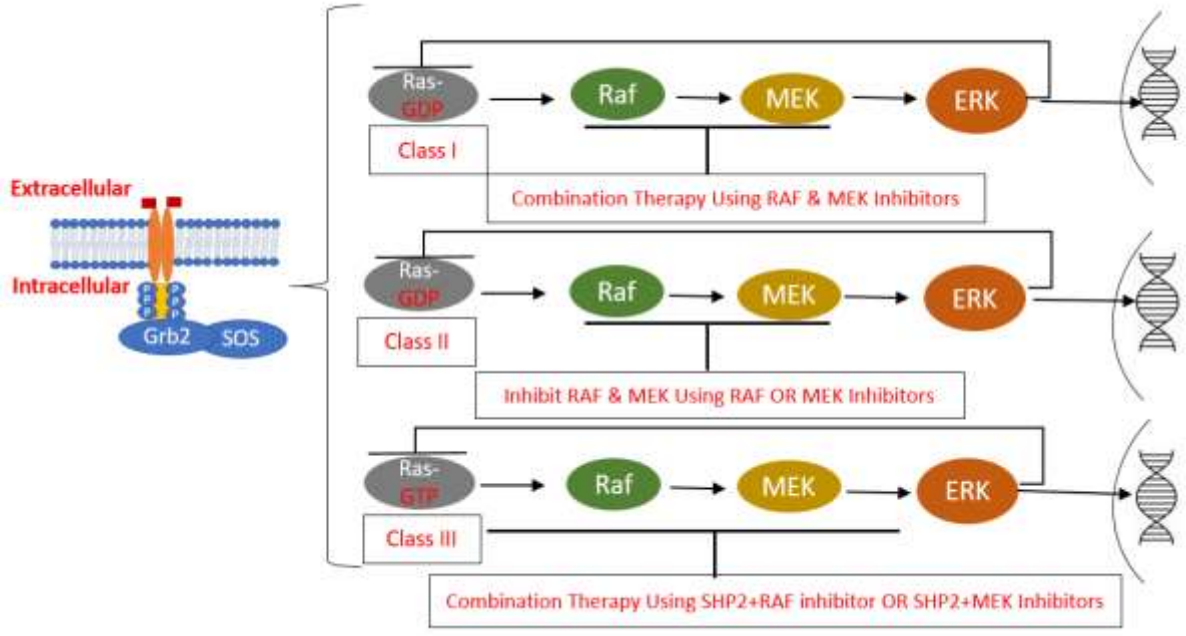

Figure 3: Functional classes of BRAF mutations for the cancer pathway

3.1 Class I BRAF Mutations

Class I BRAF mutations commonly involve the conversion of residue 600 from valine to glutamic acid, abbreviated as the $BRAF^{V600E}$-mutant, which represents over 90% of BRAF alterations reported.[5] The $BRAF^{V600E}$ mutation is found in ~50% of melanomas, ~40% of papillary thyroid cancers, and ~10% of colorectal cancers. While this mutation is the most common, in some cases valine-600 is converted to a different residue, such as lysine, arginine, or aspartic acid.[30] $BRAF^{V600}$ mutations are unique in producing a constitutively active BRAF kinase that can signal as a monomer.[34] The mutation of BRAF leads to high kinase activity followed by high levels of phosphorylated MEK and thus increased ERK phosphorylation. The ability of $BRAF^{V600}$ mutations to activate MAPK signaling is independent of RAS activity.[35] Some reports indicate RAS suppression in $BRAF^{V600}$ mutated cells due to negative feedback signals downstream of activated ERK.[36]

3.2 Class II BRAF Mutations

Scientists divide non-V600 BRAF mutants into two groups based on their properties and dependence on RAS activation.[37,38] Class II mutants, which comprise the first group with K601E[39], L597Q[40], and G469A[39] as its members, do not require RAS activation to dimerize. K601E and L597Q are located at the activation loop, or the Gly-rich loop, which contains G469A. These mutations and other MAPK mutations do not commonly occur simultaneously. High levels of ERK activation of these mutants suppress RAS activation. This type of BRAF mutant has intermediate kinase activity that leads to dimer affinity enhancement. This increase in dimer affinity triggers ERK signaling without the presence of active RAS. However, some reports demonstrated that class II BRAF mutants have shown resistance to EGFR (epidermal growth factor receptor) inhibitors among patients who suffer from lung cancer.[41]

3.3 Class III BRAF Mutations

Unlike class I and class II, class III needs an active RAS protein state to be able to activate the signaling cascade.[37] Conversely, class III mutants, including D594 and G466 mutants, result[42] in diminished BRAF kinase activity and increased ERK signaling by means of amplified signaling via wild-type RAF through mutant/wild-type RAF heterodimerization.[38] The activity of this heterodimer is critical to the abnormal signaling observed by class III BRAF mutants. Class III mutants demonstrate an ability to bind with greater affinity to RAS than wild-type BRAF and result in enhanced binding and activation of wild-type CRAF. Therefore, upstream RAS activation is required for ERK activation in tumors with these mutants. Tumors with high RTK (Receptor Tyrosine Kinase) activity have a particularly high occurrence of these alterations, leading to RAS activation and often occur simultaneously with activated RAS or NF1 (Neurofibromatosis type 1) loss-of-function mutations.[43] The frequency of concurrent mutations is observed to correlate with the basal activity of RAS, which depends on the tissue of origin. In melanoma cells, where endogenous basal RAS activity is low, the coexistence with mutant NF1 or RAS is nearly always observed. Conversely, higher basal RTK activation is seen in colorectal and lung cancers, resulting in RAS activity that is sufficient to support activation of these mutants, and coexistence with RAS/NF1 mutations is observed in only a minority of cases.[44] When RAS or NF1 are not mutated, the growth of tumors with class III mutations shows a sensitivity to the inhibition of the dominant RTK-driven RAS

hyperactivity. Consequently, EGFR (epidermal growth factor receptor) inhibitors have been shown to improve survival and tumor regression in colorectal cancers with impaired class III BRAF mutants.[45] Conversely, poor survival and an insensitivity to EGFR inhibitors is associated with activated class I and II BRAF mutations such as V600E.

3.4 RAF as a Therapeutic Target

The RAF protein family is recognized as one of the most important protein families in the MAPK signaling pathway due to its critical role. CRAF, which is also documented as one of the most important RAS effectors, was the first RAF protein identified as an oncogene.[46]

Currently, there are many FDA-approved RAF inhibitors on the market with the ability to selectively inhibit RAF monomers such as $BRAF^{V600}$ and class I RAF mutants.[34] However, most of the RAF inhibitors are often referred to as BRAF inhibitors, but in fact they do not have just selectivity for BRAF; they can inhibit other RAF mutants like ARAF and CRAF with similar potency.

4. MEK Mutations

Mitogen-activated protein kinase (MEKK or MEK) is one of the regulatory components in the RAS/ERK cascade. MEK is a dual-specificity protein kinase, which upon RAF activation, phosphorylates both of the necessary Thr and Tyr residues in ERK.[47] Unlike RAS and BRAF, MEK1/2 mutations vary, occur across both the MAPK1 and MAPK2 genes, and they are much less common in cancer genomes.[48] Generally, MEK mutations are divided into two main groups based on their pathway activation mechanism. One group activates the kinase activity of MEK by interrupting the protein's intramolecular interactions that drive inhibition, while the other group achieves the activation by increasing MEK homodimerization.[49] The resistance to inhibitors may be caused by an elevated dimer affinity in MEK proteins, as is the case in RAF proteins.

MEK 1 and 2 are 45-50 kDa proteins with 37 to 40% amino-acid sequence identity in the kinase domain and 86% sequence identity in the catalytic domain.[50] Unlike other MAPK proteins, MEK1/2 have strong leucine-rich nuclear export signals (NESs) in their N-termini.[51] As previously mentioned, MEK1/2 are dual-specificity protein kinases, which are thought to follow the same mechanism of action. However, they are actually regulated differently and act asymmetrically in the cell.[52] In 2018, several scientists suggested that MEK mutants can be divided into three classes based on their functionality: RAF-independent, RAF-regulated, and RAF-dependent (Figure 4).[53]

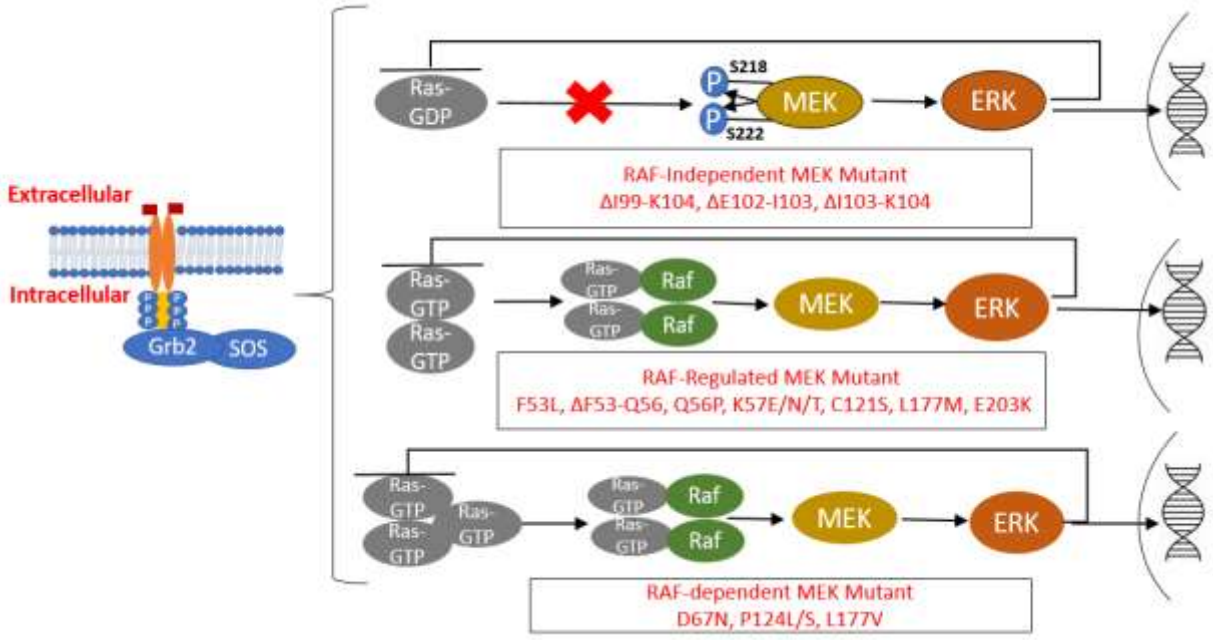

Figure 4: Physiologic MAPK signaling and functional classes of MEK mutations

4.1 RAF-Independent MEK Mutations

Independent of upstream signaling, MEK and ERK are strongly activated by RAF-independent mutants. These mutants are prone to possess in-frame deletions between the 98th and 104th amino acids that remove a potent negative regulatory segment of MEK1, contributing to its constitutive activation.[53] This region corresponds to a 3–αC loop mutation that constrains the kinase in the active "αC-in" conformation caused by the resulting shortened loop. This loss of the negative regulatory element drives autophosphorylation of the activating serine residues at positions 218 and 222 and results in a significant increase in MEK kinase activity. Expression of these MEK1 mutants can drive strong MAPK signaling and cellular transformations in "RAF-less" cells, which contain ARAF, BRAF, and CRAF (RAF1) alleles that can be deleted by an enzyme known as CRE recombinase. This transformation results in activation of MAPK signaling, regardless of the lack of RAF activity.[53] In human cancers, these mutations are found infrequently (less than 0.1% of all MAPK mutations) and, therefore, do not frequently occur with other MAPK alterations.

4.2 RAF-Regulated MEK Mutations

RAF-regulated MEK mutants result in demonstrably increased basal activity of ERK and, in the presence of activated RAF, can extend their signaling activation range. RAF-regulated MEK1 mutants can produce increased ERK phosphorylation in "RAF-less" cells compared to wild-type MEK1. However, ERK phosphorylation levels are significantly lower when functional RAF is present and are insufficient to drive cellular transformation, unlike the RAF-independent MEK1 mutants.[53] Mutation of the critical activating RAF phosphorylation sites on MEK1 (S218A and S222A) cause a reduction, but not elimination, of MEK kinase activity in these mutants. While these mutants exhibit a range of basal activity in

activating ERK, their kinase activity can be increased with the introduction of activated RAF. RAF-regulated mutants may occur with other MAPK alterations and have been observed to emerge as a potential mechanism of acquired drug resistance in patients treated with upstream inhibitors. MEK1-K57 mutants, for example, have been observed in patient groups with colorectal cancer that has formed resistance to EGFR antibodies.[54] In addition, MEK1-K57 and MEK1-F53L mutations have also been identified in patients with $BRAF^{V600E}$ colorectal cancer with an acquired resistance to RAF/MEK and/or EGFR inhibitor combinations.[55] As such, it has been shown that RAF-regulated MEK mutations possess characteristics of both activators and amplifiers.

4.3 RAF-Dependent MEK Mutations

RAF-dependent mutants increase ERK activation only in the presence of active RAF, by their affinity to bind more tightly to RAF, augmenting ERK activation.[56] These mutants do not lead to ERK phosphorylation in "RAF-less" cells and fail to drive the transformation in those environments. Similarly, mutation of the critical activating RAF phosphorylation sites on MEK1 (S218A and S222A) ceases MEK kinase activity in these mutants, making them particularly sensitive to feedback inhibition of RAF, limiting their functional output. These mutations occur nearly universally with upstream MAPK alterations such as BRAF or RAS mutations, making them amplifiers of RAF signaling.

4.4 MEK as a Therapeutic Target

Scientists did not consider MEK proteins as potential targets in the MAPK pathway for therapeutic treatments due to the rarity of MEK mutations.[57] In 1995, PD098059 was reported as the first MEK1/2 ligand with the ability to inhibit the dephosphorylated form of MEK1 and MEK1 mutants. Compound PD098059 is part of a family of allosteric inhibitors, which suggests that MEK1/2 proteins are candidates for cancer drug targeting.[58] Allosteric MEK inhibitors are highly specific for MEK, as they bind to the unique allosteric pocket, rather than the catalytic site, in MEK proteins. Notably, most allosteric MEK inhibitors are vulnerable; they show a reduced ability to inhibit MEK kinase activity in the presence of increased upstream MAPK signaling, which increases the activation of MEK through a two-step mechanism. During the first step, most of the MEK inhibitors favorably bind to the MEK inactive form, and many of them show a reduction in binding affinity to MEK when it is in the active form.[59] As a result, an increase in upstream signaling will increase the level of phosphorylation and activation of MEK and lead to the reduction in the ability of the inhibitor to bind and inhibit MEK, which results in an increase in the levels of activated MEK beyond what is needed for maximal ERK induction.[60]

Currently, there are FDA-approved MEK inhibitors in clinical use. However, patients treated with these MEK inhibitors also show drug resistance after several months of usage.[61]

5. ERK Mutations

In a comprehensive genomic study of tumors, the mutation of ERK was found to be extraordinarily rare.[62] MEK1/2 proteins are considered specific ERK1/2 activators. As ERK1/2 proteins regulate a variety of substrates, MEK1/2 plays the unique gatekeeper role in the MAPK cascade.[61] ERK1, also known as p44, and ERK2 or p42, are proteins encoded by two splice variants of the same gene.[63] ERK1/2 can be repeatedly activated by MEK1/2 upon

phosphorylation of Thr and Tyr residues, specifically Thr202 and Tyr204 of ERK1 and Thr173 and Tyr185 of ERK2.[64] Although ERK1/2 share the same gene, they have different functions for cell proliferation. For example, RAS-induced activation requires ERK2 specifically. Studies have reported that ERK1 competes with ERK2 for MEK in an antagonistic mode, which results in a weakening of ERK2 signaling. Currently, no reports indicate that the substrates for ERK1 and ERK2 are different.[65]

Upon being activated by MEK1/2, ERK1/2 proteins migrate into the nucleus and activate a significant number of transcriptional factors.[66] ERK1/2 have many substrates, and most play critical roles in the key physiological processes that control cell cycle progression, such as cell proliferation, differentiation, survival, and death.[67] Additionally, the activation of ERK1/2 substrates can result in feedback inhibition, which depends on whether a substrate can positively or negatively regulate the ERK1/2 signaling pathway.[67]

The ERK cascade is controlled by feedback loops whose effects are categorized into two main groups: rapid short-term effects and delayed long-term effects.[68] Rapid, short-term feedback is characterized by the direct effects of ERK kinase activity on its upstream effectors, namely SOS and RAF. Phosphorylation of these proteins leads to their inactivation and a subsequent decrease in pathway signaling. Delayed, long-term feedback involves activation of transcription factors by ERK that lead to the expression of proteins such as Sprouty (SPRY) and various dual-specificity phosphatases (DUSPs). SPRY prevents RAF catalytic activity and can also directly inhibit SOS and the RTK itself. DUSPs selectively cleave the phosphate groups from the phospho-threonine and phospho-tyrosine residues on ERK1/2 and result in ERK inactivation.[66–75] The ERK1/2 proteins phosphorylate both BRAF and CRAF to achieve inhibition of MEK phosphorylation.[76]

In contrast, the delayed feedback mechanism effect, which can be described as a novel communication method between Sprouty proteins (SPRY) and dual-specificity phosphatases (DUSPs), leads to normalizing the MAPK pathway by de-phosphorylating ERK1/2.

Under physiological conditions, these feedback mechanisms maintain homeostasis and ensure control of RAS-ERK signaling, indicating their importance and illustrating critical checks that can be disrupted in cancer.[77]

5.1 ERK as a Therapeutic Target

Not long ago, it was assumed that RAF and/or MEK inhibitors would be sufficient to inhibit ERK1/2 activity and that there would be no additional benefit of directly blocking ERK kinases. Therefore, the development of new direct ERK inhibitors lagged behind the development of RAF and MEK drugs. However, reports illustrate[78] that, because most resistance mechanisms to RAF and MEK drugs result in reactivation of ERK1/2, directly blocking ERK1/2 may overcome the current limitations of RAF or MEK inhibitors.

In recent years, scientists have designed and developed direct inhibitors of ERK, several of which have entered the early clinical-testing phase. Some of these direct ERK inhibitors, like SCH772984[79] by Merck, showed a dual mechanism of action, which means the ligand inhibits not only the kinase activity of ERK (inhibition of pRSK) but also the phosphorylation of ERK by MEK (inhibition of pERK) through a large shift of

the glycine-rich loop of ERK upon binding.[80]

Other reported ERK inhibitors, such as ulixertinib (BVD-523) and the structurally related tool compound Vx-11e, successfully inhibit ERK kinase activity. However, they result in significant increases in MEK-mutation via ERK phosphorylation driven by the release of negative feedback signaling.[81,82]

Yet, with all the promising results of ERK inhibitors in cancer therapy, currently, there are not any FDA-approved drugs available on the market, and this series needs more attention and time.

6. Conclusions

Through a combination of the high toxicity of currently available therapeutics and the rapid development of potentially multi-faceted resistance mechanisms cultivating in the reactivation of ERK1/2, it is an understatement to recognize that current treatments targeting the RAS-RAF-MEK-ERK signaling cascade are less than ideal. Efforts to target ERK and RAS are ongoing, and the role of these proteins in future cancer therapies cannot be ignored. However, it should be clear that, to maximize effectiveness, any therapy must be designed on a patient-by-patient basis to ensure the most rapid reduction in tumor growth and the minimization of off-target effects. Therefore, it seems unlikely that any monotherapy would be particularly useful, as has been shown in the clinical use of RAF and MEK inhibitors – rather, it is the combination of two (or more) inhibitors within this MAPK pathway that can lead to tumor regression and positive patient outcomes.

Initial testing of compounds such as SCH772984 and ulixertinib provides promising results that indicate the targeting of ERK is a crucial juncture and an important effort, but further study is needed to ensure that any compound is vetted in the clinic. Because of the critical role of both ERK1/2 signaling and RAS signaling (including other MAPK pathways such as the PI3K pathway), compounds targeting these crucial proteins must be incredibly potent to mitigate side effects. Additionally, because of the potential for pleiotropic effects of ERK or RAS inhibition, it may be advantageous to target other proteins, such as the RTK directly, or the adaptor proteins Grb2 and SOS. Importantly, these may be easier to target than RAS due to their specific and unique binding pockets, which are likely to be highly druggable. In all, it can be said that a combination of innovative RAS inhibitors, potent ERK inhibitors, inhibitors of other mechanistically important proteins, and the use of already-approved RAF and MEK inhibitors will all be crucial in the continuing fight against human cancer.

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